SOYBEAN YIELD AND QUALITY RESPONSES TO NITROGEN AND SULFUR MANAGEMENT

by

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I would like to dedicate this thesis to my husband, Jonah and to future agronomists looking to challenge themselves every day to see and learn from more fields and find solutions for growers who are looking to improve their operation.

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ABSTRACT

Reductions in atmospheric deposition of sulfur (S) coupled with increases in yields of *Glycine Max* (L.) Merr. (soybean) has led to S deficiencies in Indiana. Poor nodulation due to limited S, and thus a decrease in nitrogen (N) supply, restricts the yield and quality of soybean grain (i.e., protein). Sulfur is a key component of methionine and cysteine, which are important amino acids in the nutrition of foodstuffs. The objective of the first study is to improve yield and composition of soybean through various applications of N and S. Ten N+S fertility treatments were factored by 2 planting dates (early vs. late) at West Lafayette, IN in 2018 and 2019. The same 10 N+S fertility treatments were factored by 2 varieties (Asgrow 24x7 and 34x6) at Wanatah, IN in 2018 and 2019. Soybean yield increases among the N+S fertility treatments of the May 11th planting (early) were 380 to 1006 kg ha^{-1} over the untreated control, with no difference within the June 5th planting (late) in 2018. Cool and wet conditions that limited mineralization of N and S from the early planting are likely the source of yield improvements. Protein concentrations were maintained and even increased with N and S treatments that were coupled with yield improvements. The Wanatah location showed that protein levels were increased with the ATS and R4+ NS treatments, while the UAN Direct treatment had the lowest protein in both varieties, suggesting that having no source of S could limit protein development. Although variety did not affect yield, fertility improved yields with the V4R3 NS, Plant NS, R3 NS, R4+ NS, and V4 NS treatments. The yield improvements that developed with these treatments is interesting because each treatment contained a source of N equaling at least 44.8 kg N ha^{-1}.

Secondly, the optimal rate and timing of foliar S applications were determined at a S-deficient location (La Crosse, IN) in 2018 and 2019. Three target application timings; V4, R3, and V4 + R3, were crossed with 4 rates of foliar S at 1.12, 2.24, 4.49, 6.73 kg S ha^{-1} with each application. Therefore, the sequential application (V4 + R3) received a total of 2.24, 4.49, 8.96, and 13.44 kg S ha^{-1}. The optimal rate with 2018 yields was 4.5 kg S ha^{-1} at V4 or R3; whereas, the optimal rate was 7.9 kg S ha^{-1} with the sequential V4 + R3 treatment in 2019. Leaf tissue concentrations of S were nearly deficient (0.25%) post-V4 and post-R3. Higher rates of S had greater S concentrations in the leaf; furthermore, most cases resulted in a linear increase of S concentration with the rate of S applied. Foliar applications of S also reduced N:S ratio. Protein levels in 2018 increased at an equal rate for both the V4 and the R3 timings. In 2019, at a 6 lb ac-

[1] rate of S the protein levels were 39.5 and 39.8% for V4 and R3 timings, respectively. Foliar S applications at V4 vs. R3 timings had little variation in yield or protein levels, thereby resulting in flexibility for application timing for growers.

CHAPTER 1. REVIEW OF LITERATURE

1.1 Background

1.1.1 U.S. Soybean History

Soybean appeared in North America in 1765 when Samuel Bowen brought seeds back to Georgia from his trip to China (Hymowitz and Harlan, 1983). The crop soon found its way across the Midwestern United States when it was brought to Alton, Illinois in 1851 (Hymowitz, 1990). Although the crop spread across North America, it was not until the 1920's that soybean was used for anything more than a forage (Hymowitz, 1990). In that decade the crop made a transition of purpose from solely forage to harvesting the grain to create oil-based products and soybean meal. Through the decades soybean has continually been improved through genetics and management. In 1984, the average yield was 1896 kg ha^{-1} (28.2 bu ac^{-1}) covering 26.7 million hectares (66 million acres). This was a great improvement from the 739 kg ha^{-1} (11 bushel) average in 1924 on just 0.6 million hectares (1.5 million acres) (Hymowitz, 1990). Soybean use and production has continually increased through the decades.

1.1.2 Current U.S. Production and Usage

In 2018, soybean production hit a new record with 123 billion kilograms (4.54 billion bushels) harvested. The average yield was 3470 kg ha^{-1} (51.6 bu $acre^{-1}$) and this was produced on over 35 million hectares (88 million acres) ("USDA - National Agricultural Statistics Service - Statistics by Subject Results," 2018). The United States leads in soybean production over Brazil and is the largest exporter of raw soybean (James Karuga, 2018). Soybean has become the primary source of protein for livestock feed and soybean meal, which provides highly digestible essential amino acids like lysine, methionine, and threonine in a cost-effective manner (Willis, 2003). The preference for the swine and poultry industries for feed protein is soybean meal (Krishnan and Jez, 2008). Both industries are experiencing a greater need for higher nutritive feed, and therefore have been needing to supplement synthetic lysine, a key amino acid present in soybean, but not in great enough amounts to meet the demand (Krishnan and Jez, 2008).

As land becomes more expensive and less available, farmers feel the pressure to maximize yield in each field planted. Higher yields are beneficial from a marketing standpoint; however, as yields increase, protein and overall seed quality generally decrease (De Mello Filho et al., 2004). Soybean quality could be improved through processing and additives of amino acids that are limiting, but as a result, the cost of that processing may diminish processed meal use and effectiveness as animal feed (Dei, 2012). Other research has focused on increasing oleic acid to provide food products with healthier fats for consumption (Krishnan and Jez, 2008). Increasing oleic acid, which is lower in unhealthy trans-fat, would present more extensive food options for the growing population (Krishnan and Jez, 2008).

1.1.3 Planting Dates Responses

As of 2007, two-thirds of Indiana farmers planted soybean approximately one to three weeks earlier than was planted in 1997 (Conley and Santini, 2007). Even in the 1950's, farmers preferred to plant in the first half of May, higher yields were observed compared to late May or early June plantings (Morse, 1950). Cool temperatures during early May in the upper Midwest cause some concerns, but it is recommended that even a chance of a spring frost should not keep early planting from commencing (De Bruin and Pedersen, 2008). Early planting would differ in regions based on climate, but overall late April planted crops should miss any frost by the time emergence occurs (De Bruin and Pedersen, 2008).

Earlier planting opens an opportunity for the reproductive period to start earlier, which results in longer days and more intense lighting during this sensitive and key part of the crop's life cycle (Robinson et al., 2009). Consistently higher yields with earlier planting resulted from more seeds per square meter (De Bruin and Pedersen, 2008) or pods per square meter, not necessarily higher seed weight (Robinson et al., 2009).The higher number of seeds and pods can be connected to a longer vegetative, and more importantly, reproductive periods (Wilcox and Frankenberger, 1987). It was also found that as planting got delayed into early June, the percent of reproductive nodes continually decreased (Robinson et al., 2009).

Planting date not only affects soybean yield, but it also affects soybean seed quality. It is believed that the rate of dry matter accumulation plays a key role in determining the protein and oil concentration levels at maturity as a result of dilution increased with increased dry matter production (Muhammad et al., 2009). Specifically in early planted soybean, higher temperatures, while the soybean is developing and maturing, results in higher oil content in the seed (Muhammad et al., 2009). This study in Peshaward, Pakistan was at a latitude of 34.0º compared to West Lafayette's 40.4º that consists of warmer months compared to Indiana; however, the concept of temperature trends would translate to the southern United States. Earlier planting increased oil content compared to later plantings in the Pakistan soybean study, while protein levels were consistent until the latest planting, occurred in July (Muhammad et al., 2009). Piper and Boote found that much of the variation in protein levels is not explained by temperature alone, and the variation could be a combination of climate, genetics, and the balance of oil concentration levels (Piper and Boote, 1999). Seed oil increased with higher growth temperatures.

Under irrigated conditions in Mississippi, earlier planting produced more oil, but less protein. Conversely, late planting produced higher protein and lower oil concentrations (Bellaloui et al., 2011). One possible explanation is the resulting temperatures during seed-fill. Higher temperatures (30-40ºC) during this sensitive stage may result in higher oil, while higher protein levels in the late planting may be due to lower temperatures (25-35ºC) during the seed-fill period (Bellaloui et al., 2011).

An older study looking at drought and temperature conditions and its effects on seed composition found different results. This study was done in Ames, IA and had certain climatic conditions imposed on the potted plants, instead of being in a field where temperature and rainfall changes are common. Imposed drought stress through less soil saturation during watering and usually higher temperatures during seed-fill increased protein and decreased oil in the seed (Dornbos and Mullen, 1992). This greenhouse study had controlled air temperatures that were measured closely during seed fill and also had stress levels controlled and measured carefully. Drought stress was measured as stress degree days, adding the leaf temperature and air temperature difference during seed fill. Previous research shows that protein content decreased between 21 and 27ºC and increased at temperatures greater than 27ºC. Oil increased between 21 and 29ºC and decreased at higher temperatures. This information suggests that a critical level of 28ºC exists, at which point oil reached a maximum level and protein ended at its lowest concentration (Dornbos and Mullen, 1992).

Likewise, another study that performed a meta-analysis on data from the Uniform Soybean Test Northern Region showed that although not significant, a decrease in protein was observed with higher temperatures. The interesting component in this study was that oil content was reduced with increased temperatures from R5-R8 (Rotundo and Westgate, 2009). Researchers explained that oil was affected by current photoassimilate production, which could be negatively affected by higher temperatures because the seed fill stage is shortened. Protein is not as affected by high temperatures and senescence, because the flow is less variable and the rate of flow each day will compensate for a shorter filling time (Triboi and Triboi-Blondel, 2002). With this abundant supply, protein can continue to contribute to the seed under these more stressful temperatures.

With the inconsistencies in literature, it is hard to determine specific critical temperatures and exactly how the soybean composition will react to these changes. The literature agrees on one thing; seed composition is affected by temperature changes during the seed-fill period which is between R5 and R8 (Dornbos and Mullen, 1992; Rotundo and Westgate, 2009; Bellaloui et al., 2011).

1.1.4 Maturity Group Effects on Soybean Physiology

Without the diverse adaptation of soybean through the adoption of maturity groups, this crop would still be a very photoperiod sensitive short-day crop (Liu et al., 2017). Between groups, there are some characteristics to consider when developing farming plans. One study found that maturity group can affect phenology, physiological processes, photosynthetic active radiation interception, and allocation of nitrogen (Santachiara et al., 2016). The same study found no significant differences in biomass accumulation or seed yield between maturity groups III and V.

Egli found that total nodes per plant increased the longer the growth period (Egli, 1993). This study showed that later maturing cultivars had increased biomass and nodes; however, the shorter maturing cultivars may have had more plants in an area to contribute to total nodes, ending similar yields as the full maturity groups (Egli, 1993). Although more biomass may be a result in later maturing cultivars, there is no apparent advantage with crop growth rate (CGR) between maturity groups.

Larger plants with an excess of leaf area index can tolerate more stress than earlier maturing plants, but this does not necessarily put early-maturing soybean at a disadvantage (Egli, 1993). A longer life cycle does not necessarily transfer into more seeds, longer seed-fill duration, or a higher crop growth rate (Egli, 1993). For example, growers could plant shorter maturities in narrower rows to close the canopy much quicker than longer maturities planted in wider rows. Early-maturing varieties from maturity group II to IV have been known to have higher water use efficiencies and therefore have smaller irrigation requirements compared to longer maturing varieties when used in the midsouth (Edwards and Purcell, 2005).

1.1.5 Historical Fertilizer Practices

Fertilizer recommendations have changed considerably over the decades. In the 1920s and 1930s, fertilizer recommendations were maede based on the crop being grown and other management practices. With solely that information, varying amounts and types of fertilizer grades were recommended to growers (Warncke et al., 2009). Later in the 1940s, another level of criteria was added, and recommendations were provided based on soil texture (sandy, loamy, or clayey) and if manure had been applied within the past two years (Warncke et al., 2009). The next decade brought further advancements via soil sampling and testing. Growers were provided more accurate results (simple scale from low to high for phosphorus (P) and potassium (K)) and better recommendations. These results came from the Spurway "reserve" soil test that provided a closer evaluation of the fertility program growers were implementing (Warncke et al., 2009). Green manure crops, barnyard manure, lime, or crop residues were some fertilizer choices in the 1950s (Morse, 1950).

The Bray P1 test and ammonium acetate tests were introduced in 1960s and could divide earlier simple categories even further to very low, low, medium, high, and very high categories for phosphorus and potassium, respectively (Warncke et al., 2009). These tests are still used today to help make fertility recommendations. N research in the last couple of decades has shown that early nitrogen fertilization can inhibit nodulation and in the end, limit nitrogen fixation in soybean. Lower fixation may occur because soil nitrate is more available to plants and requires less energy for uptake compared to nitrogen fixation (Zhang and Smith, 2002).

In literature, very few past sulfur recommendations are on record for soybean. Sulfur (S) 5was not a concern due to some incidental S being applied with other fertilizer mixes, manure, and the atmospheric deposition of S (Gilbert, 1951). Sulfur deficiencies were found in several states from the 1920s to the 1940s. Other findings determined that farms near urban areas rarely encountered S deficiencies, due to the atmosphere deposition of S from urban factories (Gilbert, 1951). Fertilizers with a high analysis of N, P, or K has resulted in less S application through those fertilizers (Chen et al., 2005). With higher concentrations of these primary nutrients being applied, smaller amounts of fertilizer are used and in general, and these fertilizers tend to have a lower percentage of S overall. Suggestions to maintain or supplement S have included adding biosolids, manures, or compost to the soil. By adding these components, the S that is available to the crop from the soil will dramatically increase (Dick et al., 2008). University of Nebraska suggested that S could be a good addition to growers' corn fertility programs, if farming on sandy, low organic matter soils. This could be an application in the form of broadcasted elemental S, or a banded application of thiosulfate if the soil test (Ca $(H_2PO_4)^2$ extraction) is less than 8 ppm, depending on the irrigation water sulfate levels (Shapiro et al., 2008).

1.2 Sulfur and Nitrogen in Soybean

1.2.1 Nutrient Requirements in Soybean

Crop management and variety genetics have evolved a lot since the 1970s. Practices like planter choices, row spacing, and planting densities have all adapted to the growing need for higher yields on less land (Bender et al., 2015). Nutrient requirements were updated to provide the crop with what is needed for the highest potential yield. Over the last 80 years varieties have continued to increase biomass production, grain yield, and harvest indices resulting in higher nutrient accumulation and nutritional needs (Bender et al., 2015). In modern varieties P, N, Copper (Cu), and S have harvest indices above 60%, which is the relative proportion of grain nutrient to total nutrient accumulation (Bender et al., 2015). These nutrients could be yield limiting and adequate amounts may not be available for the crop later in the growing season.

Biological nitrogen fixation can provide anywhere from 48-93% of a soybean's nitrogen needs, with the United States averaging about 60% (Tamagno et al., 2018). Biological nitrogen fixation can provide all the nitrogen the soybean needs; however, this can reduce yield in stressful environments due to the reduction of oil or protein that makes up for the energy it takes for nitrogen fixation to occur (Tamagno et al., 2018). Harvest indices for phosphorus and overall dry weight increased during seed fill, while K decreased. With this information, it is important to note that a nutrient management plan needs to be in place during the critical seed fill stages (Bender et al., 2015).

1.2.2 Nutrient Uptake and Accumulation

All K and iron (Fe) needed for the season are taken up in the late vegetative and early reproductive stages of soybean; whereas, N, S, P calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), boron (B), and Cu are taken up throughout the entire growing season (Bender et al., 2015). This being said, these nutrients might need supplemented later in the season to meet the plant's needs. Some foliar applied S early in the season increases the S concentration in the plant and suggests that luxury uptake may occur with large applications early in the growing season (Kaiser and Kim, 2013). When N and S were looked at individually, N begins to remobilize to the seed after R5.5 (Gaspar et al., 2017). S that is accumulated late in seed fill is the primary source of the sulfur-containing amino acids that synthesize to contribute to protein formation (Sexton et al., 1998b).

Later in seed development, if S is collected by the foliage, it is automatically transported to the pods and seeds before ever entering leaf proteins, since seed growth is much faster than vegetative growth (Naeve and Shibles, 2005). Sulfur from the foliage supplies approximately 20% of the total S needed, while pods supply about 10% of that total (Naeve and Shibles, 2005). The largest expanded tissue is necessary as it plays an important role in transporting S from the roots to the newest expanding leaves; furthermore, researchers found that a quarter of this S was recycled through the root system (Sunarpi and Anderson, 1998).

If S is in abundance, the leaves higher up in the canopy often have higher S concentrations than the lower leaves, due to translocation. Once leaves have enough S, the plant will often move S higher up in new tissues (Hitsuda et al., 2008). If S is sufficient for the crop, leaves themselves by the end of vegetative development will have collected around 60% of the total plant-sulfur needed in the season (Hitsuda et al., 2008).

If S is not provided in sufficient quantities before seed fill, then enough S may not be mobilized to the seed (Naeve and Shibles, 2005). Approximately 40% of developing seeds' S needs were met by remobilized S, and that is why sulfur must be provided adequately prior to seed fill to allow time for mobilization of that sulfur to the seed (Naeve and Shibles, 2005). Sulfur is important for the utilization and efficiency of other nutrients.

1.2.3 Nitrogen's Role in the Plant

N is needed in very large amounts due to its high protein content hovering around 34% in soybean grain (Hurburgh et al., 1990). As modern varieties continue to excel at achieving high yields, the N harvest index will also continue to increase (Gaspar et al., 2017). Data has shown that assimilated N in soybean is correlated with the crop's yield (Ohyama et al., 2017). To be able to meet the N needs for soybean, the crop relies on storing the N in tissues and then remobilizing it during seed fill (Gaspar et al., 2017). Approximately 24% of N in the seed originates from the leaves of that plant that is then remobilized.

About 50-60% of the soybean's N demand is met by N fixation (Salvagiotti et al., 2008). Although it has been stated that supplementing N with fertilizer to soybean can depress nodulation and N fixation, research has shown that supplying small amounts of N consistently from soil or manure may help soybean growth and not slow fixation activity (Ohyama et al., 2017). In some cases, well-nodulated soybean growing in non-stressful conditions managed to be a high-yielding crop with a resonse to N fertilization. The best N management may include use of slow-release sources placed deep in the soil profile, which is below active nodules, or applying N during reproductive growth stages. Applications made at R3 to the soil surface showed slight numeric yield increases (Freeborn et al., 2001). A lack of response to N applications may be a result of the high soil N levels from mineralization and well-nodulated soybean. N fixation is more efficient for N supply when abiotic or biotic stresses are present (Salvagiotti et al., 2008).

1.2.4 Sulfur's Role in the Plant

Sulfur is a key component in the quality of soybean meal that is produced. Sulfur is needed in N fixation, and legumes are known to be high in protein; therefore, legumes are very susceptible to the effects of S deficiency (Dick et al., 2008). Overall protein in the seed is attributed to S, because S is a key component of cysteine and methionine (Chen et al., 2008). Cysteine alone is essential for non-ruminant animals and is one of the determining factors for nutritive value of feed (Hitsuda et al., 2008). Sulfur is involved in plant metabolic reactions and can be a constituent of coenzymes or secondary products (Chen et al., 2005).

Sulfur that is taken up during reproductive stages is converted into seed protein and continues to do so through seed fill stages (Sexton et al., 1998b). As important as S is to the quality and fill of grain in soybean, its remobilization efficiency is less than N, making it potentially the limiting factor for higher protein quality (Sexton et al., 1998a). The remobilization inefficiency of S may not be able to be reversed since it has been noted in both S sufficient and deficient environments (Sexton et al., 1998a). With deficiencies in this key nutrient, protein synthesis is slowed, and photosynthesis rates are incredibly decreased (Chen et al., 2008). Sulfur may be stored in stem tissue and not remobilize to the sink properly, resulting in deficient grain, but not visible on the tissue. This could be the result of less protein available for S utilization, or due to the inability of the plant to produce more amino acids (Sexton et al., 1998a). Yield reductions may not be obvious, but the crop may still be S deficient and lack protein that could be otherwise increased with sufficient fertilization (Hitsuda et al., 2008).

Sulfur applications may result in the crop having a deeper, lush green appearance due to increased chlorophyll production, but this factor does not always translate to yield gains (Dick et al., 2008). Chloroplast proteins in Sudangrass contained about 70% of the total protein-sulfur (Hanson, E. A.; Barrien, B. S.; Wood, 1941). The research can explain why some studies resulted in increased chlorophyll. For example, Zhao found that treatments including S increased chlorophyll content. Highest chlorophyll values were found during the pod-filling stage, which requires more photosynthesis for grain production (Zhao et al., 2008).

Another study showed that S fertilization increased the number and total weight of soybean side roots, which can help in plant absorption. Root nodules had the greatest weight during the pod filling stage, when the crop is more susceptible stress and usually requires the highest amount of nutrients (Zhao et al., 2008). Increased root mass and nodules from S fertilization can improve nutrient uptake and contribute to the darker green color as described above.

Sulfur applications increase pods plant^{-1}, seeds plant^{-1}, as well as one-hundred seed weights (Zhao et al., 2008). All of these factors contribute to and have the ability to increase soybean yield.

1.2.5 Nitrogen & Sulfur Interactions

With only minimal research studies, it is thought that N uptake is improved when soil S levels are adequate, as well indicating N and S may work hand-in-hand (Chen et al., 2008). Nitrogen use efficiency decreased if S levels were not sufficient, and as a result, this left unused N in the soil, which could then lead to more N loss through leaching and volatilization (Haneklaus et al., 2008).

In the plant, N and S interactions have been noted. For example, a study investigating the interactive effects of N and S on rape-seed-mustard (*Brassica Napus*) found that treatments fertilized with both S and N had higher leaf area index (LAI), photosynthetic rates, and biomass accumulations compared to treatments with solely N applied (Ahmad et al., 1998). Hitsuda concluded that S can control N utilization since there was a positive linear relationship between S and N concentrations in tissues during flowering (Hitsuda et al., 2004).

A narrow N to S ratio points to higher quality protein in the seed due to more sulfur-containing amino acids (Radford et al., 1977). Although done in Sudangrass, it was found that the ratio of protein-N and protein-S in the chloroplast remains relatively constant throughout the plant's lifecycle (Hanson, E. A.; Barrien, B. S.; Wood, 1941). This trend could possibly help explain the increase in greenness observed in soybean studies.

Sulfur may have effects on N fixation throughout the growing season. One study found that S increased the total number of nodules, the number of nodules per unit length of root, and increased nodule mass in clover (Trifolium) species (Anderson and Spencer, 1950). The same study found that plants deficient in S had fixation more restricted. Researchers found that increased N application rates depressed nodulation in low levels of S; however, with increased S rates, the nodulation depression was offset (Gates and Muller, 1979). Tamagno states that relative abundance of ureides (RAU) was reduced from 90% in unfertilized plots to 75% in plots with N fertilizer applications (Tamagno et al., 2018). This reduction in RAU was greater when fertilizer was applied at reproductive stages as opposed to vegetative stages.

1.2.6 Sulfur Deficiencies

Wide-spread S deficiencies are a result of fertilizer applications that contain little to no S, intensive cropping systems with high yields that remove more S than is available during the season, as well as less atmospheric deposition to provide sufficient sulfate levels to the crop (Chen et al., 2008). Sulfur removal by crops has greatly increased over the last couple of decades. In 2008, crops removed anywhere from 18-50% more S when compared to 1983 (Dick et al., 2008). In Ohio, S deposition has slowly diminished by 37% from 1979 to 2005 (Dick et al., 2008). Spots with greater S deposition can still exist today. In 1950, Bertramson found that there was a greater amount of S deposited near Gary, IN, when compared to ten other Indiana cities, as a result of greater industry activity (Bertramson B. R. et al., 1950).

A field diagnosis for S deficiency can be made by recognizing the yellowing that begins at the top of the soybean canopy and works its way down (Hitsuda et al., 2008). This yellowing is a result of lower chlorophyll and protein concentrations because the sulfur-containing amino acids are not synthesizing as quickly due to reduced photosynthetic rates. The deficiency appears very similar in appearance to other nutrients such as N, Mo, Mg, Mn, and Fe. Because of these similarities, a tissue test should be taken to confirm deficiencies (Hitsuda et al., 2008). Plant recovery is possible if S is applied and available to the plant early in the deficiency process. Recovery in deficient soybean could mean up to a 30% yield increase (Agrawal and Mishra, 1994). If a crop experiences S deficiency early and stunts the growth, a late application (R4.5) has been seen to be too late and will not compensate for the yield loss (Sexton et al., 1998b).

1.2.7 Tissue Sampling

Tissue samples sent for analysis, specifically for S concentrations are important as N:S ratio and chlorophyll-meter readings are not as accurate to diagnose S deficiency (Hitsuda et al., 2008). Tissue samples are collected from the third leaf from the top of the plant during flowering since that is the latest maturing leaf (Hitsuda et al., 2008). The deficiency level for most crops in tissue samples is less than 0.2% S (Dick et al., 2008). Purdue researchers found a higher N:S ratio and lower tissue concentration points in S deficiency, and that it is important to consider both when evaluating S availability to the plant since S works closely with N (Camberato and Casteel, 2017). In soybean, critical levels have not been as closely evaluated as other crops, where S levels between 0.2 and 0.3% in soybean are sufficient (Camberato and Casteel, 2017). The Southern Cooperative Bulletin agreed with the sufficiency level in tissues ranging from 0.2-0.6% (Campbell, 2000). The N:S ratio most institutions look for is 15:1, because this is the level where the sulfur-based amino acids occur in proteins (Campbell, 2000; Soybean Sulfur Status, 2012; Camberato and Casteel, 2017).

1.3 Soil Fertility

1.3.1 Tests for Sulfur

Soil tests for S are unreliable with high amounts of variation between labs. Labs may perform extractions differently, so labs must be calibrated in their methods to obtain similar results

(Crosland et al., 2001). The Morgan reagent, $Ca(H_2PO_4)^2$, and KH_2PO_4 were solutions that were satisfactory for determining available S due to the correlation with relative yield and S uptake of plants (Hitsuda et al., 2008). Mehlich III and weak alkaline extractants can extract part of the organic S from minerals in the soil better than weak salts; however, it is still a glimpse in time with limited accuracy and ability to make season long considerations (Dick et al., 2008). Soil analysis should be accompanied by tissue analysis, as the soil data is questionable and laborious (Hitsuda et al., 2008). These tests are not concrete because S has a gaseous biogeochemical cycle rather than a sedimentary one like Ca, P, and K (Dick et al., 2008).

Bloem shares a prediction model that has been created to determine the prognosis of S deficiency. This model uses site-specific details to calculate the S supply of that agricultural location. Some of these parameters and processes include S leaching risk, groundwater level, soil texture, crop type, precipitation, rooted soil depth, and groundwater S-concentration (Bloem et al., 2002).

1.3.2 Soil Factors as Sulfur Predictors

Soil characteristics can provide a glimpse as to the risk of S deficiency in a field. In poorly drained soils, there is slower mineralization or slower release of nutrients, as well as less intensive leaching (Lowe, 1969). Total S appears in higher amounts in dark soils with high organic nitrogen and carbon content (Bettany et al., 1973). Soybean yields did not increase with a S application if planted in a high organic matter soil that had ample supply of S from the soil and air (Chen et al., 2005). Research shows that when adding liming materials to the soil, these materials not only raise pH, but also increase the production of sulfate from the organic matter present in that soil (Freney et al., 1962). It is common that low organic matter, coarse soils that are well drained are more susceptible to leaching of sulfate and therefore, a much lower supply of available sulfate for the crop (Dick et al., 2008).

Sulfur availability is determined by the carbon (C): S ratio (C:S) and whether there is a release or immobilization of S. A C:S ratio of less than 200 usually allows for a release of plant available S (Dick et al., 2008). Likewise, a net immobilization can also occur if the C:S ratio is larger than 400.

1.4 Foliar Feeding

1.4.1 Uptake

Foliar applications are absorbed by penetrating the cuticle and cellulose wall through diffusion (Franke, 1967). After diffusing, the particles are adsorbed to the plasma membrane surface and then taken to the cytoplasm for which energy is required. Within this process, absorption is determined by multiple factors including the type of charge, the absorbability, and ion radius; furthermore, high light quality and intensity can improve absorption. Foliar uptake differs from root uptake because it must pass through the cuticle layer in leaves, which can absorb both organic and inorganic materials (Franke, 1967).

Although uptake is different in these two systems, research has shown that roots do remain active during pod-fill, as one study demonstrated, where N uptake remained constant while total recovery of N decreased with later foliar applications (Vasilas et al., 1980).

Due to the differences in plant entry, crop response time with foliar applications is usually shorter compared to soil application responses (Fageria et al., 2009). Urea-based solutions applied to the leaf usually take one to six hours for half the urea to be absorbed (Wittwer, S. H., Bukovac, M. J. and Tukey, 1963). Once activated, stomata can also prove to be an essential entry point for ions (Eichert and Rgen Burkhardt, 2001) and as a result, if stomata are open at the application time, foliar feeding is expected to be more successful (Fageria et al., 2009).

After plant entry, remobilization of nutrients may occur, mainly with N in later the growth stages. The percent of N translocated to the seed may be higher than N reaching the other parts of the plant as the seed demands more nutrients at the later growth stages (Vasilas et al., 1980). Unlike N, there are some nutrients like Ca and S that are not as mobile and as a result, may need reapplied multiple times throughout the growing season to reach new tissue that grows after the applications (Papadakis et al., 2007).

1.4.2 Application Conditions

Foliar applications later in the season can benefit the crop, but applications done improperly can damage the crop. The success of these applications is highly dependent on the conditions during the time of application. Certain rates of particular nutrients, like N, may burn leaves in a foliar application. Damage is rare when applications are done early in the morning or late evening, and when plants are not experiencing drought stress (Poole et al., 1983). Reducing plant burn would include limiting the N concentration in foliar applications (Poole et al., 1983). Other research shows that fertilizer is absorbed best when stomata are open, but when temperatures are also not excessively high after 2 or 3 o'clock in the afternoon (Fageria et al., 2009).

Applications should not be attempted in high wind conditions in order to avoid drift or off-target spraying, which could include overlap in the same field, resulting in foliage burning (Fageria et al., 2009). Dew, especially when applying a N fertilizer, can be associated with higher foliage burn (Woolfolk et al., 2002). Although the applications are to the foliage, these applications will prove less effective if soil moisture is not adequate for plant uptake and translocation (Fageria et al., 2009). Future forecast is important because most applications need at least three to four hours for proper absorption. Rain within that time period would limit the amount of fertilizer absorbed and available to the plant.

1.4.3 Plant Responses

Foliar damage and leaf burn may occur due to salt injury, or phytotoxic mixes (Parker and Boswell, 1980). Foliar burn was observed when a mix of N, P, K, and S was applied; however, when only P, K, and S was applied, no burn was noted following the application (Vasilas et al., 1980). Researchers suggested that this result was due to phytotoxic effects from the ammonia. Gooding and Davies found that ammonium sulfate and ammonium nitrate exhibited more leaf injury when compared to urea, which may be due to the source of nitrogen (Gooding and Davies, 1992).

Plant responses to foliar applications seem to vary substantially. However, early season stresses seem to increase a positive response to foliar applications. This can be seen when soil or weather conditions, which would normally reduce growth and nutrient availability, are posing a threat to the crop (Haq and Mallarino, 2000). A soybean crop S deficient during the early vegetative stages partially recovered from that deficiency when S was provided later in the life cycle (Sexton et al., 1998b).

When situations call for foliar applications, S deficiency issues could be corrected quickly. Fageria shares that a visual response from a soil applied fertilizer could take anywhere from five to six days, but responses to foliar applications can be seen within three or four days (Fageria et al., 2009). Although foliar applications may fix issues quickly, researchers argue that it is only a temporary fix and therefore many applications may be needed to maintain a healthy crop. Foliar applications of some nutrients are not transported from sprayed tissue to new growth, so the effects of that application are limited to the sprayed tissue, which usually results in a need for repeated applications. Most macronutrients are mobile; however, S and Ca have limited mobility throughout the plant, and therefore would more than likely need to be reapplied to the crop (Fageria et al., 2009).

Macronutrients are required in large quantities and needs are rarely met through foliar applications. In macronutrient applications, salt concentrations can be damaging to young plants. Foliar applications have proven to be more effective fin delivering micronutrients to the crop. Since micronutrients are needed in smaller amounts, a foliar application provides a more uniform spread of these nutrients than a soil application (Fageria et al., 2009).

1.4.4 Yield Response

There is still much debate on whether foliar applications increase yield enough to cover the cost of the application; hence, there is a need for further research in this area. Current research shows that foliar fertilizer may increase leaf concentration of N, P, and K; however, that does not always translate into considerable yield gains (Boote et al., 1978). Boote found that with fertilization, leaf area and photosynthesis was only extended slightly at the end of the season when most leaves had senesced, resulting in no further yield increases. Some of the struggles to accomplish higher yields with foliar fertilizer applications include several required sprays, possible precipitation wash-off, sufficient leaf area for interception, and foliar damage due to high concentrations. Macronutrient requirements are rarely met with these foliar applications (Fageria et al., 2009).

Researchers have reported up to a 1040 kg ha^{-1} yield increase with a N-P-K-S foliar mix applied at R5, R5.5, R6, R6.5 (Garcia L. and Hanway, 1976). This increase could be due to a positive interaction between the nutrients, because S applied alone as well as the N, P, K mix in a foliar application did not result in the same yield responses. The yield response from a late application of the N-P-K-S mix was possibly due to decreased root activity throughout pod-fill, and as a result, the roots could not supply plants with the needed nutrients during these stages (Garcia L. and Hanway, 1976). Garcia and Hanway found that S is required in higher amounts when applied as a foliar solution to obtain similar results as with a soil application at lower nutrient amounts.

Foliar applications at early vegetative stages using a N-P-K mix increased yield at some locations, but was not consistent across all sites (Haq and Mallarino, 2000). The practicality of these applications is limited because if yield increased, it rarely was enough to offset the application costs. Researchers found that leaf burn from salt injury during foliar applications may not just be cosmetic, but could have a greater impact on yield as well (Parker and Boswell, 1980; Poole et al., 1983).

1.4.5 Application Growth Stages

Basic foliar application information may guide growers to proper timing of foliar S applications. The crop should have a large enough leaf area index to maximize the spray interception and therefore efficacy of that fertilizer (Fageria et al., 2009). There have been many studies focusing on foliar applications made during the reproductive stages (Garcia L. and Hanway, 1976; Boote et al., 1978; Parker and Boswell, 1980; Poole et al., 1983), but few studies have been done investigating foliar applications at early growth stages (Haq and Mallarino, 2000).

CHAPTER 2. YIELD AND QUALITY RESPONSES OF SOYBEAN (*GLYCINE MAX* (L.) MERR.) TO NITROGEN AND SULFUR MANAGEMENT

2.1 Abstract

Reductions in atmospheric deposition of sulfur (S) coupled with increases in yields of soybean (*Glycine Max (L.) Merr*.) has led to S deficiencies in Indiana. Poor nodulation due to limited S, and thus a decrease in nitrogen (N) supply, restricts the yield and quality of soybean grain (i.e., protein). Sulfur is also a key component of methionine and cysteine, which are important amino acids of the nutrition in foodstuffs. The objective in this study is to improve yield and composition of soybean through various applications of N and S. Twenty treatments were designed in a 2x10 factorial. The 10 N+S fertility treatments were factored by 2 planting dates (early vs. late) at West Lafayette, IN in a split-plot design in 2018 and 2019. The same 10 N+S fertility treatments were factored by 2 varieties (Asgrow 24x7 and 34x6) at Wanatah, IN in a randomized complete block design (RCBD) in 2018 and 2019. Both trials were replicated 5 times. Whole plant samples were collected at R5 (first seed) and at R7 (first signs of physiological maturity) to be partitioned into stems, leaves, pods, and petioles/branches. The stems from both samplings were analyzed for ammonium, nitrate, and ureide concentrations to determine the effect of the N-S treatments on N fixation. An apparent harvest index was calculated from the R7 biomass sample. After harvest, the grain was used and analyzed for yield, protein, oil, and amino acid characterization.

Soybean yield increases among the N+S fertility treatments of the May 11th planting (early) were 380 to 1006 kg ha^{-1} over the untreated control in 2018. The same N+S fertility treatments did not show yield responses in the June 5th planting (late). The effectiveness of the early season applications may be linked to weather patterns and conditions prior to and immediately after planting. Sulfur and N fertilization can increase yield while maintaining protein levels. Treatments like STAND 20 and Plant NS improved yield (albeit, not the highest) and usually had higher protein concentrations than untreated control (UTC). It was found that with standard 20 (STAND 20) and ammonium thiosulfuate (ATS) amino acid concentrations improved with greater amounts of S applied, possibly more than plants needed to balance N source, which enhanced amino acid and protein production.

2.2 Introduction

In the last couple of years, soybean [Glycine max (L.) Merr.] has achieved record yield, where in 2018, produced yields 123.67 billion kg (4.54 billion bu), averaging 3,471 kg ha^{-1} ("USDA - National Agricultural Statistics Service - Statistics by Subject Results," 2018). Soybean is the primary source of protein in livestock feed and is essential with its array of highly digestible amino acids (Willis, 2003). Industries that prefer soybean meal require higher levels of nutrition and have been needing to supplement sulfur-containing amino acids (Krishnan and Jez, 2008). As farmers are pressured for higher yields, this results in diminished quality characteristics like protein, considering their inverse relationship (De Mello Filho et al., 2004).

In modern varieties sulfur (S), phosphorus (P), nitrogen (N), and copper (Cu) have harvest indices of about 60%. These levels are high and can limit grain production and protein content if not adequately supplied throughout the entire season (Bender et al., 2015). Sulfur has been overlooked due to the fact that S has been applied with other fertilizer mixes incidentally, as well as deposited in considerable amounts from the atmosphere (Gilbert, 1951). Higher soybean yields result in greater removal rates and high-analysis fertilizers contain less incidental sulfur, and therefore S deficiencies are becoming a larger problem in soybean production (Chen et al., 2008). As stated earlier, sulfur is a key component in cysteine and methionine production that contributes to overall protein levels (Chen et al., 2008). Sulfur is needed prior to seed fill because about 40% of that S needs time to be remobilized to the seed (Naeve and Shibles, 2005). Legumes are more susceptible to S deficiency because of such high levels of protein, which averages 34% in soybean at 13% moisture, and require S in the N fixation process (Hurburgh et al., 1990; Dick et al., 2008).

It has been found that S and N interact closely with each other in plant processes. Remobilization of S is less efficient than that of N, and therefore limit the production of high quality protein (Sexton et al., 1998a). Since there is a positive linear relationship between N and S, it has been predicted that S can direct N utilization (Hitsuda et al., 2004). Studies have shown that when soil S levels are adequate, N uptake improves (Chen et al., 2008). Nitrogen can also be left unused and lost in the soil if S is not in sufficient supply, thereby diminishing N use efficiency (Haneklaus et al., 2008).

Nitrogen is needed in considerable amounts for soybean to produce high protein content and meet the standard of a high harvest index (Gaspar et al., 2017). About 50-60% of this N is provided by N fixation, which is usually known to dwindle if N is supplemented in fertilizer applications

(Salvagiotti et al., 2008; Ohyama et al., 2017). There have been results that defy this proposal even in high-management, well-nodulated soybeans, where small amounts of N applied throughout the season have brought positive responses (Salvagiotti et al., 2008; Ohyama et al., 2017). Some results show slight positive yield responses to R3 soil applications of N (Freeborn et al., 2001). A possible application would be a slow-release N source below the nodules so to not disturb the fixation process (Salvagiotti et al., 2008). This would help supplement N if N fixation was limited because N fixation can be managed at yield and/or oil cost by lowering a soybean's harvest index. In this way, N applications could be of benefit to a soybean crop (Tamagno et al., 2018).

More information is needed to determine if supplementing N with S increases yield and efficiency, or if this addition would suppress yield due to potential effects on N fixation. More research also needs to be conducted on soybean growth and development to determine the payoff of S applications, S effects, and in what conditions S is most useful. These are the same areas that this study has targeted. The overall study objective was to investigate the influence of planting date or maturity group, timing of N and S applications, and the interaction between the two on soybean yield and seed quality. The hypothesis was that soybean yield and quality will increase with sulfur treatments and planting the soybean crop earlier in the growing season.

2.3 Materials and Methods

2.3.1 Nitrogen and Sulfur Treatments

Two studies were designed to investigate the interactions of N and S applications with either planting date or maturity group of soybean in 2018 and 2019. The N and S applications were the same across both studies and are described in Tables 2-1 and 2-2 and in detail as follows.

Ten fertility treatments consisted of an untreated control (***UTC***) and nine N+S combinations. Sulfur fertilizer was first used to meet the S target and any secondary need for N was met using urea, urea ammonium nitrate (UAN 28%), or Coron®, depending on the treatment. Two dry, pre-emergence treatments were ***STAND 2***0 at 22.4 kg S ha^{-1} in the form of granular AMS (21-0-0-24S) and ***Plant NS*** at 44.8 kg N ha^{-1} and 11.2 kg S ha^{-1} via granular AMS and urea. These dry, pre-emergence applications were spread by hand from alley center to alley center with a 3.05 m wide spread over the soil surface. One liquid pre-emergence treatment of (***ATS***) was applied to the soil surface through a broadcast spray of ATS (12-0-0-26S) at 22.4 kg S ha $^{-1}$ at a total volume of 140 L ha^{-1} (15 GPA). This treatment was applied using a 3.05 m wide boom attached to a CO_2 pressurized backpack sprayer that used TeeJet 8002XR nozzles spaced 38 cm apart.

The ***V4 NS*** treatment was 44.8 kg ha^{-1} N and 11.2 kg S ha^{-1} applied via granular AMS and urea at V4 growth stage (GS). This dry application was spread over the soil surface by hand like the pre-emergence applications. The ***R3 NS*** treatment was 44.8 kg ha^{-1} N and 11.2 kg S ha^{-1} via mixture of UAN 28% and spraygrade ammonium sulfate (AMS) that was directed to the soil surface between the soybean rows. It was applied at 281 L ha^{-1} carrier volume through a pressurized backpack sprayer. The ***V4R3 NS*** treatment was a sequential application that was the combination of V4 NS and R3 NS (see previously described) that totaled 89.7 kg ha^{-1} N and 22.4 kg S ha^{-1}.

Two more treatments were applied at R3 with drop nozzles. ***UAN Direct*** was applied at R3 at 44.8kg ha^{-1} N via UAN 28%. ***AMS Direct*** was applied at R3 at 9.8 kg ha^{-1} N and 11.2 kg S ha^{-1} via spraygrade AMS.

The ***R4+ NS*** treatment was the combination of R3 NS (see previously described) and four foliar NS applications. It totaled 89.7 kg ha^{-1} N and 22.4 kg ha^{-1} S with half from R3 timing and the other half split across four foliar applications every ~10 d between R4 and R7. Coron® (25-0-0-0.5B) (Collierville, TN) and spraygrade AMS was mixed to provide 2.8 kg S ha^{-1} and 11.2 kg N ha^{-1} with each application at a carrier volume of 140 L ha^{-1} (15 GPA). The goal was that the applications would intersect with R4, R5, R5.5, and R6 growth stages while applying to new leaves as seed formation was occurring. Some of these applications caused phytotoxicity and thus, newly formed leaves were critical for nutrient absorption and uptake. These applications were made with the backpack sprayer using the 3.05 m boom with TeeJet 8002XR tips.

2.3.2 Data Collection

Daily weather data was collected at the farm's local weather station, either at ACRE or Pinney farms depending on the study. Plant population was determined around V2 to V3 by counting plants in a 1-m length of row in each the middle rows of the plots. These counts totaled 3 different stand counts throughout the plot to then be averaged for the plot population. Population counts were also done prior to harvest.

One goal of this project is to determine the effects of N and/or S fertilization on N fixation. To detect effects, whole plant samples were collected at R5 (First Seed) and again at R7 (First or

Beginning Maturity) from different locations within the same plots. In 2018, all plots from the first planting date were sampled, but after evaluating the time and labor to harvest, dry, and partition the samples, sampling adjustments had to be made. The samples following the first planting date in 2018 excluded the R4+ NS and the UAN Direct treatments. Four of the five replications were sampled for logistics. A 1-m sample of plants from a center row was cut at the soil surface and placed in a cooler at approximately 4.4ºC until partitioning could be conducted. All plant partitions included stems, leaves, petioles and branches, and pods.

Following partitioning, plant parts were placed in a 60 ºC dryer for three to five days. After drying, all partition weights were recorded. For proper N content analysis, the stems of plants were ground down to a 0.5 mm powder and sent for ureide and N content analysis at Kansas State University. The stem partitions were ground in this stepwise fashion; stems were ground with a Wiley Mill to about 3 mm and then further ground with an Udy Cyclone to about 0.5 mm. At Kansas State University, a modified version of the Hungria and Araujo procedure was used when processing for ureides, nitrate, and calculating RAU, which is a function of ureide and nitrate concentrations (Tamagno et al., 2018; Carciochi et al., 2019).

Harvest index was calculated using the whole plant samples taken at R7. In addition to the standing plants, fallen leaves in 2018 and fallen leaves and petioles in 2019 were collected from the ground next to the 1 m of plants removed. Stems, leaves, fallen leaves, petioles and branches, and pods were dried for three to five days at 60 ºC and dry weights were recorded. Pods were then threshed with an ALMACO BT-14 Belt Thresher. Threshed seeds were weighed, which provided an accurate weight of pod shells when subtracting the seed weight from the pod. Using these dry weights, an accurate harvest index was calculated (seed/total biomass weight including seed). An apparent harvest index was also calculated to remove some variability (=seed/stems, pods, seed).

At R8, plots were end-trimmed to remove biomass sampling areas created earlier in the season, as well as to reduce border effects. The Kincaid 8-XP plot combine harvested the middle four rows for a grain subsample, plot weight, and grain moisture for each plot. In 2018, both planting dates were harvested on October 22. In 2019, the first planting date was harvested October 14 and the second planting was harvested on October 23. Grain yield was calculated and adjusted to 13% moisture. Seed weights were determined by counting two, 100-seed subsets of each plot, weighing and adjusted to 13% moisture from the as-is grain moisture. A subsample of this grain was sent to the University of Minnesota for analysis of protein, oil, and amino acid content and characterization.

2.4 STUDY 1: Planting Date x N+S

2.4.1 Site Characteristics

Field experiments were conducted in 2018 and 2019 at the Purdue Agronomy Center for Research and Education in West Lafayette, Indiana (40º29’27” N, 86º59’57”W). The 2018 and 2019 studies were located on a Drummer soil (fine-silty, mixed, superactive, mesic Typic Endoaquolls) (“USDA-NRCS Official Soil Series Description View By Name,”). Corn was the previous crop each year. The fields were chisel-plowed in the fall and field cultivated in the spring prior to planting.

A composite soil sample was collected from each replication to a depth of 20 cm. These samples were taken at the beginning of the season prior to any fertilizer applications to characterize soil fertility (Table 2-3). Soil fertility was not limiting in 2018 though phosphorus was low in 2019 (Table 2-3). Mehlich-3 extractable S was below 8 mg kg^{-1} in 2018 and below 9 mg kg^{-1} in 2019, though organic matter was 4.0 mg kg^{-1} and 3.8 mg kg^{-1}, respectively.

2.4.2 Site Design

Treatments were designed in a two-way factorial: 2 planting dates x 10 N+S treatments. These treatments were arranged in a split plot manner with planting date as the whole plot factor and fertility treatments as the subplot factor. Both factors were assigned in a randomly complete block design with five replications.

The two planting date targets were timely planting (late April to early May) and late planting (first week of June). These targets were accomplished in 2018 on May 11 and June 5. Extreme wet weather patterns and poor field conditions in 2019 forced the planting dates beyond the targets. However, two different planting dates were completed in 2019 on June 11 and June 27. Plots in both years were six, 38-cm rows wide by 12.2 m long. The two outside rows were for controlling border effect and not sampled or harvested. An Asgrow soybean variety was utilized for this study, specifically AG 34x6. In 2018, plots were seeded at 345,947 seeds ha^{-1} and in 2019, due to delayed planting, the seeding rate was increased to 395, 368 seed ha^{-1}. Plots were seeded utilizing the Kincaid Voltra planter. Herbicide applications were handled at the discretion of the farm crew, followed up by hand weeding later in the growing season once chemical applications would be considered off-label.

2.4.3 Application and Sampling Dates

Applications and samplings done throughout the season were targeted for specific growth stages. Application dates can be found in Table 2-4. The 2018 R4+ NS treatment for the first planting date was applied on July 27 (R4), August 2 (R5), August 10 (R5.5), August 26 (R5.8) and for the second planting date, treatments were applied on August 10 (R4), August 26 (R5.5), September 6 (R5.8), and September 13 (R6). The 2019 R4+ NS treatment for the first planting date was applied on August 16 (R4), August 29 (R5.5), September 9 (R5.8), September 19 (R6) and for the second planting date, applications were made on August 29 (R4), September 9 (R5.5), September 19 (R5.8), and September 30 (R6).

In 2018, both planting dates were at the R5 growth stage when sampled on August 7 and August 20, and in 2019, both planting dates were at R5.5 when sampled on August 27 and September 5, respectively. In 2018, both planting dates were at the R7 growth stage when sampled on September 14 and September 28 for planting date one and two, respectively. In 2019, the first planting date was sampled on September 30 (R7) and the second planting date was sampled on October 9 (R7.7).

2.4.4 Statistical Analysis

Years are reported individually due to weather and planting date differences in 2018 and 2019. Nitrogen fixation data was only available from the 2018 season, and although plants were collected at R5 from planting date 1 for the UAN Direct and R4+ NS treatments, these treatments were excluded from analysis since these treatments were not collected at any other sampling times or locations. Data were subjected to an analysis of variance (ANOVA) using PROC GLM in SAS (SAS Institute, version 9.4). Analysis was set up to test the two main effects of the split-plot design with the whole plot factor being planting date and the fertility treatments being the sub-plot factor. Interactions were determined using the full treatment analysis. With these analyses, planting date and fertility treatments were considered fixed while the location and year were random. Fisher's Protected LSD was used to compare the means of significant measurements.

2.5 STUDY 2: Variety x N+S

2.5.1 Site Characteristics

Field experiments were conducted in 2018 and 2019 at Pinney Purdue Agricultural Center (PPAC) in Wanatah, Indiana (41º26'31" N, 86º56'02"W). Both the 2018 and 2019 studies were conducted on a Sebewa loam, shaly sand substratum soil (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Argiaquolls) ("USDA-NRCS Official Soil Series Description View By Name,"). Corn was the previous crop each year. The fields were chisel-plowed in the fall and field cultivated in the spring prior to planting.

A composite soil sample was collected from each replication to a depth of 20 cm. These samples were taken at the beginning of the season prior to any fertilizer applications to characterize soil fertility (Table 2-5). Soil fertility was not limiting in 2018 or 2019 (Table 2-5).

2.5.2 Site Design

Treatments were designed in a two-way factorial: 2 varieties x 10 N+S treatments. These treatments were arranged in a randomized complete block design with five replications. The same two varieties were used in 2018 and 2019 where AG24x7 (2.4 relative maturity) represented the early season variety and AG34x6 (3.4 relative maturity) represented the full season variety. It is noteworthy that AG34x6 was the same variety used in the Planting Date x N+S study at West Lafayette (previously described). The N+S fertility treatments (Tables 2-1 and 2-2) were the same as the Planting Date x N+S study in West Lafayette.

In 2018 and 2019, plots were six 38-cm rows wide by 12.2 m long. The two outside rows were for controlling border effect and were not harvested or used for sampling. In both seasons, plots were seeded at 345, 947 seeds ha^{-1}. Trials were planted on May 25, 2018 and June 4, 2019, due to wet weather conditions of that spring. Plots were seeded utilizing the Kincaid Voltra planter. Herbicide applications were handled at the discretion of the farm crew, followed up by hand weeding later in the growing season once chemical applications would be considered off-label.

2.5.3 Application and Sampling Dates

Applications and samplings done throughout the season were targeted for specific growth stages. Applications dates can be found in Table 2-6. The 2018 R4+ NS treatment for AG24x7 was made on August 1 (R4), August 9 (R5), August 22 (R5.8), September 12 (R6.5) and for AG34x6, applications were made on August 9 (R5), August 22 (R5.5), September 12 (R6), and September 24 (R7). The 2019 R4+ NS treatment for AG24x7 was applied on August 8 (R4), August 20 (R5.5), August 30 (R5.8), September 9 (R6) and for AG34x6, applications were made on August 14 (R4), August 22 (R5.5), August 30 (R5.6), and September 9 (R5.8).

In 2018, AG24x7 was at R5 and AG34x6 was at the R5.5 growth stage when sampled on August 9 and August 22, respectively. In 2019, both varieties were at R5 when AG24x7 was sampled on August 14 and AG34x6 was sampled on August 20. In 2018, both varieties were between R6.5 and R7 when sampled on September 19 and September 24 for AG24x7 and AG34x6, respectively. In 2019, AG24x7 was at R7.8 and AG34x6 was at R7 when sampled on September 24.

2.5.4 Statistical Analysis

Both 2018 and 2019 are pooled together for the following analysis; except for the N fixation data since only 2018 data was available and thus, analyzed individually. Data were subjected to an analysis of variance (ANOVA) using PROC GLM in SAS (SAS Institute, version 9.4). Analysis was set up to test the two main effects of the randomized complete block including variety and fertility treatment differences. Interactions were determined using the full treatment analysis. With these analyses, variety and fertility treatments were considered fixed while the location and year were random. Fisher’s Protected LSD was used to compare the means of significant measurements.

2.6 Results and Discussion: PLANTING DATE X NITROGEN + SULFUR

2.6.1 Weather

Weather data from the past 30 years was compiled to show the season monthly averages (April-October) for temperature and precipitation totals and compared to the averages over the

2018 and 2019 seasons (Table 2-7) near West Lafayette, IN. The 2018 season experienced higher precipitation than the 30-yr average, receiving 47 mm above the average for the season. The 2018 season received greater precipitation in the final months of the growing season (August, September, and October), which aided in seed fill but provided challenges in timely harvest. April 2018 was much cooler than the 30-yr average (5.9 vs. 10.5º C); whereas, the remainder of the 2018 season was similar to the 30-yr average for temperature. Individual rainfall events and temperature fluctuations in relation to the first planting and the second planting date likely influenced early season growth, nodule formation, and organic matter mineralization in 2018 (Figure 2-1).

Frequent rains kept the fields saturated during normal planting weeks in 2019 and posed a number of challenges for timely planting (Figure 2-2). Precipitation was 27 mm and 11 mm higher in April 2019 and May 2019 compared to the 30-yr averages. Temperatures were moderate to somewhat lower during those months. In the middle of the growing season, less precipitation was recorded from June through September compared to the 30-yr average. The 2019 season received 90 mm less precipitation across the whole season compared to the 30-yr average.

2.6.2 Plant Nitrogen Partitioning

All N partitioning data is from the 2018 season. Ureide concentrations in the stem are the N form associated with N fixation, while the nitrate concentrations in the stem are associated with soil and fertilizer N uptake. Late planted soybean had greater total N concentrations at R5, but had an equal RAU percentage to the May planted soybean (Table 2-8). Late planted soybean had a higher total stem N concentration when entering the critical grain fill period, but both planting dates had equal RAU's.

Ureides and nitrate were influenced by N+S fertility. V4 + R3 NS had the lowest ureide concentrations at 279 µmol g^{-1} compared to the highest concentration with the ATS treatment containing 447 µmol g^{-1} (Table 2-8). More fertilizer N supply allowed soybean plant to take up more nitrate and limit N fixation, and thus, low levels of ureide production. V4R3 NS had 50.7 µmol g^{-1} of nitrate, and the lowest nitrate concentration was ATS at 21.7 µmol g^{-1}. This phenomenon has been seen in literature that ureide presence diminishes after a N application, with the most reduction observed after applications during reproductive stages (Tamagno et al., 2018).

The RAU was lowest for V4R3 NS at R5 and R7, which was 10% lower than the UTC (R5: 84% vs. 94% and R7: 74% vs. 84%, Table 2-8). There were no significant differences in total

N or ureide concentrations at R7. This lack of difference could be due to a diminish in overall N fixation. Soybean usually continues to develop nodules until pod-filling stages, at which point nodulation ceases and fixation starts to decrease (Pedersen, 2003; Kandel, 2015).

2.6.3 Plant Biomass and Harvest Index

In 2018, early planted soybean averaged 166 g m^{-2} more biomass than late planted soybean at R5 samplings (Table 2-9). Although both planting dates were sampled at R5, the comparison may be variable due to the exact point of R5 growth stage between planting dates. This result is most likely due to an extended vegetative growth period early in the season. Early planted soybean showed a greater stem weight that may be due to more growth and nodes on the main stem.

An apparent harvest index was used which included the dry weights of stems, pod shells, and seed. Late planted soybean harvest index was 3.5% greater compared to the early planted soybean in 2018 (Table 2-9). Although early planted soybean had greater total biomass, a greater grain mass was not detected.

In 2018, R7 leaf weights were influenced by the interaction of Planting Date x N+S Fertility (Table 2-9). Early planted soybean had the highest leaf mass with R3 NS and V4 NS that were 63.1 and 51.1 g m^{-1} greater than the UTC, respectively. The late planted soybean was not affected by the N+S fertility treatment. Greater leaf mass could have led to longer retention and a longer photosynthetically active period; thereby, prolonging seed fill and increasing yield. Greater leaf biomass did not result in higher ureide concentrations; however, ureide accumulation was not calculated.

An interaction was not found in 2019 R7 leaf mass; however, a planting date effect was observed with early planted soybean being 29.2 g m^{-2} greater than late planted soybean (Table 2-10). This weight difference could be partially due to planting date differences, and possibly due to inherent development differences, even if both sampling times were considered R7 growth stage. Early planted soybean began leaf development earlier and also able to retain the leaves longer with a couple of the N+S treatments. Sulfur has been noted to increase chlorophyll and photosynthesis, assisting in plant processes and extending leaf retention (Zhao et al., 2008). This chlorophyll increase could be documented in future studies with crop reflectance measures and tissue samples to indicate nutrient concentrations.

2.6.4 Seed Mass and Yield

The yields in 2018 were influenced by the interaction of Planting Date x N+S Fertility (Table 2-11). Most N+S fertility treatments improved the yields with the early-planted soybean (May 11th); whereas, in the late-planted soybean (June 5th), yield was not impacted by N+S fertility. Yield improvements ranged from 478 to 1,006 kg ha^{-1} over the UTC with N+S applications (Table 2-11). With the early-planted soybean, the greatest yield improvements (617 to 1,006 kg ha^{-1}) compared to the UTC were: R3 NS, V4R3 NS, V4 NS, Plant NS, UAN Direct, R4+ NS, and ATS. The next tier of yield improvement was from STAND 20, yielding 478 kg ha^{-1} greater than the UTC. Zhao (2008) found that S increased the pods $plant^{-1}$, seeds $plant^{-1}$, and seed mass. This study demonstrated increases in seed mass (Table 2-11). Fertility treatments did not impact the June 5th planted soybean, which resulted in yields from 3847 to 4223 kg ha^{-1}. This lack of similar effect could be due to the increased precipitation leading up to and after the second planting date (Figure 2-1). This increased precipitation could lead to leaching of sulfur out of the soil profile, or in other cases volatilization could be another fate for this nutrient. With these possibilities, the sulfur applied pre-emergence for the late-planted soybean could have balanced the soil levels out to what would have been present without excessive rainfall, or the applied sulfur could have leached from the profile before being utilized by the crop.

Early-planted soybean did not experience as warm of temperatures in the weeks leading up to planting, where time mineralization was most likely occurring very slowly (Figure 2-1). The Plant NS, ATS, and STAND 20 treatments provided essential S and N early in the season when these nutrients were likely limited due to slow mineralization from the soil and residue. In 2018, leaf mass in early-planted soybean, particularly in R3 NS and V4 NS, were greater than the UTC. R3 NS, V4 NS, and Plant NS all had the same amounts of N and S applied (44.8 kg N ha^{-1} and 11.2 kg S ha^{-1}), and all three were top yielding treatments in early planted soybean in 2018. It is possible that greater leaf production through R7 was a result of leaf retention, thereby increasing the seed fill period.

Nitrogen fixation at R5 in 2018 did not seem to affect overall yield as V4R3 NS had lower ureide concentrations and was one of the highest yielding treatments; however, in other seasons, the climate may play a larger role in how important N fixation or N uptake is to yield differences. In future studies, the relationship between N concentration and biomass would be beneficial to address. Ureide, or even nitrate concentrations could be perceived to be lower, if the plants had greater biomass production due to dilution. Investigating this relationship could be key in identifying differences between nitrogen fixation levels. N fixation earlier in the growing season could also be an underlying factor and should be assessed.

As mentioned previously, field conditions in 2019 were not conducive to achieving the planting targets. The first planting date in 2019 was June 11, which was a month later than the 2018 planting date 1 (May 11, 2018) and even later than the 2018 planting date 2 (June 5, 2018). Thus, drawing conclusions across the two growing seasons was fundamentally difficult. The main effects of Planting Date and N+S Fertility were observed, but there was no interaction (Table 2-11). Pooled over all fertility treatments, soybean planted on June 11 yielded 193 kg ha^{-1} greater than the June 27 planted soybean. Late-planted soybean had greater seed mass; therefore, early-planted soybean most likely has a yield advantage by more pods m^{-2}.

Pooled over both planting dates in 2019, STAND 20 yielded less than all other fertility treatments. This negative yield effect was not due to seed size, so it is likely related to fewer nodes, branches, and/or direct pod production. (Table 2-11). Luxury consumption of S by soybean plants may have caused an imbalance with N. An imbalance between nutrients could cause major reductions in nodulation, and therefore N fixation (Gates and Muller, 1979). Balanced nutrition leads to nodulation longevity and greater plant vigor.

Conversely, Plant NS did not have a negative yield effect, which may be due to a balance between N and S with the addition of urea. Soybean may have "luxury consumed" S in 2019 as it was not needed in the same way as in 2018, under cooler, wetter conditions of early-planted soybean. This result would lead to speculations that luxury consumption of S leads to higher S concentrations in the plant, lesser overall concentration of N, and an imbalanced N:S ratio. The difference between applied S amounts did not consistently affect biomass production. In both 2018 and 2019, STAND 20 had less total biomass compared to the Plant NS treatment (Table 2-9, Table 2-10). Biomass comparisons in the future could help determine if luxury consumption of S does in fact occur. Also, gathering plant height data could provide more information on balance of nutrient or lack thereof in plant development and yield.

The yield interaction observed in 2018 was not seen in 2019, and this is possibly due to warmer temperatures and better conditions for mineralization at planting in 2019. In 2018, earlier planting meant conditions were unfavorable for mineralization, and the treatments would have provided essential nutrients otherwise not available at that time. This probably results from the early-planted soybean having a longer season to put on more reproductive nodes. Early-planted soybean remained in vegetative stages longer, so more nodes could develop that would eventually put on more pods. Soybean remaining in vegetative growth stages longer could also result in greater nodulation. Sources state that nodules are visible around V2 growth stage and continue to put on new nodules up until pod-fill, with the nodules each being active for about six weeks before degrading (Pedersen, 2003; Kandel, 2015). Beginning the growing season earlier provided better temperatures during the pod development stages.

In 2018, grain moisture was 0.3% higher in the May 11 planted soybean compared to the June 5 planted soybean. Both planting dates in 2018 were harvested on the same day, but the May planted soybean had greater total biomass indicating that it could take longer to dry down compared to the late-planted soybean (Table 2-11). In 2019, late-planted soybean (June 27) moisture was 1.3% greater at harvest than the June 11 planted soybean. Late-planted soybean was harvested eight days later but may have been at a higher moisture since the plants did not mature as quickly, and therefore did not dry down as quickly.

Seed mass is an important yield component. Planting Date and N+S Fertility affected seed size, but there was no interaction in 2018. Early-planted soybean seed mas was greater than the late-planted soybean seed mass (Table 2-11). Larger seed in early-planted soybean could be a result of longer leaf retention allowing for a longer photosynthetic period and greater seed fill duration (Table 2-9). Plant NS, STAND 20, ATS, V4 NS, R3 NS, and V4R3 NS produced larger seeds (0.7-1.0 g per 100 seeds) compared to the UTC. All of these fertility treatments except for STAND 20 were the top yielding treatments for this trial. STAND 20 may have greater seed size to compensate for less reproductive nodes and/or pods.

Seed size was larger in the later planted soybean in 2019, with no effects from N+S Fertility or the Planting Date x N+S Fertility interaction. Late-planted soybean was 0.8 g 100 sd^{-1} greater than the early-planted soybean. This pattern differed from 2018 and could be the result of compensation from late-planted soybean. Robinson et. al. (2009) stated that for the last two planting dates in their study, pods m^{-2} became less important while seed mass became the leading factor for yield. In this trial, increased seed mass was still not able to generate the same yield to make up for a lack of pods. It was also noted late-planted soybean attempted to compensate with greater seed mass, but it did not translate into higher yields than the earlier planted soybean that had more time to develop, resulting in more nodes and pods. The lack of N+S fertility effect was possibly due to the weather patterns the later planted soybeans experienced. Both planting dates were in June, at which point the soil and air temperatures had warmed up (Figure 2-2). With warmer temperatures post-planting, more nutrients would already be available through mineralization, possibly making initial pre-emergence fertilization unnecessary.

2.6.5 Seed Protein and Amino Acids

In both 2018 and 2019, there was a significant protein response to planting date. Pooled over all fertility treatments, both years showed that the earlier planting date produced about a half-percent less protein than the later planting dates (Table 2-11). In 2018, STAND 20, ATS, and Plant NS had the highest protein concentrations. The 2019 season did not show the same results, but rather showed that the two treatments with the most nitrogen applied (R4+ NS and V4R3 NS) contained the greatest levels of protein. This difference between years could be a result of the growing conditions associated with large differences in planting dates. Temperature plays a role in the concentration of protein and oil. Later planting dates result in a lower temperature at R6, leading to higher protein concentrations and lower oil (Robinson et al., 2009).

Late planted soybean contained greater protein concentrations and therefore, overall greater amino acid concentrations. Pooled over both planting dates, amino acids most positively responded to STAND 20 and ATS in 2018 (Table 2-12). Only cysteine, methionine, and tryptophan positively responded to V4R3 NS. These results suggest early, pre-emergence applications of S can boost amino acid concentrations. Literature shares the importance of specifically cysteine and methionine, two sulfur-based amino acids, both of which were improved by STAND 20, Plant NS, ATS, V4 NS, and V4R3 NS in 2018 (Willis, 2003; Chen et al., 2008; Hitsuda et al., 2008). In 2019, there were only 7 positive responses to five different N+S treatments across all amino acids (7 out 90 potential responses, Table 2-13).

2.6.6 Seed Oil

Oil was influenced by the interaction of Planting Date x N+S Fertility in 2018. The interaction indicated early planted soybeans have higher oil concentrations (Table 2-11). In both planting dates, UAN Direct contained the most oil being 0.9 and 0.6% higher than AMS Direct (lowest oil concentration of both planting dates). Oil concentration in 2019 was influenced by planting date and N+S fertility, but not the interaction. Early planted soybean contained more oil than late planted soybean. Oil was affected by fertility treatments and was greatest in the two treatments that received urea and granular AMS early in the season (Plant NS & V4 NS).

Early planted soybean is expected to contain more oil, as higher temperatures at R6 are associated with higher oil levels (Robinson et al., 2009). Often with energy distribution, higher protein content will end up decreasing yield (De Mello Filho et al., 2004). This effect can be seen with treatments like STAND 20, Plant NS, and ATS that had smaller yield increases over the UTC compared to V4 NS, V4R3 NS, and R3 NS; however, each of these treatments had a greater protein level when compared to the highest yielding treatments (Table 2-11). The inverse relationship between protein and oil can also be observed in the 2018 results where UAN Direct contained the highest oil levels of all the treatments as well as the lowest protein levels (Table 2-11).

2.7 Results and Discussion: VARIETY X NITROGEN + SULFUR

2.7.1 Weather

Weather data from the past 30 years was compiled to show the season monthly averages (April-October) for temperature and precipitation totals and was compared to the averages over the 2018 and 2019 seasons in Wanatah, IN (Table 2-14). The 2018 season was drier than the 30-year average, receiving 34 mm below the average for the season. The final months of the growing season, from August through October, received more than average precipitation that assisted in grain fill. This wet fall did pose some challenges for timely harvest (Figure 2-3). Temperatures throughout the 2018 season were similar to the compiled 30-year data, with a few months having only slightly elevated temperatures (May, June, August, and September).

The spring of 2019 posed challenges for timely planting due to frequent rain events, leaving the fields saturated during normal planting time (Figure 2-4). Precipitation was 27 and 88 mm higher in April and May 2019 compared to the 30-yr averages. Precipitation was 59 and 47 lower in July and August 2019 compared to the 30-yr averages (Table 2-14). This season seemed to represent the averages for the temperatures throughout the season.

2.7.2 Plant Nitrogen Partitioning

All nitrogen partitioning data is from the 2018 season. In the R5 samples, there was a range of 410 to 515 µmol g^{-1} in ureides in AG 24x7 (Table 2-15). AG 34x6 ranged from 406 to 510 µmol g^{-1}. In both varieties, the ATS treatment had the greatest amount of ureides closely followed by STAND 20 that were 72 and 52 µmol g^{-1} greater than UTC, respectively. STAND 20 and ATS were applications that contained twice the rate of S than the Plant NS treatment, and therefore more S was supplied to the plant. This increased S availability could have boosted nodulation and N fixation, leading to increased ureide concentrations for those treatments. Plant NS had the lowest concentration of ureides in AG 34x6 (104 µmol g^{-1} less than ATS) and the second lowest in AG 24x7 (78 µmol g^{-1} less than ATS). This may suggest the negative effects of additional nitrogen fertilizer (urea) added, suppressing nodule development and fixation as other sources have found (Gates and Muller, 1979; Zhang and Smith, 2002; Ohyama et al., 2017; Tamagno et al., 2018).

Nitrate content was 20.1 µmol g^{-1} greater in samples from V4R3 NS than UTC. The V4R3 NS contained the highest amounts of nitrogen across two different growth stages. ATS contained the most ureides, and as expected, contained one of the lower concentrations of nitrate at only 5.1 µmol g^{-1} greater than UTC; however, if the plant was better able to fix N and produce high amounts of ureides, then it would rely less on mineral N uptake, resulting in smaller amounts of nitrate.

2.7.3 Plant Biomass and Harvest Index

Total biomass was not significantly affected by variety, but was numerically greater in AG 34x6 at both sampling stages (Table 2-16). V4R3 NS had the greatest apparent harvest index at 58.4%, 3.7% greater than UTC. ATS and V4 NS had apparent harvest indices of 2.7 and 2.5% greater than UTC, respectively. Nitrogen and S fertility treatments did not affect stem, pod, or total biomass weight. The UTC numerically had the lowest biomass in each category at R5 (Table 2-16). Stem weights at R5 ranged from 185 g m^{-2} (UTC) to 204 g m^{-2} (V4 NS). Pod weights at R5 ranged from 128 g m^{-2} (UTC) to 146 g m^{-2} (V4R3 NS). Total biomass at R5 ranged from 692 g m^{-2} (UTC) to 886 g m^{-2} (AMS Direct). The R5 leaf biomass was affected by N+S Fertility and resulted in STAND 20, Plant NS, V4 NS, and V4R3 NS having up to 27 more g m^{-2} compared to the UTC.

2.7.4 Seed Mass and Yield

Pooled over 2018 and 2019, the yields were influenced by the N+S fertility (Table 2-17). Variety did not have an effect on yield with AG 24x7 yielding 4025 and AG 34x6 yielding 4022 kg ha^{-1}. Five of the nine N+S fertility treatments improved yield compared to the UTC. Yield improvements ranged from 168 to 248 kg ha^{-1} over the UTC with the following treatments: V4R3 NS, Plant NS, R3 NS, R4+ NS, and V4 NS. The treatments that improved yield all had a source of N. The N and S sources in these treatments may have provided N and S that was limited and/or lost during May 2019, due to cooler temperatures and higher than normal precipitation leading up to planting. Plant NS may have stimulated early soybean growth and development by supplementing N and S that had not yet been mineralized (Figure 2-3, 2-4). The V4 and R3 treatments were applied in months that received less precipitation than the 30-yr averages, so it may have been useful for the plant to have these nutrients available near the root system, rather than trying to scavenge the nutrients (Table 2-14).

Seed mass was influenced by the interaction of Variety x N+S fertility (Table 2-17). Within each variety, N+S fertility did not differ than the UTC; however, the numeric trend was that N+S fertility applied at planting or V4 produced larger seeds. The Plant NS treatment in the AG 24x7 variety had larger seed than most treatments in AG 34x6 variety. Higher seed mass in Plant NS and V4R3 NS matches with the two highest yielding treatments pooled across varieties; and thus, a source of yield improvement. This could be due to the growing conditions in 2019 not favoring a long vegetative period, and therefore not as many nodes and pods being developed as expected.

2.7.5 Seed Protein and Amino Acids

Seed protein was influenced by the Variety x N+S Fertility interaction pooled over 2018 and 2019 (Table 2-17). This interaction indicated that comparing across N+S Fertility, AG 34x6 was lower in protein. In both varieties, R4+ NS and ATS had the highest protein levels compared to the other N+S treatments. R4+ NS could have boosted protein by providing N and S during the critical reproductive stages when more N and S is needed to build amino acids and proteins in the seed. The UAN Direct treatment also can be pulled out of this interaction and recognized as the treatment with the lowest protein concentrations in both AG 24x7 and AG 34x6. UAN Direct may have had the lowest protein concentration because it did not contain a source of S. There are indications that N and S work together in the plant, and that N uptake and movement is improved with adequate S (Chen et al., 2008).

Pooled across years and varieties, there was a positive response from every amino acid to the STAND 20 and ATS treatments (Table 2-18). It is possible that early applications of 22.4 kg S ha^{-1} helps improve not only cysteine and methionine levels, but overall amino acid and protein levels.

2.7.6 Seed Oil

In 2018, oil was influenced by the Variety x N+S fertility interaction (Table 2-17). R4+ NS had the highest protein levels, and as expected, the lowest oil concentrations in each variety. The UAN Direct treatment ended with the lowest protein level, but contained the highest concentrations of oil in each variety. The only N+S Fertility effect on AG 24x7 was R4+ NS being 0.6% less than UTC. AG 34x6 had more variation between N+S Fertility with R4+ NS being 0.9% lower than UTC. These results show the tradeoff between protein and oil concentrations.

2.8 Conclusions

Planting date can determine the effectiveness of N and S applications of soybean. Primarily due to cooler and wetter weather patterns, N and S applications to early planted soybean may help alleviate transient shortage of N and S due to limited mineralization. Sulfur has been found to aid in N fixation and nodulation. The number of nodules and nodule mass was increased in clover when S was applied (Anderson and Spencer, 1950). With this knowledge from literature and observing the effects of S on N fixation on the later stages of soybean, it would be beneficial in the future to see if early nodulation is affected by S. To determine this effect, roots could be dug to count and weigh nodules starting at the V3 growth stage and continuing until reproductive stages. With this sampling procedure having a certain amount of error regarding root digs in the field, it would also be appropriate to collect plant samples to send for ureide and nitrate testing. This testing would prove if early nodulation and also fixation is improved, and provide a better idea of when N fixation is active and begins contributing to the plant.

Ureide production at R5 was highest for the pre-emergence applications of AMS and ATS at the higher rate of S, which also had the greatest improvement in amino acids and thereby, protein. Oil production was inversely related to most protein effects observed. Just as early plantings are expected to have a higher concentration of oil due to higher temperatures at R6, early maturity groups show the same pattern possibly due to quicker maturity and experiencing higher temperatures at R6 (Robinson et al., 2009). The later plantings and early maturity group both showed higher seed mass, possibly due to compensation for having less reproductive nodes and/or fewer pods.

At both locations, it was noted that some of the largest seed weights were associated with treatments of N and S from pre-emergence to the V4 growth stage. The early season fertilization could have promoted quicker emergence and supported faster growth in the early soybean stages. If those treatments promoted faster growth into the V2-V3 growth stage, the soybeans in those treatments would have started nodule development and N fixation earlier than the other treatments. This earlier fixation development and a higher N supply could increase the overall chlorophyll content and photosynthetic activity, providing more energy for grain fill.

STAND 20 and ATS also played a role in increased amino acid production at both locations, showing that early applications of S, or more possibly, applications containing more S may affect the end result of soybean seed quality. Since these two treatments were the only treatments with a final application of 22.4 kg S ha^{-1}, it is possible that soybean had a higher S concentration than was needed to be in balance with N, and therefore could contribute more S to those amino acids and protein. It would be interesting in future studies to focus on treatments that are balanced with N in fertilizer applications, include a treatment with a higher ratio of S and a treatment with a higher ratio of N. Considering the treatments explored in this study, I would recommend utilizing the 4:1 N:S ratio for fertilizer to examine high N rates, a 1:1 N:S ratio, and a 1:4 N:S ratio to examine high S rates.

Tissue samples for nutrient analysis could be a great addition to test mid-season nutrient movement and nutrient use in the soybean. Drone footage would provide a way to determine chlorophyll differences between treatments, as well as overall plant health variations between plots.

Returning to the initial objective and hypothesis, yield can be increased while at least maintaining protein levels, if not improving them. Pre-emergence applications of N and/or S increased yield while maintaining protein. STAND 20 and Plant NS were pre-emergence applications that may have not been the very top yielding treatments, but they did exhibit improvements in protein levels while still improving yield over the UTC treatment. Higher yields and equal protein levels to the UTC were also achieved with V4 applications of N and S. Future studies that contribute towards finding the optimum rates of N and S and focusing on earlier applications with either pre-emergent or V4 stage applications, could really push soybean research forward on understanding the effects of N, S, and the interaction within the plant.

Table 2-1. Treatments partitioned by growth stage applications and rate as well as nutrient totals applied for each treatment.

Treatment	Total (kg ha^{-1})		Nitrogen (kg ha^{-1}) †				Sulfur (kg ha^{-1})			
	N	S	Pre	V4	R3	R4+	Pre	V4	R3	R4+
UTC	.	.	.	.	.	.	.	.	.	.
STAND 20	19.6	22.4	19.6	.	.	.	22.4	.	.	.
Plant NS	44.8	11.2	44.8	.	.	.	11.2	.	.	.
ATS	10.4	22.4	10.4	.	.	.	22.4	.	.	.
V4 NS	44.8	11.2	.	44.8	.	.	.	11.2	.	.
V4R3 NS	89.7	22.4	.	44.8	44.8	.	.	11.2	11.2	.
R3 NS	44.8	11.2	.	.	44.8	.	.	.	11.2	.
UAN Direct	44.8	.	.	.	44.8	.	.	.	.	.
AMS Direct	9.8	11.2	.	.	9.8	.	.	.	11.2	.
R4+	89.7	22.4	.	.	44.8	44.8	.	.	11.2	11.2

† Sulfur needs met with sulfur fertilizer, and the rest of the needed nitrogen was met with nitrogen fertilizer (urea, UAN 28%, or Coron®)

Table 2-2. Treatments partitioned by application growth stage showing nutrient totals from each treatment and the product used to accomplish those applications.

Treatment	Total (kg ha^{-1})		Nitrogen (kg ha^{-1})				Sulfur (kg ha^{-1})			
	N	S	Pre	V4	R3	R4+	Pre	V4	R3	R4+
UTC	.	.	.	.	.	.	.	.	.	.
STAND 20	19.6	22.4	.	.	.	.	AMS	.	.	.
Plant NS	44.8	11.2	urea	.	.	.	AMS	.	.	.
ATS	10.4	22.4	.	.	.	.	ATS	.	.	.
V4 NS	44.8	11.2	.	urea	.	.	.	AMS	.	.
V4R3 NS	89.7	22.4	.	urea	UAN	.	.	AMS	AMS	.
R3 NS	44.8	11.2	.	.	UAN	.	.	.	AMS	.
UAN Direct	44.8	.	.	.	UAN	.	.	.	.	.
AMS Direct	9.8	11.2	.	.	.	.	.	.	AMS	.
R4+	89.7	22.4	.	.	UAN	Coron®	.	.	AMS	AMS

Table 2-3. Soil fertility analyses for Planting Date x Fertility trials in 2018 and 2019 near West Lafayette, Indiana. Samples were taken prior to fertilizer applications and planting in each respective year and trial. Mean values are averaged over replications. (± standard deviation)

Soil Analyses	2018			2019		
OM (mg kg^{-1})	4.0	±	0.2	3.8	±	0.5
pH	6.9	±	0.2	6.6	±	0.1
CEC (cmol$_c$ kg^{-1})	26.2	±	0.6	22.6	±	1.9
P (mg kg^{-1})	29.2	±	13.6	13.8	±	1.8
K (mg kg^{-1})	186	±	18.7	151	±	6.6
Mg (mg kg^{-1})	907	±	48.3	756	±	43.8
Ca (mg kg^{-1})	3556	±	125.6	2876	±	190
S (mg kg^{-1})	7.4	±	0.9	8.6	±	1.1
Zn (mg kg^{-1})	2.4	±	0.4	2.8	±	1.3
Mn (mg kg^{-1})	21.0	±	7.3	23.2	±	2.0
Fe (mg kg^{-1})	162	±	32.1	145	±	11.7
Cu (mg kg^{-1})	4.2	±	0.2	2.4	±	0.2
B (mg kg^{-1})	0.8	±	0.2	0.8	±	0.2

Table 2-4. Planting date treatments and application schedule. Study was located near West Lafayette, IN in 2018 and 2019. Two planting dates investigated: early/ normal planting (1) and late planting (2). Planting date treatments and application schedule.

2018	Application Dates for 2018 Planting Date 1				Application Dates for 2018 Planting Date 2			
Treatment	**Pre-emerge**	**V4**	**R3**	**R4+**	**Pre-emerge**	**V4**	**R3**	**R4+**
STAND 20	5/11	.	.	.	6/6	.	.	.
Plant NS	5/11	.	.	.	6/6	.	.	.
ATS	5/12	.	.	.	6/6	.	.	.
V4 NS	.	6/18	.	.	.	7/5	.	.
V4R3 NS	.	6/18	7/13	.	.	7/5	8/2	.
R3 NS	.	.	7/13	.	.	.	8/2	.
UAN Direct	.	.	7/13	.	.	.	8/2	.
AMS Direct	.	.	7/13	.	.	.	8/2	
R4+‡	.	.	7/13	7/27-8/26	.	.	8/2	8/10-9/13
2019	**Application Dates for 2019 Planting Date 1**				**Application Dates for 2019 Planting Date 2**			
Treatment	**Pre-emerge**	**V4**	**R3**	**R4+**	**Pre-emerge**	**V4**	**R3**	**R4+**
STAND 20	6/12	.	.	.	6/27	.	.	.
Plant NS	6/12	.	.	.	6/27	.	.	.
ATS	6/12	.	.	.	6/27	.	.	.
V4 NS	.	7/15	.	.	.	7/25	.	.
V4R3 NS	.	7/15	8/6	.	.	7/25	8/21	.
R3 NS	.	.	8/6	.	.	.	8/21	.
UAN Direct	.	.	8/6	.	.	.	8/21	.
AMS Direct	.	.	8/6	.	.	.	8/21	.
R4+‡	.	.	8/6	8/16-9/19	.	.	8/21	8/29-9/30

‡ R4+ treatments were applied approximately every 10 days to target growth stages around R4, R5, R5.5, and R6; however, with phytotoxicity new growth had to be allowed to grow before the next treatment, thus sprays were done about every 10 days starting at R4 growth stage

Table 2-5. Soil fertility for Variety x N+S Fertility in 2018 and 2019 near Wanatah, Indiana. Samples were taken prior to fertilizer applications and planting in each respective year. Mean values are averaged over replications. (+ standard deviation)

Soil Analyses	2018			2019		
OM (mg kg^{-1})	3.7	±	0.2	2.8	±	0.2
pH	6.2	±	0.3	6.3	±	0.2
CEC (cmol$_c$ kg^{-1})	19.8	±	1.7	16.3	±	1.6
P (mg kg^{-1})	31.8	±	5.0	35.4	±	3.3
K (mg kg^{-1})	137	±	8.3	133	±	12.2
Mg (mg kg^{-1})	601	±	56.6	496	±	28.5
Ca (mg kg^{-1})	2266	±	161	1939	±	108
S (mg kg^{-1})	11.4	±	3.0	9.6	±	1.9
Zn (mg kg^{-1})	2.9	±	0.4	2.4	±	0.6
Mn (mg kg^{-1})	49.6	±	4.4	5.6	±	0.9
Fe (mg kg^{-1})	162	±	5.3	173	±	15.1
Cu (mg kg^{-1})	2.9	±	0.1	3.2	±	0.3
B (mg kg^{-1})	0.4	±	0.1	0.3	±	0.1

Table 2-6. Maturity group treatments and application schedule. Study was located near Wanatah, IN in 2018 and 2019. Two maturity groups were investigated: AG 24x7 (2.4) and AG 34x6 (3.4).

2018	**Application Dates for 2018 AG24x7**				**Application Dates for 2018 AG34x6**			
Treatment	**Pre-emerge**	**V4**	**R3**	**R4+**	**Pre-emerge**	**V4**	**R3**	**R4+**
STAND 20	6/2	.	.	.	6/2	.	.	.
Plant NS	6/2	.	.	.	6/2	.	.	.
ATS	6/2	.	.	.	6/2	.	.	.
V4 NS	.	6/25	.	.	.	6/25	.	.
V4R3 NS	.	6/25	7/27	.	.	6/25	8/1	.
R3 NS	.	.	7/27	.	.	.	8/1	.
UAN Direct	.	.	7/27	.	.	.	8/1	.
AMS Direct	.	.	7/27	.	.	.	8/1	
R4+‡	.	.	7/27	8/1-9/12	.	.	8/1	8/9-9/24
2019	**Application Dates for 2019 AG24x7**				**Application Dates for 2019 AG34x6**			
Treatment	**Pre-emerge**	**V4**	**R3**	**R4+**	**Pre-emerge**	**V4**	**R3**	**R4+**
STAND 20	6/4	.	.	.	6/4	.	.	.
Plant NS	6/4	.	.	.	6/4	.	.	.
ATS	6/4	.	.	.	6/4	.	.	.
V4 NS	.	7/9	.	.	.	7/9	.	.
V4R3 NS	.	7/9	8/2	.	.	7/9	8/7	.
R3 NS	.	.	8/2	.	.	.	8/7	.
UAN Direct	.	.	8/2	.	.	.	8/7	.
AMS Direct	.	.	8/2	.	.	.	8/7	.
R4+‡	.	.	8/2	8/8-9/9	.	.	8/7	8/14-9/9

‡ R4+ treatments were applied approximately every 10 days to target growth stages around R4, R5, R5.5, and R6; however, with phytotoxicity new growth had to be allowed to grow before the next treatment, thus sprays were done about every 10 days starting at R4 growth stage

Table 2-7. Mean monthly air temperature, total monthly precipitation, and 30-yr averages (1989 to 2019) for a typical growing season at West Lafayette, IN.

	2018	2019	30-yr
Air Temperature		——— °C ———	
April	5.9	9.8	10.5
May	20.8	16.1	16.7
June	22.6	20.9	21.7
July	22.4	24.3	22.9
August	22.6	21.6	21.9
September	20.4	20.4	18.4
October	11.7	12.0	12.0
Precipitation		——— mm ———	
April	65	128	101
May	94	137	122
June	126	84	122
July	62	47	109
August	155	65	94
September	100	67	78
October	149	86	78

Table 2-8. Soybean stem nitrogen analysis in response to planting date across fertility treatment and response to fertility treatment across planting date. Only 2018 results are shown.

Main Effect	R5								R7							
	Ureide		**Nitrate**		**Total N**		**RAU¶**		**Ureide**	**Nitrate**				**Total N**	**RAU¶**	
	µmol g^{-1}		**µmol g^{-1}**		**µmol g^{-1}**		**%**		**µmol g^{-1}**	**µmol g^{-1}**				**µmol g^{-1}**	**%**	
Planting Date																
May 11	332	B	26.1	B	358	B	92		52.5	12.0‡				64.6	80	
June 5	455	A	34.8	A	490	A	92		60.4	8.3				68.7	85	
Fertility										§ PD 1		PD 2				
UTC	387	a	21.9	c	409	a	94	ab	63.9	13.6	ab	8.4	gh	74.8	84	a
STAND 20	429	a	24.9	c	454	a	94	ab	55.2	12.7	abc	7.3	h	65.2	82	a
Plant NS	389	a	28.5	bc	418	a	93	ab	66.0	12.3	abcd	8.2	gh	76.3	85	a
ATS	447	a	21.7	c	469	a	95	a	57.0	11.0	cdef	8.7	fgh	66.8	84	a
V4 NS	387	a	29.2	bc	416	a	92	ab	51.9	10.4	defg	7.8	h	61.0	83	a
V4R3 NS	279	b	50.7	a	329	b	84	c	37.6	11.2	cde	9.3	efgh	47.9	74	b
R3 NS	400	a	36.5	b	436	a	91	b	57.8	13.9	a	7.6	h	68.5	83	a
AMS Direct	431	a	30.1	bc	461	a	92	ab	62.2	11.5	bcde	9.4	efgh	72.7	85	a
Planting Date	*		x		*		ns		ns	**				ns	ns	
Fertility	***		***		**		***		ns	ns				ns	*	
Pdate x Fertility	ns		ns		ns		ns		ns	**				ns	ns	
CV (%)	16.2		32.5		14.7		3.3		34.4	15.4				30.2	7.5	

†Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively; ns, not significant.
‡see pdate x fertility interaction
§ CV for interactions: Nitrate = 15.7
¶ Relative Abundance of Ureides

Table 2-9. 2018 soybean biomass responses to planting date across fertility treatment and response to fertility treatment across planting date from the trial conducted near West Lafayette, IN.

Main Effect	R5					R7				
	Stems g m^{-2}	**Leaves** g m^{-2}	**Pods** g m^{-2}	**Total ¶** g m^{-2}	**Pods:Total**	**Stems** g m^{-2}	**Leaves** g m^{-2}		**Total ¶** g m^{-2}	**Apparent HI |** %
Planting Date										
May 11	310 A	237	156 A	921 A	16.8 A	321 A	92.0‡		1505 A	45.2 B
June 5	252 B	223	109 B	755 B	14.3 B	242 B	7.8		1178 B	48.7 A
Fertility							§ PD 1	PD 2		
UTC	263	217	125	791	15.5	270 bc	64 c	10.1 d	1271	45.9
STAND 20	289	237	136	863	15.7	268 bc	91 bc	6.4 d	1297	47.5
Plant NS	307	242	144	894	15.8	303 ab	92 bc	4.7 d	1402	47.0
ATS	274	221	135	816	16.3	283 abc	72 c	11.2 d	1318	48.4
V4 NS	302	255	145	927	15.2	318 a	116 ab	4.1 d	1511	45.6
V4R3 NS	270	218	121	793	14.9	290 ab	81 c	11.0 d	1350	47.2
R3 NS	280	228	133	832	15.8	268 bc	128 a	3.6 d	1311	46.5
AMS Direct	261	222	122	792	15.1	252 c	89 bc	11.1 d	1271	47.3
Planting Date	x	ns	*	x	**	*	***		*	**
Fertility	ns	ns	ns	ns	ns	*	ns		ns	ns
Pdate x Fertility	ns	ns	ns	ns	ns	ns	*		ns	ns
CV (%)	14.8	18.1	25.0	18.4	7.8	12.7	39.0		14.6	6.4

†Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively; ns, not significant.
‡see pdate x fertility interaction
§ CV for interactions: Leaves = 39.7
¶ Total biomass includes the total of dry biomass from stems, leaves, pods, petioles/branches, and in the case of R7 seed and fallen leaves are also included
| Apparent harvest index is calculated as the dry weights of seed/ (stem + pod + seed) without using the biomass of the fallen leaves and attached leaves

Table 2-10. 2019 soybean biomass responses to planting date and fertility treatment across planting date from West Lafayette, IN study.

Main Effect	R5 Stems g m^{-2}	R5 Leaves g m^{-2}	R5 Pods g m^{-2}	R5 Total ¶ g m^{-2}	R5 Pods:Total	R7 Stems g m^{-2}		R7 Leaves g m^{-2}	R7 Total ¶ g m^{-2}		R7 Apparent HI | %
Planting Date											
June 11	187	225 A	94 B	659 A	14.2 B	174‡		40.2 A	1053‡		54.3
June 27	182	216 B	104 A	626 B	16.5 A	161		11.0 B	902		55.2
Fertility						***§ PD 1***	***PD 2***		***§ PD 1***	***PD 2***	
UTC	178	210	98	621	15.7	155 bcd	161 bcd	22.4	969 cde	929 def	55.4
STAND 20	187	221	95	639	14.8	179 abc	148 d	31.9	1132 abc	888 def	55.6
Plant NS	200	233	105	688	15.3	171 abcd	182 ab	16.4	1021 abcd	1004 abcd	55.3
ATS	191	225	101	659	15.2	201 a	150 cd	25.8	1149 ab	824 ef	55.7
V4 NS	184	225	100	647	15.4	161 bcd	170 bcd	22.3	1000 abcd	972 cde	54.7
V4R3 NS	184	226	99	654	15.1	185 ab	142 d	26.1	1161 a	784 f	52.9
R3 NS	174	211	96	613	15.7	172 abcd	168 bcd	27.5	990 bcde	866 def	54.0
AMS Direct	179	213	99	622	15.8	163 bcd	167 bcd	32.1	1005 abcd	946 def	54.7
Planting Date	ns	x	*	x	***	x		**	*		ns
Fertility	ns	ns	ns	ns	ns	ns		ns	ns		ns
Pdate x Fertility	ns	ns	ns	ns	ns	x		ns	**		ns
CV (%)	13.5	12.3	17.4	13.0	6.7	15.4		72.0	11.7		6.7

†Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively; ns, not significant. §CV for interactions: Stems = 15.2, Total = 12.2
‡see pdate x fertility interaction ¶Total of dry biomass and in the case of R7 seed and fallen leaves are also included | seed/ (stem + pod + seed) using dry weights

Table 2-11. Soybean seed yield and quality responses to planting date across fertility treatment and response to fertility treatment across planting dates. Two interactions were found in 2018 as shown in the table. Study was located near West Lafayette, IN in 2018 and 2019.

Main Effect	2018 Yield		2018 Seed Size	2018 Moisture	2018 Protein	2018 Oil		2019 Yield	2019 Seed Size	2019 Moisture	2019 Protein	2019 Oil
Planting Date⁋	kg ha^{-1}		g 100 sd^{-1}	%	% dry basis	% dry basis		kg ha^{-1}	g 100 sd^{-1}	%	% dry basis	% dry basis
Pdate 1	4842‡		16.4 A	11.4 A	39.4 B	22.1‡		3927 A	17.5 B	10.5 B	38.8 B	22.0 A
Pdate 2	4018		16.2 B	11.1 B	40.0 A	21.9		3734 B	18.3 A	11.8 A	39.4 A	21.5 B
Fertility	***§ PD 1***	***PD 2***				***§ PD 1***	***PD 2***					
UTC	4194 cd	3984 d	15.7 c	11.4 a	39.6 bcd	22.4 ab	21.9 efg	3904 a	17.8	10.9	39.0 bc	21.7 bc
STAND 20	4672 b	4081 d	16.4 ab	11.1 c	40.1 a	22.1 bcd	21.8 efgh	3649 b	17.7	11.3	39.0 bc	21.6 c
Plant NS	4989 ab	4223 cd	16.6 a	11.3 abc	39.9 ab	22.2 bc	21.8 efgh	3778 ab	17.8	11.2	38.6 d	22.1 a
ATS	4811 ab	4160 d	16.4 ab	11.3 ab	40.0 ab	21.7 gh	21.8 efgh	3868 a	18.1	11.1	39.0 bc	21.8 bc
V4 NS	5103 a	3901 d	16.4 ab	11.2 bc	39.9 abc	22.3 ab	21.9 efg	3923 a	18.0	10.9	38.8 cd	21.9 ab
V4R3 NS	5116 a	3874 d	16.7 a	11.1 c	39.5 cd	22.2 bcd	22.0 cdef	3829 a	18.0	11.4	39.3 b	21.8 bc
R3 NS	5200 a	4026 d	16.4 ab	11.2 abc	39.6 bcd	22.0 cdef	22.0 cde	3800 ab	18.0	11.6	39.1 bc	21.7 bc
UAN Direct	4892 ab	4070 d	16.0 bc	11.2 bc	39.0 e	22.6 a	22.2 bc	3875 a	17.8	11.1	39.1 bc	21.8 bc
AMS Direct	4578 bc	4014 d	16.2 abc	11.2 bc	39.8 abcd	21.7 fgh	21.6 h	3777 ab	18.1	11.0	38.9 cd	21.7 bc
R4+	4866 ab	3847 d	16.0 bc	11.2 bc	39.4 d	21.9 defg	21.7 fgh	3900 a	18.1	11.0	40.0 a	21.3 d
Planting Date	***		*	***	**	*		**	***	***	***	***
Fertility	ns		**	*	***	***		x	ns	ns	***	***
Pdate x Fertility	x		ns	ns	ns	x		ns	ns	ns	ns	ns
CV (%)	9.0		3.5	1.7	1.1	1.2		5.0	2.5	4.9	1.0	1.3

†Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively; ns, not significant. ‡see pdate x fertility interaction

§ CV for interactions: yield = 8.8 , oil = 1.2 ⁋ 2018 planting date 1: 5/11, planting date 2: 6/5; 2019 planting date 1: 6/11, planting date 2: 6/27

Table 2-12. 2018 amino acid response to N+S fertility treatments in West Lafayette, IN trial.

ACRE	2018				
	STAND 20	*Plant NS*	*ATS*	*V4 NS*	*V4R3 NS*
<u>Amino Acids</u>	In Comparison to UTC				
Lysine	NS	NS	NS	NS	NS
Cysteine	+	+	+	+	+
Methionine	+	+	+	+	+
Threonine	+	+	+	+	NS
Tryptophan	+	+	+	+	+
Isoleucine	NS	NS	NS	NS	-
Leucine	+	NS	+	NS	NS
Histidine	+	+	+	NS	NS
Phenylalanine	+	NS	NS	NS	-
Valine	NS	NS	NS	NS	NS
Alanine	+	+	+	NS	NS
Arginine	+	NS	+	NS	NS
Asparagine	+	NS	+	+	NS
Glutamine	+	+	+	+	NS
Glycine	+	+	+	NS	NS
Proline	+	NS	+	NS	NS
Serine	+	+	+	NS	NS
Tyrosine	+	NS	NS	NS	NS

† + is a significant positive response of the amino acid to the fertility treatment
‡ - is a significant negative response of the amino acid to the fertility treatment
§ Not Significant
¶ Cysteine and Methionine are S-containing amino acids

Table 2-13. 2019 amino acid response to N+S fertility treatments in West Lafayette, IN trial.

ACRE	2019				
	STAND 20	*Plant NS*	*ATS*	*V4 NS*	*V4R3 NS*
Amino Acids	In Comparison to UTC				
Lysine	NS	NS	NS	NS	NS
Cysteine	+	NS	NS	NS	+
Methionine	NS	NS	+	NS	+
Threonine	NS	NS	NS	NS	NS
Tryptophan	NS	+	+	NS	+
Isoleucine	NS	-	NS	-	NS
Leucine	NS	-	NS	NS	NS
Histidine	NS	NS	NS	NS	NS
Phenylalanine	NS	-	NS	-	NS
Valine	NS	-	NS	NS	NS
Alanine	NS	-	NS	NS	NS
Arginine	NS	NS	NS	NS	NS
Asparagine	NS	NS	NS	NS	NS
Glutamine	NS	-	NS	NS	NS
Glycine	NS	NS	NS	NS	NS
Proline	NS	NS	NS	NS	NS
Serine	NS	NS	NS	NS	NS
Tyrosine	NS	-	NS	NS	NS

† + is a significant positive response of the amino acid to the fertility treatment
‡ - is a significant negative response of the amino acid to the fertility treatment
§ Not Significant
¶ Cysteine and Methionine are S-containing amino acids

Table 2-14. Mean monthly air temperature, total monthly precipitation, and 30-yr averages (1989 to 2019) for a typical growing season at Wanatah, IN.

	2018	2019	30-yr
Air Temperature		——— °C ———	
April	4.0	8.9	8.7
May	19.1	14.4	15.1
June	21.8	19.8	20.6
July	22.4	23.8	22.2
August	22.2	20.8	21.0
September	19.2	19.5	17.3
October	10.4	10.6	10.7
Precipitation		——— mm ———	
April	51	114	87
May	123	189	101
June	89	128	119
July	72	48	107
August	141	67	114
September	88	175	85
October	110	110	95

Table 2-15. 2018 Pinney soybean stem nitrogen in response to variety and fertility treatment.

Main Effect	R5 Ureide µmol g^{-1}		R5 Nitrate µmol g^{-1}	R5 Total N µmol g^{-1}		R5 RAU %		R7 Ureide µmol g^{-1}	R7 Nitrate µmol g^{-1}	R7 Total N µmol g^{-1}	R7 RAU %
Variety											
AG 24x7	458		44.6 A	502		91.1 B		43.4	7.1	50.5	84.5 A
AG 34x6	443		24.5 B	468		94.7 A		29.3	8.3	37.7	76.3 B
Fertility	**⁋*AG24x7***	***AG34x6***		**⁋*AG24x7***	***AG34x6***	**⁋*AG24x7***	***AG34x6***				
UTC	443 cdef	406 ef	25.2 d	476 cdefg	424 g	93.2 bc	95.7 a	42.3	7.3	49.5	84.1 a
STAND 20	495 abc	483 abcd	32.5 bc	537 ab	506 abcde	92.2 c	95.3 a	48.8	7.7	56.5	83.0 ab
Plant NS	437 cdef	406 f	36.7 bc	488 bcdef	428 fg	89.6 e	94.7 ab	35.0	8.2	43.2	80.5 ab
ATS	515 a	510 a	30.3 cd	553 a	533 abc	93.2 bc	95.7 a	30.8	7.6	38.4	78.7 bc
V4 NS	446 bcdef	398 f	38.7 ab	495 abcde	426 g	90.1 de	93.3 bc	35.4	8.6	43.9	78.9 bc
V4R3 NS	465 abcde	417 ef	45.5 a	520 abcd	453 efg	89.4 e	92.0 cd	25.8	7.7	33.4	74.8 c
R3 NS	448 bcdef	424 def	32.6 bc	489 bcde	448 efg	91.6 cd	94.7 ab	32.0	7.6	39.6	79.8 ab
AMS Direct	410 ef	504 ab	34.6 bc	460 defg	524 abc	89.2 e	96.2 a	40.9	7.3	48.2	83.5 a
Variety†	ns		**	Ns		**		ns	ns	ns	*
Fertility	**		***	**		***		ns	ns	ns	**
Var x Fertility	**		ns	**		x		ns	ns	ns	ns
CV (%)	7.0		20.8	6.3		1.7		53.9	15.5	45.2	6.4

†Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively; ns, not significant.
⁋ CV for interactions: Ureide = 9.2, total N = 8.7, RAU = 1.7

Table 2-16. 2018 and 2019 pooled Pinney soybean biomass responses to variety across fertility treatment and response to fertility treatment across variety from the trial conducted near Wanatah, IN.

Main Effect	R5					R7				
	Stems	**Leaves**	**Pods**	**Total ¶**	**Pods:Total**	**Stems**	**Leaves**	**Grain**	**Total ¶**	**Apparent HI |**
	g m^{-2}	**g m^{-2}**	**g m^{-2}**	**g m^{-2}**		**g m^{-2}**	**g m^{-2}**	**g m^{-2}**	**g m^{-2}**	**%**
Variety										
AG 24x7	167 B	227 B	117	672	17.2	129 B	12.8	418	894	58.5
AG 34x6	219 A	242 A	157	847	18.5	185 A	31.6	423	1086	54.3
Fertility										
UTC	185	220 c	128	692	17.8	158	25.5	405	988	54.7 d
STAND 20	201	242 ab	143	762	18.3	152	21.5	403	959	56.4 bcd
Plant NS	197	240 ab	132	745	17.5	161	21.4	405	983	54.9 d
ATS	187	228 bc	135	711	18.1	153	20.4	431	987	57.4 ab
V4 NS	204	244 ab	144	772	18.2	160	20.1	438	1006	57.2 abc
V4R3 NS	200	247 a	146	773	18.0	153	20.6	434	983	58.4 a
R3 NS	186	234 abc	135	732	18.0	158	23.5	418	1006	55.4 cd
AMS Direct	186	222 c	132	886	16.8	159	24.2	429	1006	56.5 abcd
Variety†	**	*	ns	ns	ns	*	ns	ns	ns	ns
Fertility	ns	x	ns	ns	ns	ns	ns	ns	ns	x
Var x Fertility	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Year x Var	ns	ns	***	*	***	ns	***	ns	***	*
Year x Fertility	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Year x Var x Fertility	**	x	*	ns	ns	ns	ns	ns	ns	ns
CV (%)	11.4	11.9	15.8	37.4	11.1	15.1	66.0	13.8	10.6	5.1

†Significance at $P \leq 0.10$, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively; ns, not significant.
¶ Total biomass includes the total of dry biomass from stems, leaves, pods, petioles/branches, and in the case of R7 seed and fallen leaves are also included
| Apparent harvest index is calculated as the dry weights of seed/ (stem + pod + seed) without using the biomass of the fallen leaves and attached leaves.

Table 2-17. 2018 & 2019 pooled Pinney soybean seed yield and quality responses to variety across fertility treatment and response to fertility treatment across variety.

Main Effect	2018 & 2019														
Variety	**Yield kg ha^{-1}**		**Seed Size g 100 seed^{-1}**				**Moisture %**	**Protein % dry basis**				**Oil % dry basis**			
AG 24x7	4025		17.0‡				11.4	40.4‡				22.1‡			
AG 34x6	4022		16.3				11.5	39.6				21.7			
Fertility			AG24x7		AG34x6			AG24x7		AG34x6		AG24x7		AG34x6	
UTC	3900	cd	16.7	abc	16.0	c	11.5	39.8	ef	38.5	g	22.2	abc	22.1	abcde
STAND 20	4002	abcd	17.2	abc	16.6	abc	11.4	40.5	bc	39.9	def	22.2	abcd	21.6	def
Plant NS	4105	a	17.8	a	16.6	abc	11.4	40.4	bcd	39.8	ef	22.2	abcd	21.7	bcdef
ATS	3874	d	17.1	abc	16.4	c	11.4	40.6	b	40.1	cde	22.0	abcde	21.7	cdef
V4 NS	4068	ab	17.3	abc	16.5	abc	11.4	40.4	bc	39.6	f	22.0	abcde	21.8	abcde
V4R3 NS	4148	a	17.4	ab	16.4	bc	11.4	40.4	bc	39.7	ef	22.0	abcde	21.8	abcde
R3 NS	4087	ab	16.8	abc	16.0	c	11.5	40.5	bc	39.7	ef	21.9	abcde	21.7	cdef
UAN Direct	4027	abc	16.8	abc	16.3	bc	11.5	39.6	f	38.4	g	22.3	a	22.3	ab
AMS Direct	3938	bcd	16.9	abc	16.1	bc	11.5	40.5	bc	39.7	ef	22.2	ab	21.5	ef
R4+	4082	ab	16.5	abc	16.1	bc	11.5	41.2	a	40.5	bc	21.6	ef	21.2	f
Variety†	ns		ns				ns	ns				ns			
Fertility	x		**				ns	***				ns			
Var x Fertility	ns		x				ns	x				*			
Year x Var	ns		***				ns	*				***			
Year x Fertility	ns		ns				ns	**				***			
Year x Var x Fertility	ns		ns				ns	ns				ns			
CV (%)	8.5		2.5				ns	1.1				1.2			

†Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively; ns, not significant. ‡see var x fertility interactions

Table 2-18. Pinney 2018 and 2019 pooled amino acid response to N+S Fertility treatments at the Wanatah, IN trial.

Pinney	2018 & 2019				
	STAND 20	*Plant NS*	*ATS*	*V4 NS*	*V4R3 NS*
Amino Acids	In Comparison to UTC				
Lysine	+	+	+	+	+
Cysteine	+	+	+	+	+
Methionine	+	+	+	+	+
Threonine	+	+	+	+	+
Tryptophan	+	+	+	+	NS
Isoleucine	+	+	+	+	NS
Leucine	+	NS	+	NS	NS
Histidine	+	+	+	+	+
Phenylalanine	+	+	+	+	+
Valine	+	+	+	+	+
Alanine	+	+	+	+	+
Arginine	+	+	+	+	+
Asparagine	+	+	+	+	+
Glutamine	+	+	+	+	+
Glycine	+	+	+	+	+
Proline	+	NS	+	+	+
Serine	+	+	+	+	+
Tyrosine	+	+	+	+	+

† + is a significant positive response of the amino acid to the fertility treatment
‡ - is a significant negative response of the amino acid to the fertility treatment
§ Not Significant
¶ Cysteine and Methionine are S-containing amino acids

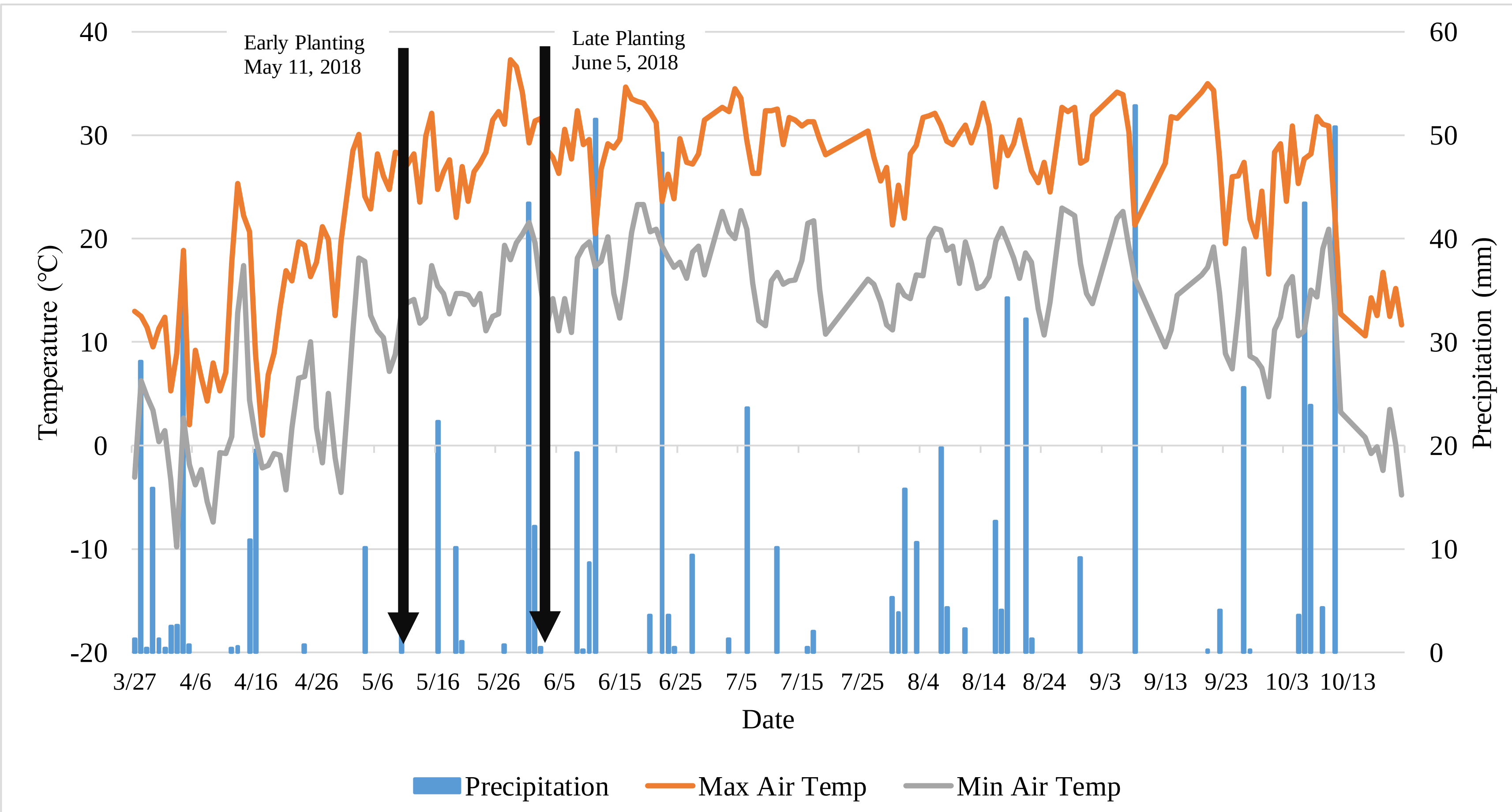

Figure 2-1. 2018 Air temperature and precipitation patterns for Planting Date x N+S Fertility trial in West Lafayette, I

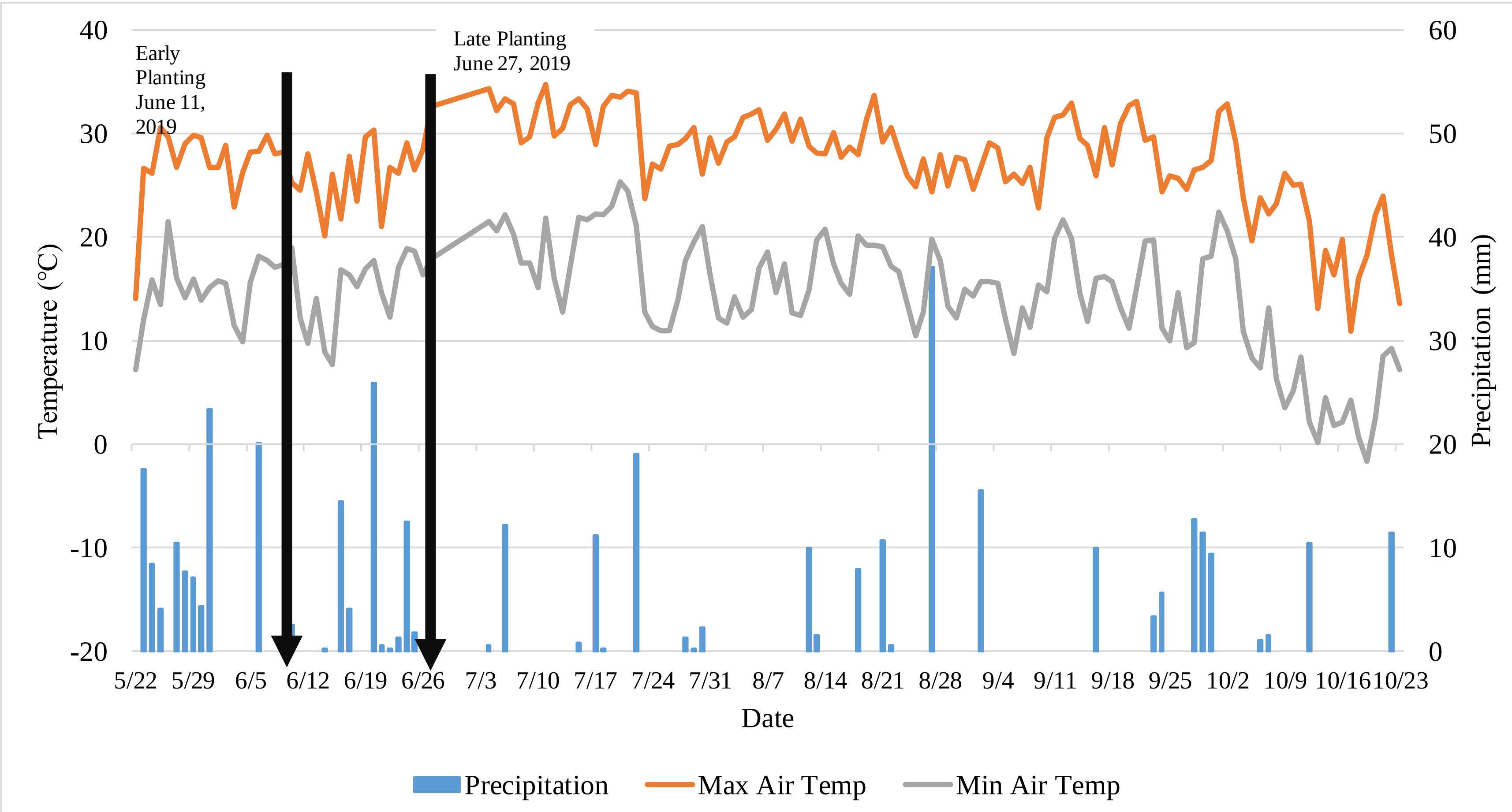

Figure 2-2. 2019 Air temperature and precipitation patterns for Planting Date x N+S Fertility trial in West Lafayette, IN

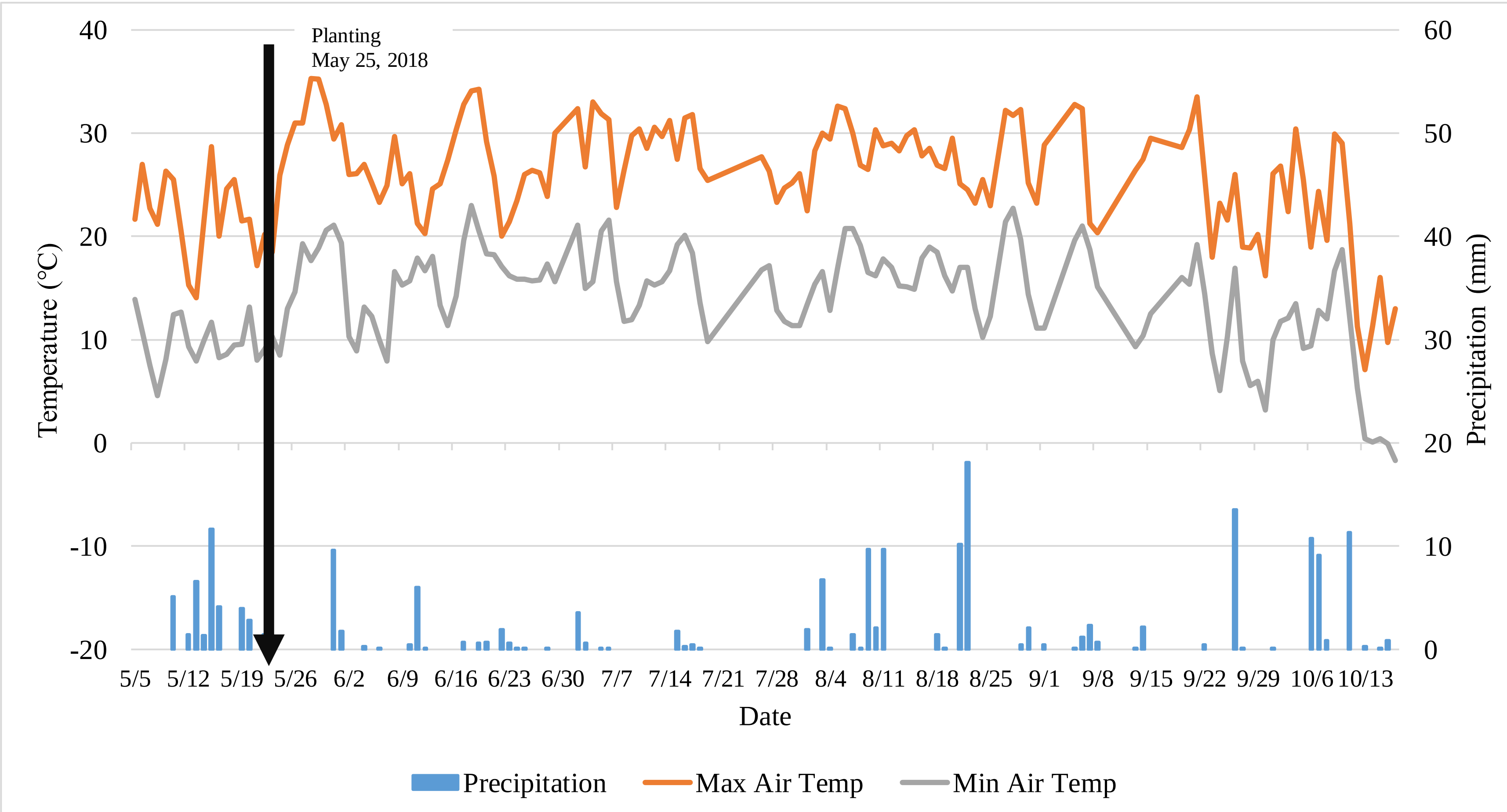

Figure 2-3. 2018 Air temperature and precipitation patterns for Variety x N+S Fertility trial in Wanatah, IN.

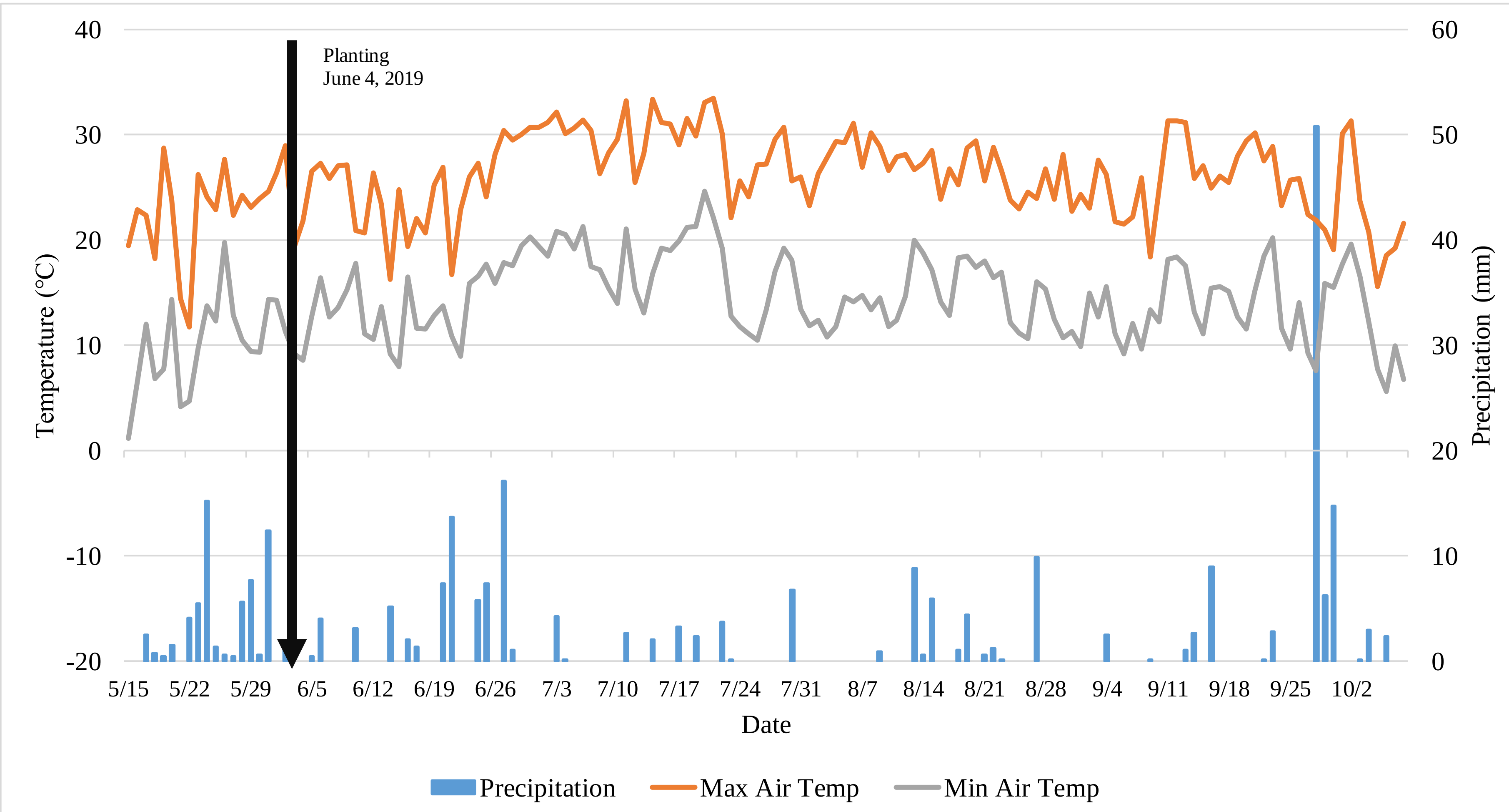

Figure 2-4. 2019 Air temperature and precipitation patterns for Variety x N+S Fertility trial in Wanatah, IN.

CHAPTER 3. SOYBEAN (*GLYCINE MAX* (L.) MERR.) RECOVERY RESPONSE TO VARYING RATES AND TIMINGS OF FOLIAR SULFUR APPLICATIONS

3.1 Abstract

The key objective of this study was to see if foliar applications of S could help soybean (*Glycine Max* (L.) Merr.) recover from S deficiency. If foliar applications can aid in the recovery of soybean, it is essential to know the optimum rate of S for that crop to thrive, yet not have economic or environmental repercussions. This study took place for two years at the Mary Rice farm in La Crosse, IN, mostly comprised of sandy loam soils mixed with fine sands, soils more prone to S deficiency. This study was a randomized complete block design with five replications both years.

The treatments included a pre-emergence application of granular ammonium sulfate (AMS) to the soil and untreated checks as comparison treatments for the foliar applications. Foliar applications of spraygrade AMS were made at V4, R3, and a sequential application was done at V4 followed by R3 growth stage. The rates included 1, 2, 4, and 6 pounds of S per acre for each foliar application timing. To see if these applications were effective, tissue samples were collected ~14 days following each application to see if the S reached the newest growth in the plant, foliar burn damage photos were documented, and plot comparison photos were taken when treatment differences were observed. At harvest, grain from the middle rows of the plots were collected to calculate yield and analyze seed size, protein, and oil content. With this data, the hope was to determine the optimum growth stage and S rate to apply to soybean that are predicted to be deficient in S but can still recover to end with a higher yield than if no S had been applied throughout the season.

The study showed that the seasons differed in an optimal rate with 2018 yields being maximized at approximately 4 lb S ac^{-1}. In 2019, the optimum rate was 7 lb S ac^{-1} with the sequential V4R3 treatments. Some tissue results approached deficient levels, but never were truly deficient. With this in mind, S concentrations still improved with applications. Applications at different growth stages (V4 or R3) did not result in much variation in yield or protein levels, thereby resulting in flexibility for application timing.

3.2 Introduction

Sulfur deficiencies are sometimes hard to diagnose but are more common in certain field conditions. This deficiency is hard to diagnose with visible symptomology because it looks very similar to N, Mo, Mg, Mn, and Fe deficiencies (Hitsuda et al., 2008). Symptomology appears as a general yellowing that starts at the top of the canopy and then proceeds its way down into the canopy due to lower chlorophyll and protein concentrations, resulting in a pale green or yellow canopy. Due to these similar appearances, a leaf tissue nutrient analysis is needed to identify the specific nutrient issue in that field. Soil tests can be helpful to an extent, but soil tests for S are not reliable due to the amount of variation between labs (Crosland et al., 2001). Sulfur is relatively mobile in the soil profile, and therefore is hard to sample and difficult to obtain an accurate value on the S level when the crop is growing versus the day the soil sample was collected (Camberato and Casteel, 2017). Using a combination of soil tests, tissue tests, and a prediction model, growers may be able to be prepared for S deficiency on certain farms.

This prediction comes from evaluating the leaching risk, groundwater level, soil texture, crop, precipitation levels, and the S-concentration in the groundwater (Bloem et al., 2002). All of these factors contribute to S deficiency, but a large contributor is soil type. It is most common that low organic matter, coarse textured soils that are well drained will be more susceptible to S deficiency due to increased leaching potential (Dick et al., 2008). Atmospheric deposition of S has diminished while crop removal rates have continued to increase (Chen et al., 2008). For example, in Ohio from 1979 to 2005, S deposition has decreased by 37% and crops have removed 18-50% more S in 2008 compared to 1983. There has been a 90% decrease in sulfur dioxide nationally from 1990 to 2019 (US EPA, 2019).

Foliar feeding is a popular method to apply micronutrients to a growing crop throughout the season. Macronutrients are rarely applied through foliar applications because these nutrients are required in such large quantities that the need is rarely met by a single foliar application without crop damage (Fageria et al., 2009). One benefit of foliar applications is how the nutrients enter the crop, where a crop response is closer to 3-4 days as compared to the response of soil applications, which may take up to 6 days. This quick response means that if S deficiency appears to be in the field, it could be corrected; however, with S being a relatively immobile, macronutrient in the plant, repeated applications might be necessary.

Repeated applications in a short period of time or using phytotoxic mixes may have a negative impact on the crop. Foliar burn has been noted in a number studies, and most often, due to the weather or a foliar mix that contains some form of nitrogen, where ammonium appeared to be the primary cause of the burn (Vasilas et al., 1980; Gooding and Davies, 1992). According to Gooding and Davies (1992), injury could be the result of the higher salt index of ammonium compared to urea.

One main challenge with foliar applications is the lack of research conducted on the proper application conditions. Most agree that it is best to do foliar applications on various crops in the morning or later afternoon when the temperatures are not excessive, but still applying when stomata are open (Fageria et al., 2009). The other factor to consider is that increased dew or humidity levels cause foliage damage during foliar N applications, and less than sufficient soil moisture could make the application ineffective (Woolfolk et al., 2002; Fageria et al., 2009).

A timely and successful foliar application is a difficult task to accomplish, and due to the lack of research, it is unclear if foliar applications pay off in yield. Although tissue concentrations may increase, this does not always translate to greater yield due to remobilization and other factors (Boote et al., 1978). Garcia reported soybean yields increased up to 31% with a N-P-K-S foliar mixture, but these results were not consistent across all sites or other studies (Garcia L. and Hanway, 1976; Haq and Mallarino, 2000). Haq and Mallarino found that even if there were yield increases with N-P-K foliar applications, these yield increases were not great enough to offset the cost of the application. It has been found that foliar applications of S (AMS) alone and S in combination with N (urea) can improve wheat yield and increase harvest index (SAEED et al., 2013).

With these inconsistencies in the literature and lack of research in foliar S applications in soybean, more research is needed to determine if foliar applications of S can help soybean recover along with increasing soybean yields. The objectives of this study were to determine: if foliar applications of S could help soybeans recover from S deficiencies, the optimum rate of S, and at what growth stage S applications have the greatest impact on yield and protein. The hypothesis is that the optimum rate of sulfur is at 4.5 kg S ha^{-1} and that this rate will be further optimized being applied to the foliage at R3, a stage where large quantities of nutrients are needed for seed production.

3.3 Materials and Methods

3.3.1 Site Characteristics

Field experiments were conducted in 2018 and 2019 at the Mary Rice Purdue Farm in La Crosse, Indiana (41º19’38” N, 86º48’28”W). The 2018 trial was conducted on a Gilford fine sandy loam (Coarse-loamy, mixed, superactive, mesic Typic Endoaquolls) with some Maumee loamy fine sand (Sandy, mixed, mesic Typic Endoaquolls) (“USDA-NRCS Official Soil Series Description View By Name,”). The 2019 trial was conducted on similar soils that were more evenly distributed throughout the field.

Soybean was bulk planted in 38-cm rows with a Case IH 1245 at 353, 353 seeds ha^{-1} under no-till conditions with the previous crop of corn planted in 76-cm rows. Pioneer variety P27T59 was planted on May 9, 2018 and Pioneer variety P26T07L on May 20, 2019. The seeds of these varieties were treated with LumiGEN fungicide. Individual plots were created prior to emergence at 3 m wide x 13.7 m long.

In-season herbicide program for 2018 included an early post application of Flexstar – fomesafen (8.6 pt ha^{-1}), dimethenamid (34.6 oz ha^{-1}), Fluazifop and Fenoxaprop (19.8 oz ha^{-1}), luftech (2 qt 100 gal^{-1} water). There was an early post spray in 2019 of glufosinate (2.5 qt ha^{-1}), dimethenamid (34.6 oz ha^{-1}), Fluazifop and Fenoxaprop (19.8 oz ha^{-1}), ENYA (4.9 qt ha^{-1}), NIS (1pt 100 gal^{-1} water).

3.3.2 Treatments

Fifteen treatments were designed in a 3 x 4 factorial + 3 individual treatments. Three target application timings were V4, R3, and V4 + R3 were crossed with 4 rates of foliar S at 1, 2, 4, 6 lb S ac^{-1} (1.12, 2.24, 4.49, 6.73 kg S ha^{-1}) with each application (Table 3-1). Therefore, the sequential application (V4 + R3) received a total of 2, 4, 8, 12 lb S ac^{-1} (2.24, 4.49, 8.96, and 13.44 kg S ha^{-1}). Individual treatment additions were two untreated controls and one standard S application of 20 lb S ac^{-1} (22.4 kg S ha^{-1}) as granular AMS (21-0-0-24S) pre-emergence. This application was broadcast spread alley-center to alley-center by hand over the soil surface. Treatments were arranged in a randomized complete block design with five replications.

In 2018, the early post herbicide spray of Flexstar caused crop injury (e.g., leaf burn, hardening of leaf tissue); therefore, the targeted V4 application was delayed until V6 where the new growth was not damaged. Spraygrade AMS was slowly dissolved into the proper rate of hot water and applied at 140 L ha^{-1} (15 GPA) with the Lee Avenger sprayer (Lubbock, TX). The vegetative application was made on June 25, 2018 and the R3 (first pod) application on July 17, 2018. The boom was held approximately 51 cm above the soybean canopy for each application.

In 2019, foliar applications were completed with a CO_2 pressurized backpack sprayer on July 2 (V5) and July 23 (R3). Nozzle spacing (38-cm) was the same as the previous year with TeeJet 8002 at 140 L ha^{-1} (15 GPA). The boom was held approximately 51 cm above the soybean canopy. The same rates of AMS were applied at V4 and R3 as those applied in the 2018 season, which also included the V4 + R3 sequential treatment.

3.3.3 Data Collection

Plant population was calculated early in the season around V2 by counting plants in a 1-m length of row in each the middle rows of the plots. This totaled three different counts that were later averaged for the plot stand count. Prior to harvest, plant population was again calculated.

Soil samples were collected to a depth of 20 cm at the beginning of the season to characterize general fertility and S concentrations present in the soil prior to emergence and fertilizer S applications. Approximately 12-15 cores were collected per replication to provide proper representation. See Table 3-2 for fertility information.

To determine plant uptake of fertilizer S, leaf tissue samples were collected approximately 14 days after each spray treatment. Standard AMS, untreated control (UTC), and topically treated plots were sampled. These leaf tissue samples were the most recent mature leaf (MRML), which is the largest, fully developed trifoliate at the top of the canopy (usually the 3rd or 4th node from the apical meristem). Plots that were sampled had 20 trifoliates collected, which were dried at 60ºC for three to five days. After drying in labeled bags, plant tissue was ground to 1mm in a Wiley Mill grinder, labeled, packaged, and sent to A&L Laboratories (Ft. Wayne, IN) for nutrient analyses. This provided a snapshot in time of the nutrient concentration differences between treatments. The ~14 day delay after application allows time for the S to move from sprayed foliage and translocate throughout the plant as it continues to develop new leaves. In 2018, the MRML samples were collected on July 10 (15 DAA) following the vegetative application and August 1 (15 DAA) following the R3 application. In 2019, the post-vegetative MRML samples were collected on July 16 (14 DAA) and the post-R3 MRML samples were collected on August 8 (16 DAA).

At R8, plots were end-trimmed to cut out possible border effects. The Kincaid 8-XP plot combine harvested the middle four rows for a grain subsample, sample weight, and moisture for each plot. This information was used to calculate yield adjusted to 13% grain moisture. Trials were harvested on October 9, 2018 and October 1, 2019. Grain subsamples were used to determine protein, oil, and seed size.

3.3.4 Statistical Analysis

Years are reported individually due to weather and condition differences in 2018 and 2019. Data were subjected to an analysis of variance (ANOVA) using PROC GLM in SAS (SAS Institute, version 9.4). Analysis was set up to test the main effect of rate and time of S fertilizer of the randomized complete block. With this analysis, fertility treatments and location were considered fixed, while year was random. Data were subjected to PROC REG with Linear and Quadratic models followed by PROC NLIN with Quadratic-Plateau model to determine the best fit using SAS (SAS Institute, version 9.4).

3.4 Results and Discussion: FOLIAR SULFUR TIMING & RATE

3.4.1 Weather

Air temperature data from the past 30 years from the Wanatah, IN location was compiled to show the season monthly averages (April-October) and was compared to the averages over the 2018 and 2019 seasons (Table 3-3). Monthly precipitation totals over the past 22 years from a North Judson, IN station was compiled to show the season averages (April-October) and was compared to the averages over the 2018 and 2019 seasons (Table 3-3). Two separate locations were used because no station was present in La Crosse, IN where the study took place. North Judson was closer than Wanatah, but only had precipitation data, so as a result, temperature data had to be used from the Wanatah station.

The 2018 season experienced more precipitation than the 22-year average, receiving 56.3 mm more than the average for the season. The crop was limited on water in July only receiving 31.5 mm compared to the 111 mm average over 22 years. October was very wet and posed challenges for timely harvest in 2018 (Figure 3-1). Temperatures throughout the 2018 season were similar to the compiled 30-year data with only a few months with slightly elevated temperatures (May, June, August, September).

Timely planting for the 2019 season was challenging due to the frequent rain events, which meant fields were saturated during optimal planting time (Figure 3-2). Precipitation was 26.4 and 64 mm higher than the 22-year average in April and May, respectively (Table 3-3). Temperatures represented the average for the season with little variation.

3.4.2 Applications and Phytotoxicity Observations

In 2018, there was some herbicide damage to the soybean crop at the V4 growth stage. FlexStar was applied on June 12th and damage was noticeable on June 13th. Damage included necrosis and, in some places where overlap occurred, the apical meristem was occasionally injured. As a result, the target V4 foliar application was delayed to allow new growth to emerge prior to foliar S applications.

The R3 foliar S application on July 17, 2018 caused some phytotoxic symptomology (i.e., necrotic burn), especially in the plots with the highest rate of AMS (6.7 kg S ha^{-1}). The same visual observations were noted in the 2019 season in plots that had 6.7 kg S ha^{-1} from the R3 foliar applications of spraygrade AMS. It is speculated that burn injury can be a result of the phytotoxicity of ammonia, so the ammonium in the AMS could have similar injury symptoms (Vasilas et al., 1980). It has been noted in literature that high temperatures leading up to 2:00 pm could cause the burning injury that was observed in some plots as well (Fageria et al., 2009).

3.4.3 Tissue Analysis

Tissue samples were collected approximately 14 days following each foliar application to determine leaf nutritional status (i.e., N, S). Leaf N concentrations from the post-V4 application sampling did not differ in 2018. Leaf S after the V4 application was sufficient regardless of S rate; however, leaf S was very close to critical (0.25% S) after the R3 applications (Table 3-4). In both leaf samplings and application timings, leaf S concentrations linearly increased as S rate increased (Figure 3-3). The N:S ratio in 2018 inversely plateaued at 15.2 after the V4 application of 5.6 lb S ac^{-1} (Table 3-5). The N:S ratio following the R3 application decreased linearly for the V4 treatments, and decreased in a quadratic fashion with the lowest value of 16.8 at a rate of 6 lb S ac^{-1} for the R3 timing, with the V4R3 treatments trending the same to the lowest value of 16.2 at the 12 lb S ac^{-1} total rate (Figure 3-4).

In 2019, post-V4 application samples showed that leaf N increased linearly 0.10% N for every 1 lb S ac^{-1} added with the V4 timing (Table 3-6). In 2019, leaf S concentration increased linearly (0.01% S for every 1 lb S ac^{-1} that was applied) following the V4 foliar application (Table 3-6). The N:S ratio decreased linearly (0.23 decrease per additional 1 lb S ac^{-1}) following the V4 application in 2019 to a bottom of 15.6.

Leaf S concentrations were low following the R3 applications (~0.23 %S) in 2019 (Table 3-7). Though, leaf S increased linearly for the V4 applications, increased quadratically for R3 applications, and increased quadratically for V4R3 applications (Figure 3-5). The N:S ratio decreased linearly for V4 and R3 applications (17.6 and 17.4, respectively), and quadratically for V4R3 reaching the lowest value of 16.5 at a total rate of 12 lb S ac^{-1} (Figure 3-6).

3.4.4 Seed Mass and Yield

In 2018, both the V4 and the R3 foliar timing had similar effects on yield. The V4 timing plateaued at 63.5 bu ac^{-1} at a rate of 4.1 lb S ac^{-1}, whereas the R3 timing plateaued at 62.2 bu ac^{-1} at a rate of 4.5 lb S ac^{-1} (Table 3-5). This compares well with the nutrient data discussed above. Leaf S was sufficient post-V4 applications, but increased linearly as S rate increased. Leaf S was close to the critical level, yet S concentrations increased linearly as S rate increased (Tables 3-4, 3-5). The leaf N:S was lower following foliar S applications at V4 (15.2 at 5.6 lb S ac^{-1}) than following the R3 and V4R3 applications (16.8 at 6 lb S ac^{-1} and 16.2 at 12 lb S ac^{-1}). The leaf N:S of the V4 application sampled post-R3 was determined to be 18.1, this suggests that the S need and the balance of N:S is more critical during earlier vegetative growth than the middle of reproductive development. The 2018 data showed that the V4 applications were still increasing leaf S concentration linearly when the post-R3 samples were collected, and at a similar rate of increase to the V4R3 and R3 applications (Table 3-5). Early vegetative applications of S (i.e., V4) had longer-lasting effects on soybean tissue concentrations that translate into higher yields.

In 2018, seed size was significant for the V4 and V4R3 applications and increased through the highest rate of S applied within each of those treatment regimes (Table 3-5). Seed size increased at a 6 lb S ac^{-1} rate with the V4 timing, and a 12 lb S ac^{-1} rate with the V4R3 timing.

Seed size was a contributor to yield, but was likely balanced with the number of pods and/or number of seeds since seed size continued to increase with additional S, yet yield plateaued with approximately 4 lb S ac^{-1}.

In 2019, the V4R3 applications of S caused a yield plateau at 49.1 bu ac^{-1} when 7.5 lb S ac^{-1} was applied (Table 3-6). R3 treatments increased yield linearly 1 bu for each additional pound of S applied (Table 3-6). Following the R3 application in 2019, there were fewer rain events compared to following the V4 application (Figure 3-2). If there was less water moving into the plant and bringing in less nutrients from the root system, this is where the R3 foliar application may have been beneficial in providing nutrients. The soils in 2019 were more deficient in S compared to 2018, and this could help explain why a sequential application of S would succeed be needed (Table 3-2).

In 2019, R3 foliar applications resulted in a maximum seed mass of 17.2 g 100 sd^{-1} when 5.7 lb S ac^{-1} was applied (Table 3-6). This rate of S at R3 (beginning pod) could assist the soybean in having S stored for proper seed fill. Seed size increased quadratically as S rate increased with the V4 applications, with the highest value being 18.1 g 100 sd^{-1} at the 6 lb S ac^{-1} rate (Table 3-8). This rate is similar to what the maximum yield reached. Yield increased linearly with the S rates at R3, with a maximum yield of 47.8 bu ac^{-1} with 5.7 lb S ac^{-1}.

Comparing the single applications versus the sequential applications of S can determine whether S needs applied in multiple passes, or just a single pass. In 2018, the application of 2 lb S ac^{-1} at V4 and again at R3 revealed a yield that was about 6 bu ac^{-1} off the pace compared to the V4 and R3 single applications of 4 lb S ac^{-1} (65 and 64.3 bu ac^{-1}, respectively). 2019 yields were all very similar in each timing category. Seed weights in both 2018 and 2019 differed only slightly between all three timings. With the sequential application not providing significant improvements, it is not worth the extra S application. This leaves the V4 or R3 application options, which allows growers the flexibility of applying S when conditions are adequate for foliar applications.

3.4.5 Seed Protein

Despite the very different growing seasons, 2018 and 2019 protein levels did plateau at similar rates. In 2018, both the V4 and R3 treatments increased protein linearly at the same rate (0.25% for every 1 lb S added). The V4R3 foliar applications caused a plateau of protein (38.3%) at a total rate of 6.2 lb S ac^{-1} (Table 3-5).

In 2019, the application only at R3 reached a maximum protein level of 39.8% at a rate of 6.0 lb S ac^{-1}; whereas, the V4R3 treatment plateaued at a total rate of 10.8 lb S ac^{-1} (Table 3-6). It is promising too to see that the V4 treatments increased protein linearly (0.29 points for every 1 lb S ac^{-1}). In both 2018 and 2019 no matter the growth stage, foliar applications of S improved protein compared to the UTC (Table 3-8). This information suggests that there is flexibility in timing of application to improve protein and most likely yield. These data were consistent for two growing seasons with different weather patterns, but an additional year of data should determine if the pattern is true, and possibly pinpoint an optimum S rate for reaching maximum protein potential.

Fertilizer S and N work together to form essential sulfur-based amino acids, and especially in a sandy soil, S would be needed to maximize the use of available N (Hitsuda et al., 2008; Scherer, 2008). An interesting addition in the future would be to investigate whether an application of only N at R3 would have similar protein increases. To do this, one could apply 1.75 gal ac^{-1} of N-28-SRN (28-0-0, 72% SRN) to reach the equivalent of N received from 25 lb AMS applied in this study (~5.25 lb N ac^{-1}) ("Plant Food Company, Inc.," 2014).

3.4.6 Seed Oil

In 2018, the V4R3 application of foliar S lowered the oil concentration in an inverse-plateau down to 23.9% at a 12.9 lb S ac^{-1} (Table 3-5). In 2019, the V4R3 application lowered the oil concentration in an inverse-plateau trend down to 22.0% at a rate of 9.7 lb S ac^{-1}. Literature shows that there is an inverse relationship between soybean seed protein and seed oil (Dornbos and Mullen, 1992). That relationship was observed in this study as well, noting the protein levels from the V4 treatments in both 2018 and 2019 increased linearly and the oil from the V4 treatments decreased linearly (Table 3-5, Table 3-6). Overall, the 2018 season had higher oil and lower protein levels as compared to the 2019 season (Table 3-8).

3.5 Conclusions

Foliar applications of S at this S-deficient site improved S concentration in the leaves following the V4 and R3 applications in 2018 and 2019. This increase in S concentrations along with smaller N:S ratios translated to grain yield and protein improvements. Literature has shown that N:S ratios wider than 16:1 may limit protein formation and ratios above 20:1 could indicate S deficiency (Agrawal and Mishra, 1994). These foliar applications led to a 9 bu ac^{-1} increase compared to the UTC in 2018. With this knowledge, it is important that future studies also focus on the quality component of soybeans to determine if these management strategies can continue to advance soybean quality.

The amount of foliar S needed to enable soybean recovery was a key objective of this study. In 2018, the optimal rate applied to reach maximum grain yield was approximately 4 lb S ac^{-1}. In 2019, the optimal rate was closer to 7 lb S ac^{-1} as seen with the sequential V4R3 treatments. Although the two seasons did not reveal the same optimal rate, this does provide a narrower range of rates to study in the future. This also brings to question why these differences occurred between seasons. It could be speculated that weather patterns could have contributed towards these differences; however, that is a factor that would need to be more closely monitored and examined in the future.

The other part of this study to consider is when to apply foliar applications of S. Although tissues in 2018 and 2019 were never truly deficient in S when plots were sampled, the tissues were near critical levels after R3 applications in 2019. Even though tissues were not deficient, foliar applications at both timings still improved the S concentrations, yield, and protein. Since yield and protein did not vary considerably between the V4 and R3 application timing, I would recommend a V4 application of foliar S. At this growth stage, the row is most likely not canopied, which would result in less damage to the crop. Earlier in the season, it is easier to apply in conditions more appropriate for foliar fertilizers to prevent leaf damage (i.e. less dew in the canopy, and cooler temperatures). Applying S early means less trips through the field during reproductive stages when soybean may be more sensitive to stress.

With this recommendation in mind, it is important to note that the flexibility of application timing is possible for growers. This flexibility may be important for growers who spray their own herbicides or fungicides and may want to apply fertilizer in the tank mix they are spraying already. When tank mixing AMS with herbicides, the AMS should be dissolved first in water by utilizing warm water and using constant agitation to prevent AMS from precipitating out and clogging screens. Adding the other products after the AMS is dissolved is the preferred order (Manucherhri, 2018). AMS is utilized in herbicide mixes already with weak-acid herbicides to lower the pH of hard water, and it has also been found to bind with the herbicide and be more readily absorbed by the plant (Voight, 2017). This flexibility allows growers to apply S when conditions are favorable, or possibly more importantly, avoid poor conditions for spraying and/or driving through the field. The flexibility observed in this study would allow a grower to address the S inadequacy with foliar applications across a wide range of development of soybean stages.

In future similar studies, an economic analysis would be extremely beneficial for growers to determine the return on investment (ROI). It would also be interesting to place this study in more locations that are S deficient in order to look at the effects of applications on soybean truly deficient in S during tissue testing. These studies could focus on the optimum rate range found in this study. Other foliar studies could investigate N only foliar applications along with foliar applications of AMS to investigate differences between N and S and the synergistic effect on soybean.

Table 3-1. Treatments partitioned by growth stage applications and amount as well as the showing nutrient totals applied for each treatment throughout entire growing season.

Timing	Total Rate		Rate at Timing (kg S ha^{-1})		
	lb S/ac	kg S/ha	Pre	V4	R3
UTC	0	.	.	.	.
Pre-emerge	20	22.4	22.4	.	.
V4	1	1.1	.	1.1	.
V4	2	2.2	.	2.2	.
V4	4	4.5	.	4.5	.
V4	6	6.7	.	6.7	.
R3	1	1.1	.	.	1.1
R3	2	2.2	.	.	2.2
R3	4	4.5	.	.	4.5
R3	6	6.7	.	.	6.7
twice†	2	2.2	.	1.1	1.1
twice†	4	4.4	.	2.2	2.2
twice†	8	9.0	.	4.5	4.5
twice†	12	13.4	.	6.7	6.7

† Twice denotes two sequential applications of the same rate of S at V4 and again at R3 target growth stages.

Table 3-2. Soil fertility for foliar sulfur trials in 2018 and 2019 near La Crosse, Indiana. Samples were taken prior to fertilizer application and planting in each respective year and trial. Mean values are averaged over replications. (± standard deviation).

Soil Analyses	2018			2019		
OM (mg kg^{-1})	2.3	±	0.2	2.2	±	0.3
pH	6.5	±	0.1	6.5	±	0.2
CEC (cmol$_c$ kg^{-1})	10.7	±	0.7	10.5	±	1.1
P (mg kg^{-1})	78.4	±	15.1	29.4	±	2.1
K (mg kg^{-1})	99.0	±	14.9	130	±	14.5
Mg (mg kg^{-1})	300	±	15.8	299	±	33.6
Ca (mg kg^{-1})	1351	±	108	1292	±	159
S (mg kg^{-1})	8.0	±	0.7	3.4	±	1.5
Zn (mg kg^{-1})	1.3	±	0.1	2.0	±	0.4
Mn (mg kg^{-1})	11.4	±	2.6	6.6	±	1.1
Fe (mg kg^{-1})	108	±	6.1	183.6	±	21.6
Cu (mg kg^{-1})	1.4	±	0.1	1.6	±	0.2
B (mg kg^{-1})	0.3	±	0.04	0.3	±	0.1

Table 3-3. Mean monthly air temperature, and 30-yr averages (1989 to 2019) for a growing season at Wanatah, IN. Total monthly precipitation and 22-yr average (1997-2019) for a growing season in North Judson, IN.

	2018	**2019**	**30-yr**
Air Temperature		°C	
April	4.0	8.9	8.7
May	19.1	14.4	15.1
June	21.8	19.8	20.6
July	22.4	23.8	22.2
August	22.2	20.8	21.0
September	19.2	19.5	17.3
October	10.4	10.6	10.7
	2018	**2019**	**22-yr**
Precipitation		mm	
April	66.8	115	88.6
May	127	168	104
June	167	123	111
July	31.5	82.0	111
August	144	84.6	132
September	98.0	149	79.7
October	142	98.6	93.7

Table 3-4. 2018 most recent mature leaf sampling from post-V4 application results of macronutrients on the left. Samples were collected on 7/10/2018 which was 15 days post-application. The most recent mature leaf sampling from post-R3 application results of macronutrients on the right. Samples were collected on 8/1/2018 which was 15 days post-application.

		Post – V4 ‡							Post – R3 ¶						
Timing	Rate	N	P	K	S	Ca	Mg	N:S	N	P	K	S	Ca	Mg	N:S
	lb S/acre	%						Ratio	%						Ratio
Pre-emerge	20	5.4	0.41	2.0	0.40	1.3	0.59	13.5	5.5	0.36	1.6	0.34	1.2	0.46	16.4
UTC	0	5.4	0.41	2.0	0.32	1.3	0.62	17.2	4.9	0.34	1.5	0.26	1.3	0.50	19.1
V4	1	5.3	0.41	2.1	0.32	1.3	0.59	16.8	4.8	0.35	1.7	0.25	1.2	0.45	19.1
V4	2	5.3	0.40	2.2	0.33	1.3	0.58	16.2	5.2	0.36	1.7	0.28	1.3	0.46	18.5
V4	4	5.4	0.40	2.1	0.36	1.3	0.64	15.2	5.4	0.36	1.7	0.29	1.2	0.49	19.0
V4	6	5.4	0.40	2.1	0.36	1.3	0.60	15.3	5.4	0.37	1.7	0.31	1.2	0.54	17.7
UTC	0	5.4	0.41	2.0	0.32	1.3	0.62	17.2	4.9	0.34	1.5	0.26	1.3	0.50	19.1
R3	1	.	.	.	.	.	.	.	5.4	0.36	1.6	0.29	1.3	0.51	18.8
R3	2	.	.	.	.	.	.	.	5.7	0.36	1.6	0.30	1.2	0.51	19.2
R3	4	.	.	.	.	.	.	.	5.5	0.36	1.6	0.30	1.3	0.48	17.9
R3	6	.	.	.	.	.	.	.	5.7	0.36	1.6	0.34	1.3	0.51	16.8
UTC	0	5.4	0.41	2.0	0.32	1.3	0.62	17.2	4.9	0.34	1.5	0.26	1.3	0.50	19.1
twice†	1	5.4	0.41	2.1	0.33	1.3	0.64	16.6	5.4	0.37	1.8	0.28	1.2	0.48	19.2
twice†	2	5.4	0.40	2.0	0.33	1.3	0.66	16.3	5.5	0.35	1.6	0.29	1.3	0.52	18.9
twice†	4	5.4	0.39	2.0	0.35	1.3	0.60	15.5	5.6	0.36	1.7	0.32	1.2	0.50	17.4
twice†	6	5.4	0.40	2.1	0.36	1.2	0.61	15.1	5.9	0.38	1.8	0.36	1.1	0.44	16.2

† twice denotes the sequential application treatment with target applications at V4 and again at R3 with the same stated rate applied at each. The first sampling data shows results from a single application of the planned two application (the first application at V4).

‡ Tissue sampling of most recent mature leaves collected on 7/10/2018 (15 days post V4 application). Soybean was at R3 when sampled.

¶ Tissue sampling of most recent mature leaves collected on 8/1/2018 (15 days post R3 application). Soybean was at R5 growth stage when samples were collected.

Table 3-5. 2018 Foliar S Regression models of best fit: Linear, Quadratic, and Quadratic Plateau.

Data	Timing	Model	Linear		Quad	Quad + Plateau		Model
			Y-int.	**Rate**	**Rate SQ**	**x0**	**Plateau**	**Significance**
Yield	V4	Quad+Plateau	56.2	3.6	-0.43	4.1	63.5	*
	R3	Quad+Plateau	55.4	3.1	-0.34	4.5	62.2	*
	V4R3	Linear	57.3	0.68	.	.	.	*
Seed Size	V4	Quadratic	16.5	-0.09	0.04	.	.	*
	R3	.	.	.	.	.	.	ns
	V4R3	Linear	16.8	0.09	.	.	.	x
Protein	V4	Linear	36.6	0.25	.	.	.	**
	R3	Linear	36.9	0.25	.	.	.	**
	V4R3	Quad+Plateau	36.5	0.58	-0.05	6.2	38.3	***
Oil	V4	Linear	25.7	-0.22	.	.	.	***
	R3	Linear	25.6	-0.24	.	.	.	***
	V4R3	Quad+Plateau	25.7	-0.28	0.01	12.9	23.9	***
Leaf N 1 †	V4	.	.	.	.	.	.	ns
	V4R3	.	.	.	.	.	.	ns
Leaf S 1 †	V4	Linear	0.31	0.008	.	.	.	**
	V4R3	Linear	0.32	0.004	.	.	.	***
Leaf N:S 1 †	V4	Quad+Plateau	17.4	-0.76	0.07	5.6	15.2	**
	V4R3	Quadratic	17.2	-0.40	0.009	.	.	***
Leaf N 2 ‡	V4	Quad+Plateau	4.8	0.21	-0.02	5.8	5.4	***
	R3	Quad+Plateau	4.9	0.71	-0.19	1.9	5.6	***
	V4R3	Quad+Plateau	5.0	0.13	-0.005	12.3	5.8	***
Leaf S 2 ‡	V4	Linear	0.26	0.008	.	.	.	***
	R3	Linear	0.27	0.01	.	.	.	***
	V4R3	Linear	0.26	0.008	.	.	.	***
Leaf N:S 2 ‡	V4	Linear	19.2	-0.19	.	.	.	x
	R3	Quadratic	19.1	0.04	-0.07	.	.	***
	V4R3	Quadratic	19.3	-0.06	-0.02	.	.	***

† Tissue sampling of most recent mature leaves collected on 7/10/2018 (15 days post V4 application). Soybean was at R3 growth stage when samples were collected.

‡ Tissue sampling of most recent mature leaves collected on 8/1/2018 (15 days post R3 application). Soybean was at R5 growth stage when samples were collected.

Table 3-6. 2019 Foliar S Regression models of best fit: Linear, Quadratic, and Quadratic Plateau.

Data	Timing	Model	Linear		Quad	Quad + Plateau		Model
			Y-int.	**Rate**	**Rate SQ**	**x0**	**Plateau**	**Significance**
Yield	V4	.	.	.	.	.	.	ns
	R3	Linear	42.1	1.0	.	.	.	x
	V4R3	Quad+Plateau	40.2	2.4	-0.16	7.5	49.1	*
Seed Size	V4	Quadratic	16.4	0.23	0.01	.	.	***
	R3	Quad+Plateau	16.3	0.29	-0.03	5.7	17.2	**
	V4R3	Quad+Plateau	16.2	0.32	-0.01	12.5	18.3	***
Protein	V4	Linear	37.8	0.29	.	.	.	***
	R3	Quad+Plateau	37.7	0.67	-0.06	6.0	39.8	***
	V4R3	Quad+Plateau	37.7	0.42	-0.02	10.8	39.9	***
Oil	V4	Linear	23.0	-0.12	.	.	.	***
	R3	Quadratic	23.1	-0.33	0.03	.	.	***
	V4R3	Quad+Plateau	23.1	-0.22	0.01	9.7	22.0	***
Leaf N 1 †	V4	Linear	4.5	0.10	.	.	.	*
	V4R3	Linear	4.4	0.05	.	.	.	*
Leaf S 1 †	V4	Linear	0.26	0.01	.	.	.	**
	V4R3	Linear	0.26	0.003	.	.	.	*
Leaf N:S 1 †	V4	Linear	17.0	-0.23	.	.	.	*
	V4R3	.	.	.	.	.	.	ns
Leaf N 2 ‡	V4	Linear	4.2	0.10	.	.	.	**
	R3	Linear	4.3	0.15	.	.	.	***
	V4R3	Quad+Plateau	4.2	0.31	-0.02	7.0	5.3	***
Leaf S 2 ‡	V4	Linear	0.22	0.009	.	.	.	***
	R3	Quadratic	0.23	0.008	0.0007	.	.	***
	V4R3	Quadratic	0.23	0.01	-0.0005	.	.	***
Leaf N:S 2 ‡	V4	Linear	19.3	-0.29	.	.	.	*
	R3	Linear	19.1	-0.28	.	.	.	*
	V4R3	Quadratic	18.9	-0.26	0.005	.	.	***

†Tissue sampling of most recent mature leaves collected on 7/16/2019 (14 days post V4 application). Soybean was at R2 growth stages when samples were collected.

‡ Tissue sampling of most recent mature leaves collected on 8/8/2019 (16 days post R3 application). Soybean was at R5 growth stages when samples were collected.

Table 3-7. 2019 most recent mature leaf sampling from post-V4 application results of macronutrients on the left. Samples were collected on 7/16/2019 which was 14 days post-application. The most recent mature leaf sampling from post-R3 application results of macronutrients on the right. Samples were collected on 8/8/2019 which was 16 days post-application.

		Post – V4 ‡							Post – R3 ¶						
Timing	Rate lb S/acre	N	P	K	S	Ca	Mg	N:S Ratio	N	P	K	S	Ca	Mg	N:S Ratio
		%							%						
Pre-emerge	20	4.5	0.30	1.7	0.32	1.2	0.51	14.2	4.9	0.29	1.7	0.31	0.99	0.32	16.1
UTC	0	4.4	0.31	1.8	0.25	1.2	0.54	17.3	4.2	0.29	1.6	0.22	1.0	0.32	18.9
V4	1	4.6	0.32	1.8	0.28	1.2	0.55	16.4	4.3	0.28	1.7	0.22	1.1	0.32	19.7
V4	2	4.7	0.32	1.8	0.29	1.2	0.53	16.5	4.4	0.29	1.7	0.24	1.0	0.32	18.5
V4	4	4.7	0.32	2.0	0.29	1.2	0.51	16.2	4.5	0.29	1.8	0.25	1.0	0.29	17.8
V4	6	5.1	0.33	1.8	0.33	1.1	0.52	15.6	4.9	0.29	1.9	0.27	0.94	0.30	17.8
UTC	0	4.4	0.31	1.8	0.25	1.2	0.54	17.3	4.2	0.29	1.6	0.22	1.0	0.32	18.9
R3	1	.	.	.	.	.	.	.	4.4	0.30	1.8	0.23	1.1	0.31	19.0
R3	2	.	.	.	.	.	.	.	4.7	0.30	1.7	0.25	1.1	0.32	18.6
R3	4	.	.	.	.	.	.	.	4.7	0.30	1.8	0.27	1.1	0.33	17.8
R3	6	.	.	.	.	.	.	.	5.3	0.31	1.8	0.30	1.0	0.32	17.4
UTC	0	4.4	0.31	1.8	0.25	1.2	0.54	17.3	4.2	0.29	1.6	0.22	1.0	0.32	18.9
twice†	1	4.6	0.32	1.8	0.27	1.2	0.56	16.9	4.7	0.31	1.8	0.26	1.1	0.33	18.4
twice†	2	4.7	0.32	1.8	0.28	1.2	0.52	16.7	5.2	0.32	1.8	0.28	1.0	0.32	18.1
twice†	4	4.7	0.31	1.9	0.28	1.2	0.52	16.8	5.2	0.31	1.9	0.31	0.90	0.30	17.0
twice†	6	5.1	0.32	1.7	0.30	1.1	0.52	17.2	5.4	0.31	1.8	0.33	0.91	0.32	16.5

† Twice denotes the sequential application treatment with target applications at V4 and again at R3 with the same stated rate applied at each. The first sampling data shows results from a single application of the planned two application (the first application at V4).
‡ Tissue sampling of most recent mature leaves collected on 7/16/2019 (14 days post V4 application). Soybean was R2 when sampled.
¶ Tissue sampling of most recent mature leaves collected on 8/8/2019 (16 days post R3 application). Soybean was R5 when sample

Table 3-8. Soybean seed yield and quality means in response to sulfur fertilizer application times and rates in 2018 and 2019.

Main Effect Timing	Rate lb S/acre	2018 Yield bu ac^{-1}	2018 Seed Size g 100 sd^{-1}	2018 Moisture %	2018 Protein % dry basis	2018 Oil % dry basis	2019 Yield bu ac^{-1}	2019 Seed Size g 100 sd^{-1}	2019 Moisture %	2019 Protein % dry basis	2019 Oil % dry basis
Pre-emerge	20	68.9	18.3	12.2	38.2	24.3	44.5	18.0	13.4	39.5	22.2
UTC	0	55.9	16.4	12.1	36.6	25.7	40.0	16.4	13.5	37.7	23.1
V4	1	60.1	16.8	12.0	36.7	25.5	45.6	16.6	13.5	38.1	22.9
V4	2	60.8	16.2	12.1	37.5	25.1	42.4	16.8	13.2	38.4	22.8
V4	4	65.0	16.9	12.1	37.4	24.8	46.9	17.5	13.4	38.8	22.5
V4	6	62.1	17.5	12.3	38.1	24.4	44.5	18.1	13.5	39.5	22.4
UTC	0	55.9	16.4	12.1	36.6	25.7	40.0	16.4	13.5	37.7	23.1
R3	1	57.3	16.6	12.3	37.6	25.3	44.3	16.5	13.4	38.2	22.8
R3	2	59.9	16.8	12.1	37.5	25.0	45.5	17.0	13.4	39.0	22.5
R3	4	64.3	16.8	12.0	37.3	24.7	46.5	17.1	13.3	39.4	22.2
R3	6	60.7	16.7	12.1	38.6	24.1	47.4	17.2	13.1	39.8	22.1
UTC	0	55.9	16.4	12.1	36.6	25.7	40.0	16.4	13.5	37.7	23.1
twice†	1	61.8	17.6	12.1	37.4	25.2	44.7	16.6	13.5	38.3	22.7
twice†	2	58.3	17.0	12.0	38.2	24.6	46.9	17.3	13.4	39.0	22.4
twice†	4	62.7	17.4	12.0	38.1	24.3	49.4	18.1	13.2	39.8	22.0
twice†	6	65.6	17.9	12.0	38.5	23.9	48.8	18.2	13.2	39.9	22.0
CV %		8.1	4.2	2.3	1.8	2.1	8.7	2.2	2.2	1.3	1.1

† Twice denotes the sequential application treatment with target applications at V4 and again at R3 with the same stated rate applied at V4 and again at R3.

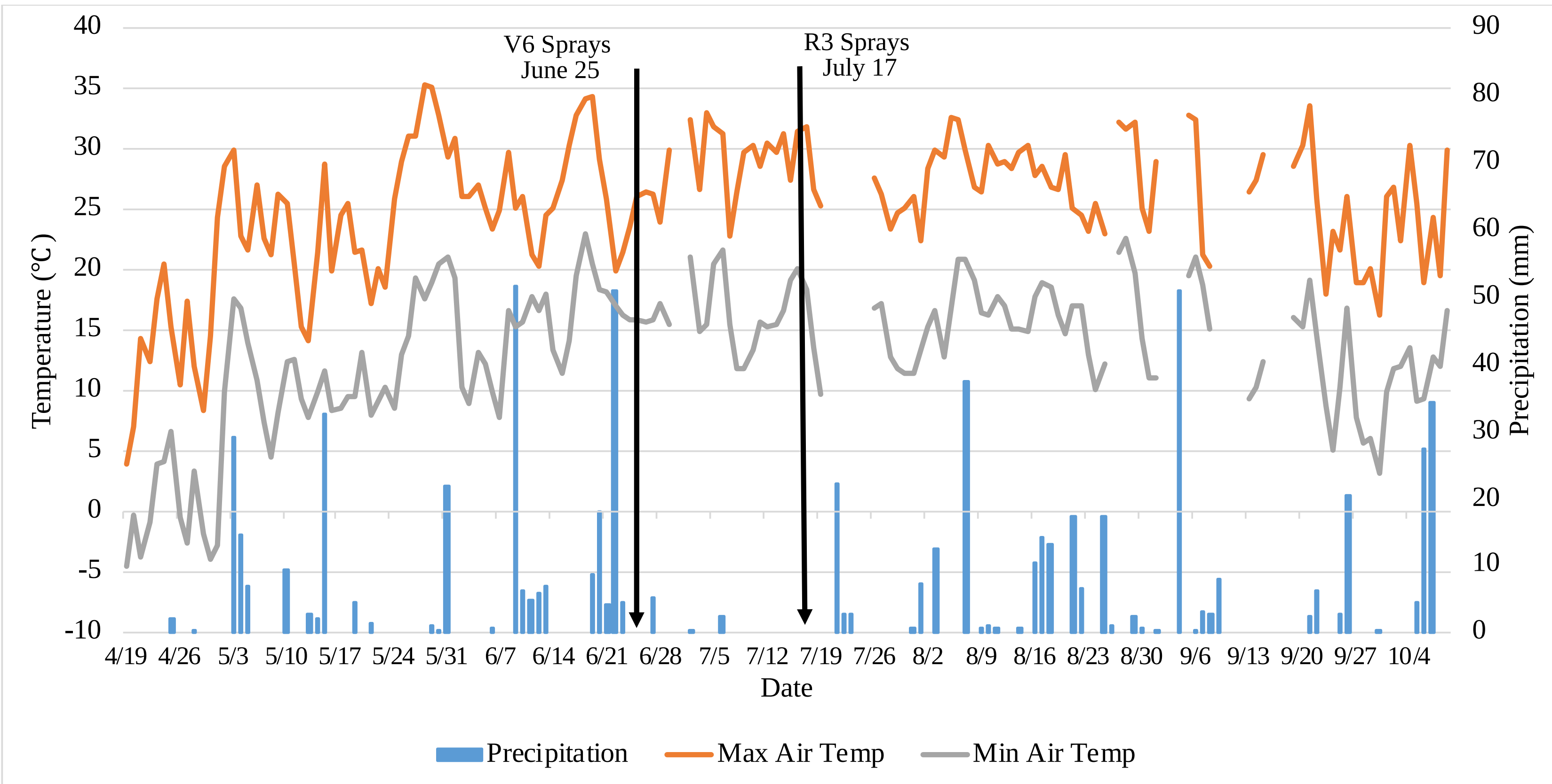

Figure 3-1. 2018 precipitation and air temperature data leading up to and following planting and in-season applications. †Precipitation data came from weather station located in North Judson, IN and temperature data was taken from Wanatah, IN location due to no temperature data at North Judson.

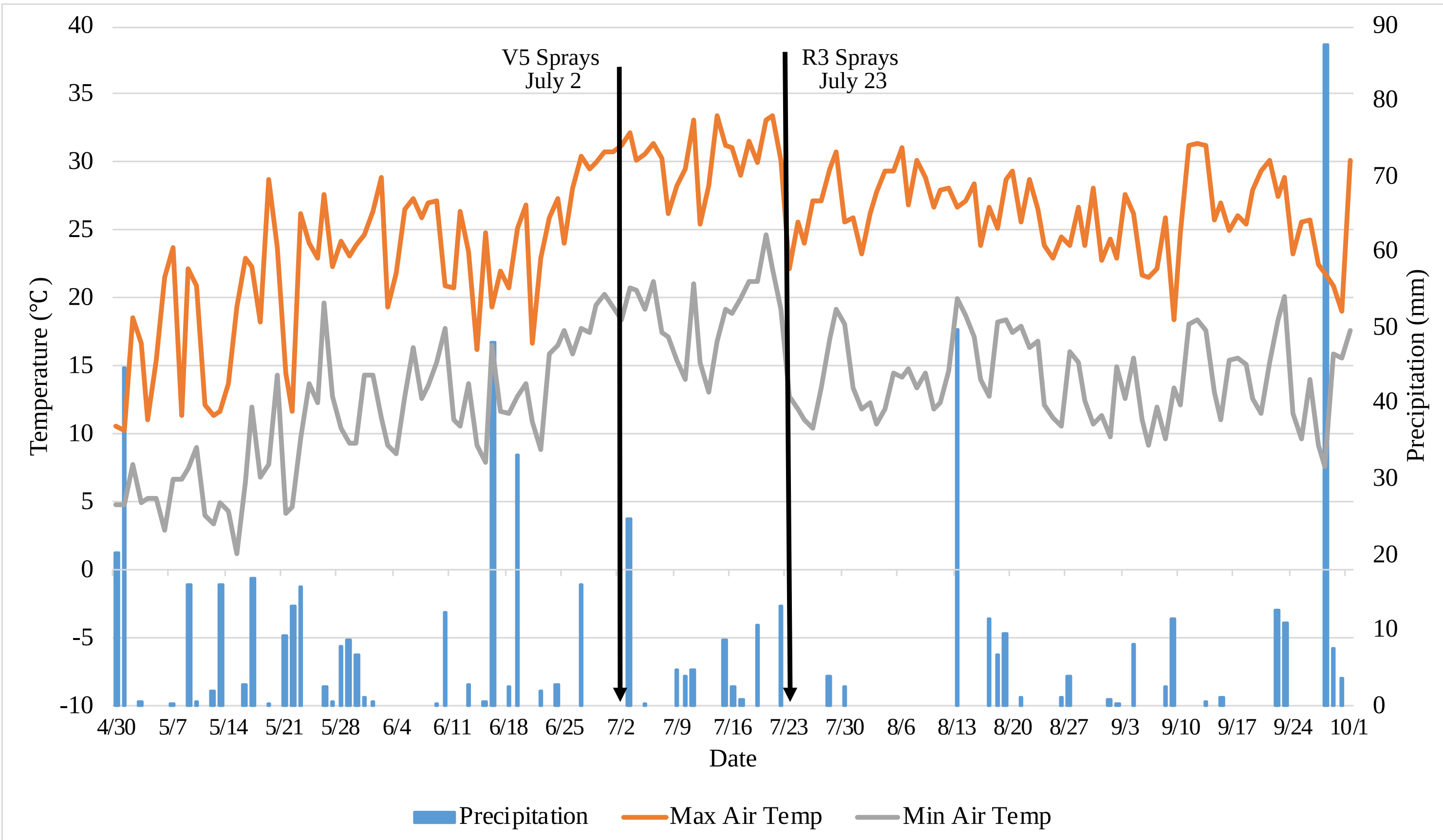

Figure 3-2. 2019 precipitation and air temperature data leading up to and following planting and in-season applications.

†Precipitation data came from weather station located in North Judson, IN and temperature data was taken from Wanatah, IN location due to no temperature data at North Judson.

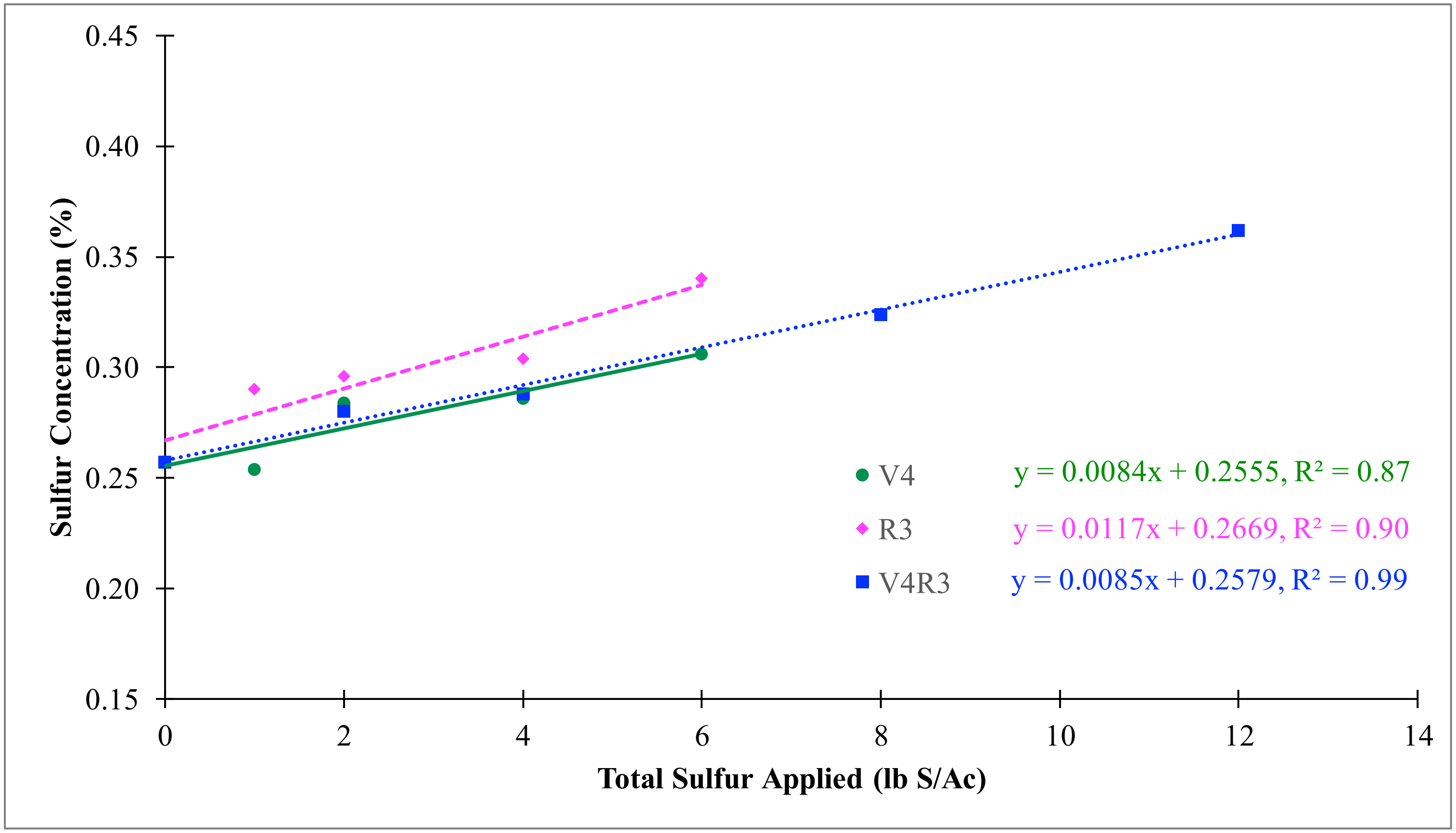

Figure 3-3. 2018 sulfur concentrations (%) in tissues collected 8/1/2018 (15 DAA: Post-R3).

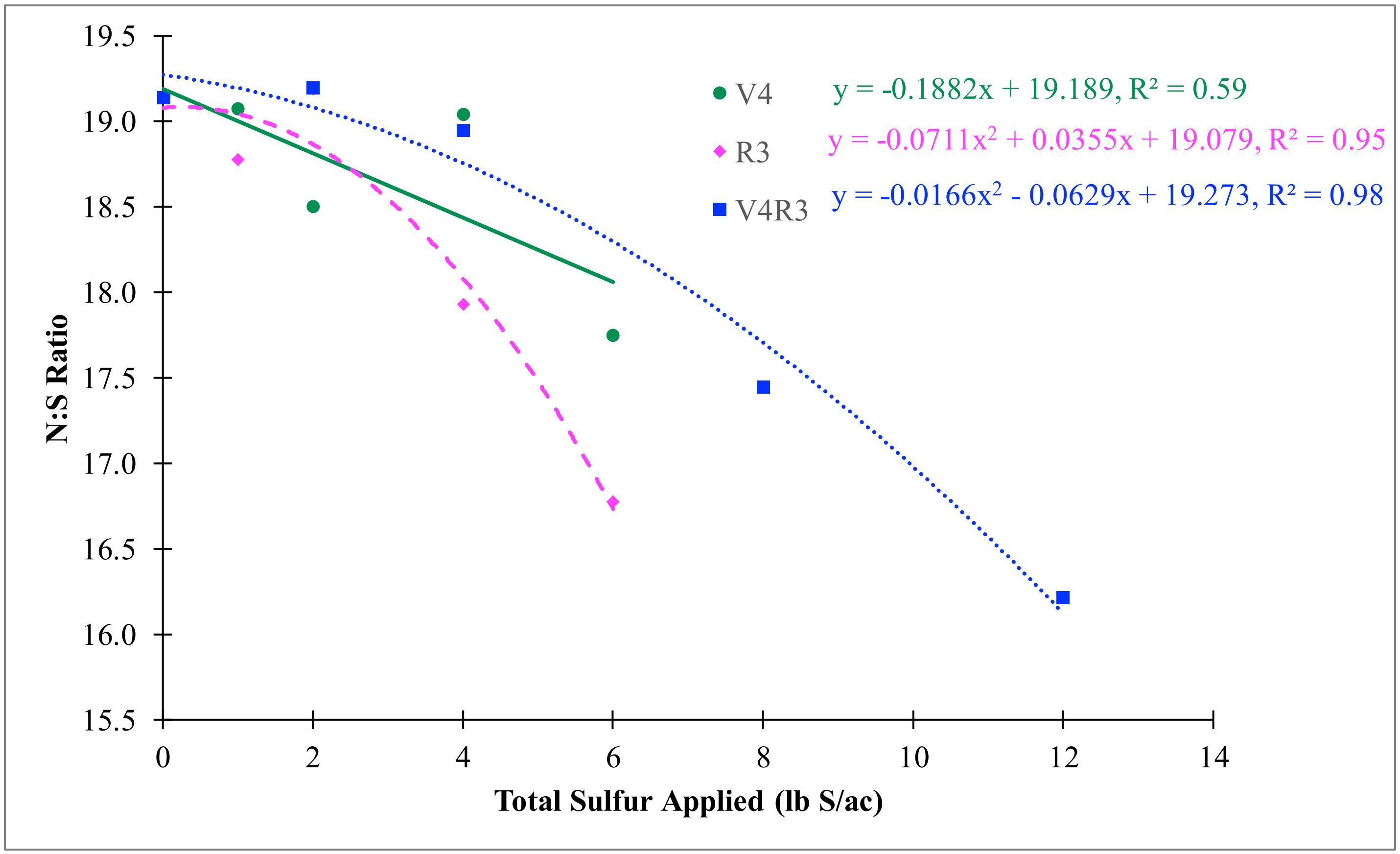

Figure 3-4. 2018 N:S ratio in tissues collected 8/1/2018 (15 DAA: Post-R3).

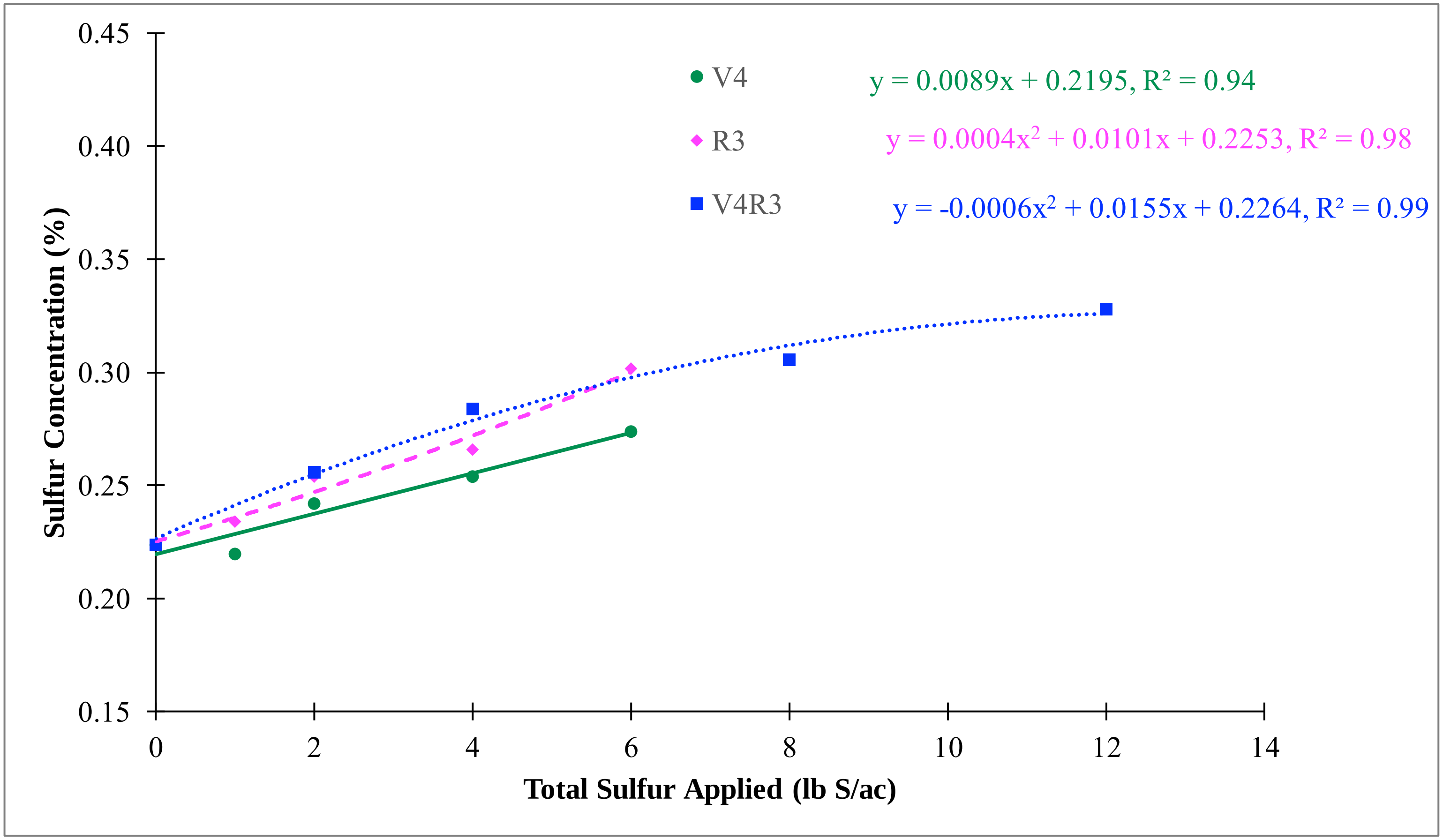

Figure 3-5. 2019 sulfur concentrations (%) in tissues collected 8/8/2019 (16 DAA: Post-R3).

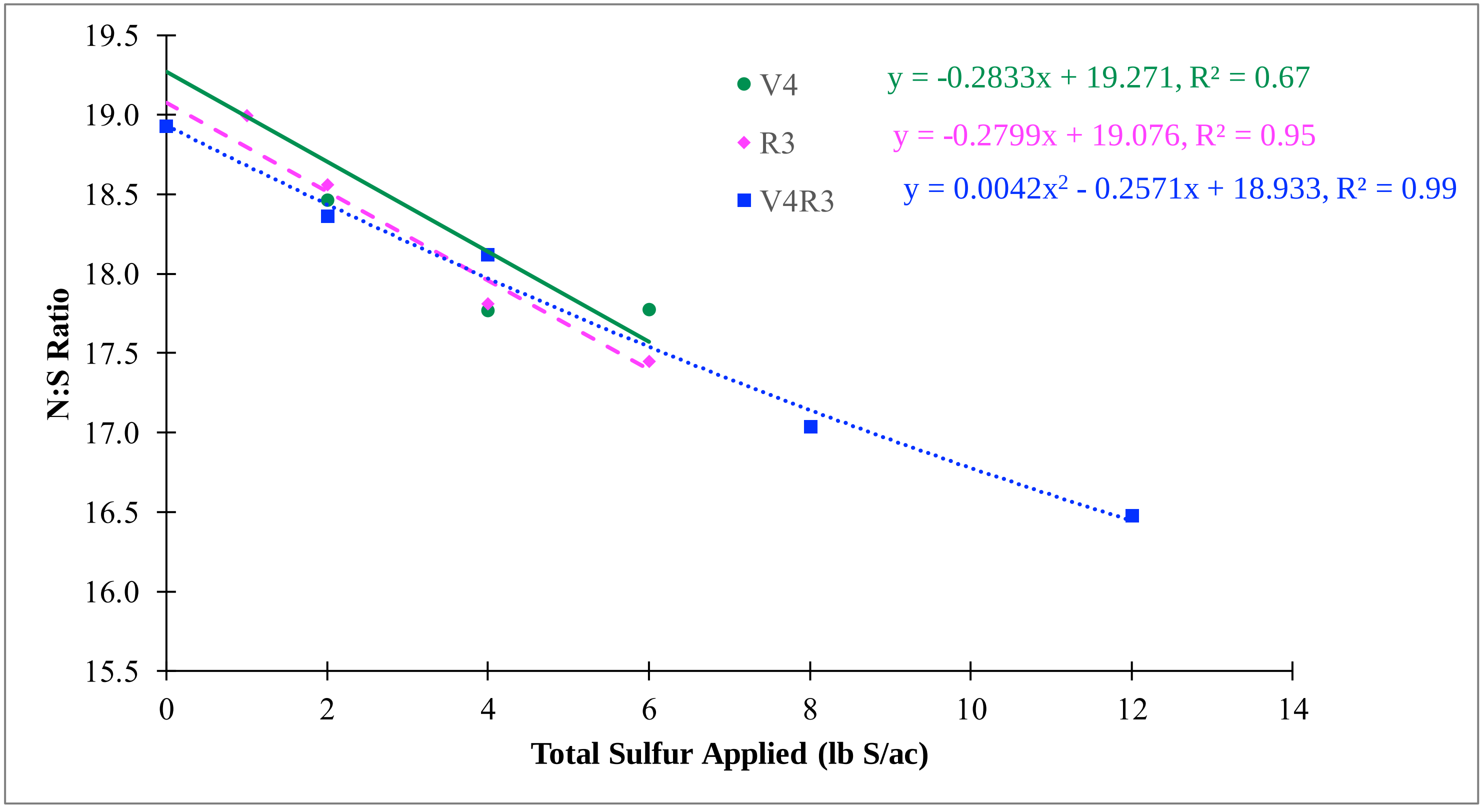

Figure 3-6. 2019 N:S ratio in tissues collected 8/8/2019 (16 DAA: Post-R3

APPENDIX A

Table A-1. Soybean fatty acid responses to planting date across fertility treatments and response to fertility treatments across planting dates. Study was located near West Lafayette, IN in 2018.

Main Effect	2018															
	Linoleic				**Linolenic**				**Oleic**		**Palmitic**		**Stearic**			
	%															
Planting Date																
May 11	51.2‡				9.0				21.3	B	10.5	A	4.1			
June 5	49.9				8.8				22.8	A	10.4	B	4.2			
Fertility	***§ PD 1***		***PD 2***		***PD 1***		***PD 2***						***PD 1***		***PD 2***	
UTC	53.1	a	50.6	cdef	8.5	g	9.2	abcd	21.4	cde	10.3	bc	4.2	bcde	4.1	de
STAND 20	50.3	def	49.4	f	8.7	efg	8.8	cdefg	22.8	ab	10.4	bc	4.2	ab	4.2	bcd
Plant NS	50.0	defg	50.3	def	9.2	ab	8.6	fg	22.5	abc	10.4	bc	4.1	cde	4.2	abcd
ATS	49.7	efg	50.0	defg	9.4	a	9.2	abc	22.4	abcd	10.5	bc	4.2	abc	4.1	bcde
V4 NS	51.8	abc	49.4	fg	8.8	bcdefg	8.5	g	22.7	ab	10.3	c	4.1	de	4.2	bcde
V4R3 NS	50.5	cdef	48.7	g	9.0	abcdef	8.4	g	23.0	a	10.6	ab	4.1	bcde	4.2	abc
R3 NS	51.9	abc	49.5	fg	9.4	a	9.0	abcdef	21.1	e	10.8	a	4.1	bcde	4.3	a
UAN Direct	52.5	ab	51.2	bcd	9.0	abcdef	8.8	bcdefg	21.2	de	10.3	bc	4.0	e	4.2	abcd
AMS Direct	51.0	cde	49.6	fg	9.1	abcde	9.1	abcd	22.0	abcde	10.5	bc	4.2	bcd	4.1	bcde
R4+	51.1	bcde	50.1	def	8.7	defg	8.6	efg	21.7	bcde	10.5	abc	4.1	bcde	4.3	a
Planting Date	***				*				**		*		ns			
Fertility	***				**				**		*		*			
Pdate x Fertility	*				x				ns		ns		**			
CV (%)	2.2				4.8				6.1		2.9		2.4			

†Significance at P ≤ 0.10, 0.05, 0.01, ≤ 0.001 denoted by x, *, **, and ***, respectively; ns, not significant. ‡see pdate x fertility interaction

§ CV for interactions: Linoleic = 2.2, Linolenic = 4.7, Stearic = 2.5

Table A-2. Soybean fatty acid responses to planting date across fertility treatments and response to fertility treatments across planting dates. Study was located near West Lafayette, IN in 2019.

Main Effect	2019									
	Linoleic		Linolenic		Oleic		Palmitic		Stearic	
					%					
Planting Date										
June 11	52.2		6.3	B	20.2		11.8		4.66	B
June 27	51.2		6.8	A	20.6		12.0		4.74	A
Fertility										
UTC	52.0	abc	6.8		20.0	bcd	11.8		4.7	
STAND 20	51.0	cd	6.7		21.0	ab	11.8		4.8	
Plant NS	52.1	abc	6.4		20.1	bcd	12.1		4.6	
ATS	52.1	abc	6.3		20.7	bc	11.6		4.7	
V4 NS	51.1	bc	6.4		20.9	ab	11.8		4.7	
V4R3 NS	52.2	abc	6.3		19.6	cd	12.0		4.7	
R3 NS	52.7	a	6.4		20.0	bcd	11.8		4.7	
UAN Direct	52.6	ab	6.9		18.9	d	12.3		4.7	
AMS Direct	51.5	abc	6.6		20.6	bc	12.0		4.6	
R4+	49.5	d	6.9		22.0	a	11.8		4.8	
Planting Date	ns		*		ns		ns		**	
Fertility	**		ns		***		ns		ns	
Pdate x Fertility	ns		ns		ns		ns		ns	
CV (%)	3.3		11.7		6.6		4.1		3.4	

†Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively; ns, not significant.

Table A-3. ANOVA summary of planting date (Pdate) and fertility (Trt_NS) main effects and their interaction on the seed amino acid profile from 2018 and 2019 at the study near West Lafayette, IN.

Year	———— 2018 ————			———— 2019 ————		
ACRE	Planting Date (Pdate)	Fertility (Trt_NS)	Pdate*Trt_NS	Planting Date (Pdate)	Fertility (Trt_NS)	Pdate*Trt_NS
Amino Acid	Level of Significance					
Lysine	***	***	x	**	***	ns
Cysteine	**	***	*	**	**	ns
Methionine	***	***	ns	*	***	ns
Threonine	**	***	ns	**	***	ns
Tryptophan	*	***	ns	**	x	*
Isoleucine	*	***	ns	**	***	ns
Leucine	**	***	ns	**	***	ns
Histidine	**	***	ns	**	***	ns
Phenylalanine	**	***	ns	**	***	ns
Valine	**	**	ns	***	***	ns
Alanine	**	***	ns	***	***	ns
Arginine	**	***	ns	***	***	ns
Asparagine	**	***	ns	***	***	ns
Glutamine	**	***	ns	***	***	ns
Glycine	**	***	ns	***	***	ns
Proline	***	***	ns	**	***	ns
Serine	**	***	ns	**	***	ns
Tyrosine	***	***	ns	**	***	ns

†Significance at $P \leq 0.10$, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively; ns, not significant.

Table A-4. West Lafayette planting date effect on amino acid composition compared to UTC.

Year	—— 2018 ——		—— 2019 ——
ACRE			
<u>Amino Acid</u>	Effect of Early Planting		
Lysine	-		-
Cysteine	-		-
Methionine	-		-
Threonine	-		-
Tryptophan	-		-
Isoleucine	-		-
Leucine	-		-
Histidine	-		-
Phenylalanine	-		-
Valine	-		-
Alanine	-		-
Arginine	-		-
Asparagine	-		-
Glutamine	-		-
Glycine	-		-
Proline	-		-
Serine	-		-
Tyrosine	-		-

† **-** is a significant negative effect of early planting compared to late planting date

Table A-5. West Lafayette amino acid means as a % dry basis for 2018.

Year	2018					
ACRE	*UTC*	*STAND 20*	*Plant NS*	*ATS*	*V4 NS*	*V4R3 NS*
Amino Acids		Means (% dry basis)				
Lysine	2.63	2.65	2.64	2.65	2.65	2.63
Cysteine	0.64	0.67	0.67	0.66	0.66	0.66
Methionine	0.55	0.56	0.56	0.56	0.56	0.56
Threonine	1.55	1.57	1.57	1.57	1.57	1.56
Tryptophan	0.47	0.48	0.48	0.48	0.48	0.48
Isoleucine	1.92	1.93	1.91	1.92	1.92	1.89
Leucine	3.00	3.04	3.03	3.04	3.03	3.00
Histidine	1.00	1.02	1.01	1.02	1.01	1.00
Phenylalanine	2.07	2.09	2.08	2.07	2.08	2.05
Valine	1.90	1.91	1.91	1.90	1.90	1.88
Alanine	1.68	1.70	1.69	1.69	1.69	1.68
Arginine	2.77	2.81	2.79	2.81	2.78	2.74
Asparagine	4.45	4.53	4.50	4.52	4.50	4.46
Glutamine	6.76	6.89	6.87	6.87	6.85	6.76
Glycine	1.71	1.74	1.73	1.74	1.72	1.72
Proline	1.87	1.90	1.89	1.90	1.89	1.88
Serine	1.78	1.81	1.80	1.80	1.80	1.78
Tyrosine	1.49	1.50	1.50	1.50	1.50	1.49

Table A-6. West Lafayette amino acid means as a % dry basis for 2019.

Year	2019					
ACRE	*UTC*	*STAND 20*	*Plant NS*	*ATS*	*V4 NS*	*V4R3 NS*
Amino Acids	Means (% dry basis)					
Lysine	2.59	2.60	2.57	2.60	2.58	2.61
Cysteine	0.64	0.65	0.65	0.65	0.65	0.65
Methionine	0.57	0.58	0.57	0.58	0.57	0.59
Threonine	1.53	1.53	1.52	1.53	1.53	1.54
Tryptophan	0.33	0.33	0.34	0.33	0.33	0.34
Isoleucine	1.77	1.76	1.74	1.76	1.75	1.77
Leucine	2.22	2.22	2.18	2.22	2.20	2.23
Histidine	1.03	1.02	1.02	1.03	1.02	1.04
Phenylalanine	2.00	1.99	1.96	1.98	1.97	1.99
Valine	1.83	1.82	1.80	1.82	1.82	1.83
Alanine	1.65	1.66	1.64	1.66	1.65	1.66
Arginine	2.82	2.81	2.79	2.82	2.80	2.83
Asparagine	4.34	4.36	4.31	4.35	4.32	4.37
Glutamine	6.58	6.58	6.47	6.55	6.53	6.61
Glycine	1.65	1.66	1.65	1.66	1.65	1.66
Proline	2.10	2.10	2.08	2.11	2.10	2.13
Serine	1.77	1.77	1.76	1.77	1.77	1.78
Tyrosine	1.42	1.42	1.41	1.43	1.42	1.42

Table A-7. Fatty acid levels pooled over 2018 and 2019 from Wanatah, IN in response to variety and N+S fertility treatment.

Main Effect	§ 2018 & 2019					
	Linoleic	**Linolenic**	**Oleic**	**Palmitic**	**Stearic**	
			%			
Variety						
AG 24x7	48.4	7.7	24.6	11.0	4.3	B
AG 34x6	50.4	8.2	21.8	11.3	4.4	A
Fertility						
UTC	51.5	8.0	21.5	11.0	4.3	bcde
STAND 20	48.5	8.0	23.8	11.2	4.3	abcde
Plant NS	49.6	8.0	23.2	11.0	4.3	de
ATS	48.4	8.1	23.8	11.2	4.4	abcd
V4 NS	49.2	7.8	23.5	11.2	4.4	abcd
V4R3 NS	49.0	7.8	23.4	11.3	4.5	a
R3 NS	49.3	8.0	23.6	11.3	4.5	abc
UAN Direct	51.2	7.9	22.1	10.9	4.2	e
AMS Direct	49.3	8.2	22.9	10.9	4.3	cde
R4+	48.5	8.0	23.7	11.2	4.5	ab
Variety†	ns	ns	ns	ns	x	
Fertility	ns	ns	ns	ns	*	
Var x Fertility	ns	ns	ns	ns	ns	
Year x Var	***	***	***	ns	ns	
Year x Fertility	*	ns	*	ns	x	
Year x Var x Fertility	x	ns	ns	*	ns	
CV (%)	4.4	8.1	10.1	4.4	3.9	

†Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively; ns, not significant.
§Pooled over 2018 and 2019 at the Wanatah, IN location

Table A-8. ANOVA summary of variety (Var) and fertility (Trt_NS) main effects and their interaction on the seed amino acid profile pooled over 2018 and 2019 at the study near Wanatah, IN.

Year	———————— 2018 & 2019 ————————		
Pinney	Variety (Var)	Fertility (Trt_NS)	Var*Trt_NS
Amino Acid	Level of Significance		
Lysine	x	***	ns
Cysteine	*	***	ns
Methionine	ns	***	ns
Threonine	x	***	ns
Tryptophan	ns	x	ns
Isoleucine	*	*	ns
Leucine	x	**	ns
Histidine	*	**	ns
Phenylalanine	*	**	ns
Valine	ns	**	ns
Alanine	x	***	ns
Arginine	ns	**	ns
Asparagine	*	***	ns
Glutamine	*	***	ns
Glycine	*	***	ns
Proline	x	**	ns
Serine	*	**	ns
Tyrosine	**	**	ns

†Significance at $P \leq 0.10$, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively; ns, not significant.

Table A-9. Wanatah amino acid means as a % dry basis pooled over 2018 and 2019.

Year	2018 & 2019					
Pinney	*UTC*	*STAND 20*	*Plant NS*	*ATS*	*V4 NS*	*V4R3 NS*
Amino Acids	Means (% dry basis)					
Lysine	2.60	2.65	2.64	2.65	2.64	2.64
Cysteine	0.63	0.66	0.66	0.66	0.67	0.67
Methionine	0.56	0.58	0.58	0.58	0.58	0.58
Threonine	1.53	1.56	1.55	1.56	1.56	1.56
Tryptophan	0.39	0.40	0.40	0.40	0.40	0.40
Isoleucine	1.83	1.86	1.86	1.86	1.85	1.85
Leucine	2.60	2.67	2.65	2.66	2.64	2.64
Histidine	1.01	1.04	1.03	1.04	1.03	1.03
Phenylalanine	2.03	2.06	2.05	2.06	2.05	2.04
Valine	1.85	1.89	1.88	1.89	1.88	1.87
Alanine	1.66	1.70	1.69	1.70	1.69	1.69
Arginine	2.78	2.87	2.85	2.88	2.86	2.85
Asparagine	4.37	4.50	4.48	4.49	4.47	4.48
Glutamine	6.58	6.81	6.77	6.79	6.77	6.77
Glycine	1.67	1.72	1.72	1.72	1.72	1.72
Proline	2.00	2.06	2.03	2.05	2.05	2.04
Serine	1.76	1.80	1.80	1.79	1.79	1.80
Tyrosine	1.45	1.48	1.47	1.48	1.47	1.47

APPENDIX B

Table B-1. 2018 most recent mature leaf sampling from post-V4 application results of micronutrients. Samples were collected on 7/10/2018 which was 15 days post-application. Most recent mature leaf sampling from post-R3 application results of micronutrients. Samples were collected on 8/1/2018 which was 15 days post-application.

Timing	Rate lb S/acre	Zn	Mn	Fe	Cu	B	Zn	Mn	Fe	Cu	B
				ppm ‡					ppm ¶		
Pre-emerge	20	44	42	108	12	29	34	51	105	10	27
UTC	0	47	43	103	13	32	37	49	91	11	25
V4	1	46	45	101	13	30	36	50	87	10	24
V4	2	44	38	98	13	30	36	45	97	11	26
V4	4	44	44	106	13	34	33	48	98	10	28
V4	6	44	45	107	14	31	33	50	102	11	28
UTC	0	47	43	103	13	32	37	49	91	11	25
R3	1	.	.	.	.	.	35	46	99	11	29
R3	2	.	.	.	.	.	32	48	103	11	30
R3	4	.	.	.	.	.	35	52	103	11	28
R3	6	.	.	.	.	.	33	45	106	11	30
UTC	0	47	43	103	13	32	37	49	91	11	25
twice†	1	46	39	101	13	33	34	44	101	11	27
twice†	2	44	42	109	13	34	33	48	100	10	25
twice†	4	42	43	108	13	32	33	50	104	11	26
twice†	6	45	44	102	13	31	34	50	112	11	26

† twice denotes the sequential application treatment with target applications at V4 and again at R3 with the same stated rate applied at V4 and again at R3. The first sampling data shows results from a single application of the planned two application (the first application at V4). The second set of data shows nutrient results after having both the V4 and R3 applications done.
‡ Tissue sampling of most recent mature leaves collected on 7/10/2018 (15 days post V4 application)
¶ Tissue sampling of most recent mature leaves collected on 8/1/2018 (15 days post R3 application). Soybean was at R5 growth stages when samples were collected.

Table B-2. 2019 most recent mature leaf sampling from post-V4 application results of micronutrients on the left. Samples were collected on 7/16/2019 which was 14 days post-application. The most recent mature leaf sampling from post-R3 application results of micronutrients on the right. Samples were collected on 8/8/2019 which was 16 days post-application.

Timing	Rate lb S/acre	Zn	Mn	Fe ppm ‡	Cu	B	Zn	Mn	Fe ppm ¶	Cu	B
Pre-emerge	20	56	73	100	10	30	38	33	171	8	23
UTC	0	60	66	97	10	33	37	28	101	8	26
V4	1	52	66	107	10	32	37	26	103	8	24
V4	2	55	68	103	11	33	37	29	84	9	23
V4	4	50	65	96	10	34	35	29	82	8	23
V4	6	57	73	113	11	33	36	31	89	8	22
UTC	0	60	66	97	10	33	37	28	104	8	26
R3	1	.	.	.	.	.	37	29	114	9	25
R3	2	.	.	.	.	.	33	26	86	8	24
R3	4	.	.	.	.	.	35	27	95	8	26
R3	6	.	.	.	.	.	35	31	131	9	26
UTC	0	60	66	97	10	33	37	28	101	8	26
twice†	1	55	66	102	11	31	39	29	118	9	21
twice†	2	51	66	99	10	32	42	30	98	9	23
twice†	4	54	68	105	10	33	36	32	395	9	23
twice†	6	49	61	103	10	34	38	31	96	9	24

† twice denotes the sequential application treatment with target applications at V4 and again at R3 with the same stated rate applied at V4 and again at R3. The first sampling data shows results from a single application of the planned two application (the first application at V4). The second set of data shows nutrient results after having both the V4 and R3 applications done.
‡ Tissue sampling of most recent mature leaves collected on 7/16/2019 (14 days post V4 application). Soybean was at R2 growth stages when samples were collected.
¶ Tissue sampling of most recent mature leaves collected on 8/8/2019 (16 days post R3 application). Soybean was at R5 growth stages when samples were collected

REFERENCES

Agrawal, H.P., and A.K. Mishra. 1994. Sulphur nutrition of soybean. Commun. Soil Sci. Plant Anal. 25(9–10): 1303–1312. doi: 10.1080/00103629409369116.

Ahmad, A., G. Abraham, N. Gandotra, Y.P. Abrol, and M.Z. Abdin. 1998. Interactive Effect of Nitrogen and Sulphur on Growth and Yield of Rape-seed-Mustard (Brassica juncea L. Czern. and Coss. and Brassica campestris L.) Genotypes. J. Agron. Crop Sci. 181(4): 193–199. doi: 10.1111/j.1439-037X.1998.tb00417.x.

Anderson, A.J., and D. Spencer. 1950. SULPHUR IN NITROGEN METABOLISM OF LEGUMES AND NON-LEGUMES. Aust. J. Sci. Res. Ser. B 3(4): 431–449. https://www.publish.csiro.au/bi/BI9500431 (accessed 16 April 2020).

Bellaloui, N., K.N. Reddy, A.M. Gillen, D.K. Fisher, and A. Mengistu. 2011. Influence of Planting Date on Seed Protein, Oil, Sugars, Minerals, and Nitrogen Metabolism in Soybean under Irrigated and Non-Irrigated Environments *. Am. J. Plant Sci. 2: 702–715. doi: 10.4236/ajps.2011.25085.

Bender, R.R., J.W. Haegele, and F.E. Below. 2015. Nutrient uptake, partitioning, and remobilization in modern soybean varieties. Agron. J. 107(2): 563–573. doi: 10.2134/agronj14.0435.

Bertramson B. R., M. Fried, and S.L. TIsdale. 1950. Sulfur Studies of Indiana Soils and Crops. Soil Sci. 70(1): 27–42. https://journals.lww.com/soilsci/Citation/1950/07000/Sulfur_Studies_of_Indiana_Soils_and_Crops.5.aspx#pdf-link.

Bettany, J.R., J.W.B. Stewart, and E.H. Halstead. 1973. Sulfur Fractions and Carbon, Nitrogen, and Sulfur Relationships in Grassland, Forest, and Associated Transitional Soils1. Soil Sci. Soc. Am. J. 37(6). doi: 10.2136/sssaj1973.03615995003700060034x.

Bloem, E., S. Haneklaus, and E. Schnug. 2002. Model based prognosis of sulphur deficiency.

Boote, K.J., R.N. Gallaher, W.K. Robertson, K. Hinson, and L.C. Hammond. 1978. Effect of Foliar Fertilization on Photosynthesis, Leaf Nutrition, and Yield of Soybeans1. Agron. J. 70(5): 787. doi: 10.2134/agronj1978.00021962007000050022x.

De Bruin, J.L., and P. Pedersen. 2008. Soybean seed yield response to planting date and seeding rate in the upper midwest. Agron. J. 100(3): 696–703. doi: 10.2134/agronj2007.0115.

Camberato, J., and S. Casteel. 2017. Purdue University Department of Agronomy Soil Fertility Update Sulfur deficiency.

Campbell, C.R. 2000. reference sufficiency ranges for plant analysis in the southern region of the united states southern cooperative series Bulletin #394. South. Coop. Ser. Bull. (394). www.ncagr.gov/agronomi/saaesd/scsb394.pdf (accessed 3 December 2019).

Carciochi, W.D., L.H.M. Rosso, M.A. Secchi, A.R. Torres, S. Naeve, et al. 2019. Soybean yield, biological N2 fixation and seed composition responses to additional inoculation in the United States.

Chen, L., W. Dick, and S. Nelson. 2005. Flue Gas Desulfurization Products as Sulfur Sources for Alfalfa and Soybean. Agron. J. 97(1): 265–271. doi: 10.2134/agronj2005.0265.

Chen, L., D. Kost, and W.A. Dick. 2008. Flue Gas Desulfurization Products as Sulfur Sources for Corn. Soil Sci. Soc. Am. J. 72(5): 1464. doi: 10.2136/sssaj2007.0221.

Conley, S.P., and J.B. Santini. 2007. Crop Management Practices in Indiana Soybean Production Systems. Crop Manag. 6: 1–9. doi: 10.1094/cm-2007-0104-01-rs.

Crosland, A.R., F.-J. Zhao, and S.P. McGrath. 2001. INTER-LABORATORY COMPARISON OF SULPHUR AND NITROGEN ANALYSIS IN PLANTS AND SOILS. Commun. Soil Sci. Plant Anal. 32(5–6): 685–695. doi: 10.1081/CSS-100103901.

Dei, H.K. 2012. Soybean as a Feed Ingredient for Livestock and Poultry. Recent Trends for Enhancing the Diversity and Quality of Soybean Products. InTech

Dick, W.A., D. Kost, L. Chen, and J. Jez. 2008. Availability of Sulfur to Crops from Soil and Other Sources. Sulfur: A Missing Link between Soils, Crops, and Nutrition. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. p. 59–82

Dornbos, D.L., and R.E. Mullen. 1992. Soybean Seed Protein and Oil Contents and Fatty Acid Composition Adjustments by Drought and Temperature. JAOCS 69(3): 228–231.

Edwards, J.T., and L.C. Purcell. 2005. Soybean Yield and Biomass Responses to Increasing Plant Population Among Diverse Maturity Groups. Crop Sci. 45(5): 1770. doi: 10.2135/cropsci2004.0564.

Egli, D.B. 1993. Cultivar maturity and potential yield of soybean. F. Crop. Res. 32(1–2): 147–158. doi: 10.1016/0378-4290(93)90027-K.

Eichert, T., and J.È. Rgen Burkhardt. 2001. Quantification of stomatal uptake of ionic solutes using a new model system.

Fageria, N.K., M.P.B. Filho, A. Moreira, and C.M. Guimarães. 2009. Foliar Fertilization of Crop Plants. J. Plant Nutr. 32(6): 1044–1064. doi: 10.1080/01904160902872826.

Franke, W. 1967. MECHANISMS OF FOLIAR PENETRATION OF SOLUTIONSl Further ANNUAL REVIEWS.

Freeborn, J.R., D.L. Holshouser, M.M. Alley, N.L. Powell, and D.M. Orcutt. 2001. Soybean yield response to reproductive stage soil-applied nitrogen and foliar-applied boron. Agron. J. 93(6): 1200–1209. doi: 10.2134/agronj2001.1200.

Freney, J.R., N.J. Barrow, and K. Spencer. 1962. A review of certain aspects of sulphur as a soil constituent and plant nutrient. Plant Soil 17(3): 295–308. doi: 10.1007/BF01377669.

Garcia L., R., and J.J. Hanway. 1976. Foliar Fertilization of Soybeans During the Seed-filling Period1. Agron. J. 68(4): 653. doi: 10.2134/agronj1976.00021962006800040030x.

Gaspar, A.P., C.A.M. Laboski, S.L. Naeve, and S.P. Conley. 2017. Dry matter and nitrogen uptake, partitioning, and removal across a wide range of soybean seed yield levels. Crop Sci. 57(4): 2170–2182. doi: 10.2135/cropsci2016.05.0322.

Gates, C.T., and W.J. Muller. 1979. Nodule and Plant Development in the Soybean, Glycine max (L.) Merr.: Growth Response to Nitrogen, Phosphorus and Sulfur.

Gilbert, F.A. 1951. THE PLACE OF SULFUR IN PLANT NUTRITION. Bot. Rev. 17(9): 671–691. https://link.springer.com/article/10.1007/BF02879757 (accessed 6 January 2020).

Gooding, M.J., and W.P. Davies. 1992. Foliar urea fertilization of cereals: a review.

Haneklaus, S., E. Bloem, E. Schnug, and J. Jez. 2008. History of Sulfur Deficiency in Crops. In: Jez, J., editor, Sulfur: A Missing Link Between Soils, Crops, and Nutrition. p. 45–58

Hanson, E. A.; Barrien, B. S.; Wood, J.G. 1941. RELATIONS BETWEEN PROTEIN-NITROGEN, PROTEIN-SULPHUR AND CHLOROPHYLL IN LEAVES OF SUDAN GRASS. Aust. J. Exp. Biol. Med. Sci. 19(3): 231–234. https://web.b.ebscohost.com/abstract?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=0004945X&asa=Y&AN=17298972&h=w8%2BCyIkboxlTUbZcfRRHKwQXY4rlB5GkmGwZM4IJER5%2BwddAy5lUZkzjMdbcpuc%2F0%2BHF2vH6CD3vJj3SOkue3A%3D%3D&crl=c&resultNs=AdminWebAuth&r (accessed 17 April 2020).

Haq, M.U., and A.P. Mallarino. 2000. Soybean Yield and Nutrient Composition as Affected by Early Season Foliar Fertilization. Agron. J. 92(1): 16. doi: 10.2134/agronj2000.92116x.

Hitsuda, K., G.J. Sfredo, and D. Klepker. 2004. Diagnosis of Sulfur Deficiency in Soybean using Seeds. Soil Sci. Soc. Am. J. 68(4): 1445. doi: 10.2136/sssaj2004.1445.

Hitsuda, K., K. Toriyama, G. V Subbarao, and O. Ito. 2008. Sulfur Management for Soybean Production. : 117–142.

Hurburgh, C.R., T.J. Brumm, J.M. Gulnn, and R.A. Harhnig. 1990. Protein and Oil Patterns in U.S. and World Soybean Markets.

Hymowitz, T. 1990. Soybeans: The Success Story. https://hort.purdue.edu/newcrop/proceedings1990/V1-159.html (accessed 30 May 2019).

Hymowitz, T., and J.R. Harlan. 1983. Introduction of Soybean to North America by Samuel Bowen in 17651. Econ. Bot. 37(4): 371–379. https://link.springer.com/content/pdf/10.1007%2FBF02904196.pdf (accessed 10 July 2019).

James Karuga. 2018. 10 Countries With Largest Soybean Production - WorldAtlas.com. https://www.worldatlas.com/articles/world-leaders-in-soya-soybean-production-by-country.html (accessed 10 July 2019).

Kaiser, D.E., and K.-I. Kim. 2013. Soybean Response to Sulfur Fertilizer Applied as a Broadcast or Starter Using Replicated Strip Trials. Agron. J. 105(4): 1189. doi: 10.2134/agronj2013.0023.

Kandel, H. 2015. Soybean Nodulation — Crop & Pest Report.

Krishnan, H.B., and J. Jez. 2008. Improving the Sulfur-Containing Amino Acids of Soybean to Enhance its Nutritional Value in Animal Feed. Sulfur: A Missing Link between Soils, Crops, and Nutrition. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. p. 235–249

Liu, X., J.-A. Wu, H. Ren, Y. Qi, C. Li, et al. 2017. Genetic variation of world soybean maturity date and geographic distribution of maturity groups. Breed. Sci. 67(3): 221–232. doi: 10.1270/jsbbs.16167.

Lowe, L.E. 1969. Lowe.soil_factors.

De Mello Filho, O.L., C.S. Sediyama, M.A. Moreira, M.S. Reis, G.A. Massoni, et al. 2004. Grain yield and seed quality of soybean selected for high protein content. Pesqui. Agropecu. Bras. 39(5): 445–450.

Morse, W.J. 1950. No Title. In: Markley, K., editor, History of Soybean Production. Interscience Publisher, INC, New York. p. 3–59

Muhammad, A., S.K. Khalil, K. Marwat, A.Z. Khan, I.H. Khalil, et al. 2009. Integrated use of organic and inorganic fertilizers in wheat and their residual effect on subsequent mung bean View project Crop Modelling View project.

Naeve, S.L., and R.M. Shibles. 2005. Distribution and mobilization of sulfur during soybean reproduction. Crop Sci. 45(6): 2540–2551. doi: 10.2135/cropsci2005.0155.

Ohyama, T., K. Tewari, S. Ishikawa, K. Tanaka, S. Kamiyama, et al. 2017. Role of Nitrogen on Growth and Seed Yield of Soybean and a New Fertilization Technique to Promote Nitrogen Fixation and Seed Yield. Soybean - The Basis of Yield, Biomass and Productivity. InTech

Papadakis, I.E., T.E. Sotiropoulos, and I.N. Therios. 2007. Mobility of Iron and Manganese within Two Citrus Genotypes after Foliar Applications of Iron Sulfate and Manganese Sulfate. doi: 10.1080/01904160701555754.

Parker, M.B., and F.C. Boswell. 1980. Foliage Injury, Nutrient Intake, and Yield of Soybeans as Influenced by Foliar Fertilization1. Agron. J. 72(1): 110. doi: 10.2134/agronj1980.00021962007200010022x.

Pedersen, P. 2003. Soybean seed inoculation. Integr. Crop Manag. News. http://lib.dr.iastate.edu/cropnewshttp://lib.dr.iastate.edu/cropnews/1669 (accessed 27 April 2020).

Piper, E.L., and K.I. Boote. 1999. Temperature and cultivar effects on soybean seed oil and protein concentrations. J. Am. Oil Chem. Soc. 76(10): 1233–1241. doi: 10.1007/s11746-999-0099-y.

Plant Food Company, Inc. 2014. https://www.plantfoodco.com/media/1883/28-0-0-label.pdf (accessed 1 July 2020).

Poole, W.D., G.W. Randall, and G.E. Ham. 1983. Foliar Fertilization of Soybeans. I. Effect of Fertilizer Sources, Rates, and Frequency of Application1. Agron. J. 75(2): 195. doi: 10.2134/agronj1983.00021962007500020010x.

Radford, R.L., C. Chavengsaksongkram, and T. Hymowitz. 1977. Utilization of Nitrogen to Sulfur Ratio for Evaluating Sulfur-Containing Amino Acid Concentrations in Seed of Glycine max and G. soja1. Crop Sci. doi: 10.2135/cropsci1977.0011183x001700020011x.

Robinson, A.P., S.P. Conley, J.J. Volenec, and J.B. Santini. 2009. Analysis of High Yielding, Early-Planted Soybean in Indiana. Agron. J. 101(1): 131. doi: 10.2134/agronj2008.0014x.

Rotundo, J.L., and M.E. Westgate. 2009. Meta-analysis of environmental effects on soybean seed composition. F. Crop. Res. 110: 147–156. doi: 10.1016/j.fcr.2008.07.012.

SAEED, B., A.Z. KHAN, S.K. KHALIL, H.U. RAHMAN, F. ULLAH, et al. 2013. RESPONSE OF SOIL AND FOLIAR APPLIED NITROGEN AND SULFUR TOWARDS YIELD AND YIELD ATTRIBUTES OF WHEAT CULTIVARS.

Salvagiotti, F., K.G. Cassman, J.E. Specht, D.T. Walters, A. Weiss, et al. 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. F. Crop. Res. 108: 1–13. doi: 10.1016/j.fcr.2008.03.001.

Santachiara, G., L. Borrás, and J. Rotundo. 2016. Physiological Processes Leading to Similar Yield in Contrasting Soybean Maturity Groups Grain yield genetic gain in corn for early and late planting dates during the las three decades in Argentina View project. doi: 10.2134/agronj2016.04.0198.

Scherer, H.W. 2008. Impact of Sulfur on N 2 Fixation of Legumes. In: Khan N.A., Singh S., U.S., editor, Sulfur Assimilation and Abiotic Stress in Plants. Springer, Berlin, Heidelberg. p. 43–54

Sexton, P.J., N.C. Paek, and R. Shibles. 1998a. Soybean sulfur and nitrogen balance under varying levels of available sulfur. Crop Sci. 38(4): 975–982. doi: 10.2135/cropsci1998.0011183X003800040016x.

Sexton, P.J., N.C. Paek, and R.M. Shibles. 1998b. Effects of nitrogen source and timing of sulfur deficiency on seed yield and expression of 11S and 7S seed storage proteins of soybean. F. Crop. Res. 59(1): 1–8. doi: 10.1016/S0378-4290(98)00101-4.

Shapiro, C.A., R.B. Ferguson, G.W. Hergert, and C.S. Wortmann. 2008. Know how. Know now. Fertilizer Suggestions for Corn.

Soybean Sulfur Status. 2012.

Sunarpi, and J.W. Anderson. 1998. Direct evidence for the involvement of the root in the redistribution of sulfur between leaves. J. Plant Nutr. 21(6): 1273–1286. doi: 10.1080/01904169809365481.

Tamagno, S., V.O. Sadras, J.W. Haegele, P.R. Armstrong, and I.A. Ciampitti. 2018. Interplay between nitrogen fertilizer and biological nitrogen fixation in soybean: implications on seed yield and biomass allocation OPEN.

Triboi, E., and A.M. Triboi-Blondel. 2002. Productivity and grain or seed composition: A new approach to an old problem - Invited paper. Eur. J. Agron. 16(3): 163–186. doi: 10.1016/S1161-0301(01)00146-0.

US EPA. 2019. Sulfur Dioxide Trends. Natl. Air Qual. Status Trends Key Air Pollut. https://www.epa.gov/air-trends/sulfur-dioxide-trends (accessed 1 July 2020).

USDA-NRCS Official Soil Series Description View By Name. https://soilseries.sc.egov.usda.gov/osdname.aspx (accessed 15 November 2019).

USDA - National Agricultural Statistics Service - Statistics by Subject Results. 2018. https://www.nass.usda.gov/Statistics_by_Subject/result.php?3B23E8A5-9894-3863-9CED-825D3B9B3A49&sector=CROPS&group=FIELD CROPS&comm=SOYBEANS (accessed 21 May 2019).

Vasilas, B.L., J.O. Legg, and D.C. Wolf. 1980. Foliar Fertilization of Soybeans: Absorption and Translocation of 15N-Labeled Urea1. Agron. J. 72(2): 271. doi: 10.2134/agronj1980.00021962007200020006x.

Warncke, D., J. Dahl, and L. Jacobs. 2009. Nutrient Recommendations for Field Crops in Michigan.

Wilcox, J.R., and E.M. Frankenberger. 1987. Indeterminate and Determinate Soybean Responses to Planting Date1. Agron. J. 79(6): 1074. doi: 10.2134/agronj1987.00021962007900060025x.

Willis, S. 2003. The use of Soybean Meal and Full Fat Soybean Meal by the Animal Feed Industry.

Wittwer, S. H., Bukovac, M. J. and Tukey, H.B. 1963. Fertilizer Technology and Usage (G.L.B. and L.B.N. M. H. McVickar, editor). Soil Sci. Soc. Am. Inc., Madison.

Woolfolk, C.W., W.R. Raun, G. V Johnson, W.E. Thomason, R.W. Mullen, et al. 2002. Influence of Late-Season Foliar Nitrogen Applications on Yield and Grain Nitrogen in Winter Wheat. Agron. J. 94(3). doi: 10.2134/agronj2002.4290.

Zhang, F., and D.L. Smith. 2002. INTERORGANISMAL SIGNALING IN SUBOPTIMUM ENVIRONMENTS: THE LEGUME-RHIZOBIA SYMBIOSIS.

Zhao, Y., X. Xiao, D. Bi, and F. Hu. 2008. Effects of Sulfur Fertilization on Soybean Root and Leaf Traits, and Soil Microbial Activity. J. Plant Nutr. 31(3): 473–483. doi: 10.1080/01904160801895001.