SOYBEAN YIELD AND QUALITY RESPONSE TO FLUID STARTER SULFUR FERTILIZER

by

Nicholas Roysdon

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STATEMENT OF COMMITTEE APPROVAL

Dr. Shaun Casteel, Chair
Department of Agronomy

Dr. Corey Gerber
Department of Agronomy

Dr. James Camberato
Department of Agronomy

Approved by:
Dr. Ron Turco

Dedicated to my grandfather, Doris Roysdon, whose love and pride in me was a constant source of joy in my life.

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ABSTRACT

Sulfur (S) demand has increased as atmospheric deposition of S decreased and soybean (*Glycine max* (L.) Merr.) production has increased. Soybean growers have invested into agronomic practices to maximize production and alleviate potential S shortfalls including the use of starter fertilizer. For this reason, this study was designed to quantify and qualify the effects of fluid starter S fertilizer on soybean yield. The objectives were to determine an optimum source, rate, and placement of fluid starter fertilizer. A split-plot design of S source-rate and placement was used in 2019 and 2020 at West Lafayette and Wanatah, Indiana. Three starter S fertilizers were used: ammonium thiosulfate (ATS, 12-0-0-26S, Hydrite Chemical), potassium thiosulfate (KTS, 0-0-25-17S, Hydrite Chemical), and K-Fuse (derived from potassium acetate, ammonium thiosulfate and urea 6-0-12-12S, NACHURS) as well as broadcast granular ammonium sulfate (AMS, 21-0-0-24S), and an untreated control. Starter S products were applied at four S rates: 5.6, 11.2, 16.8, and 22.4 kg S ha^{-1} to determine optimal S rate and in two placements (single: 0x5x1-cm; dual: 0x5x2-cm). AMS was broadcast at 22.4 kg S ha^{-1}

Placement did not affect a majority of the factors analyzed and was largely factored out when not significant. Leaf concentrations of essential macro-nutrients, including S, were above critical levels and were not affected by starter fertilizer at any site-year. ATS increased manganese (Mn) in 2019 and 2020 and Wanatah. In West Lafayette 2020 (timely planting), all three starter sulfur fertilizers increased yield and protein, while broadcast AMS did not. Yield and protein did not change with starter S fertilizer in the remaining site-years, which was likely due to plantings later than recommended.

To evaluate and quantify the effects of fluid starter fertilizer across early and late planting dates, a split-plot design was used with an earlier (May 13, 2020) and late (June 8, 2020)

planting dates at West Lafayette, IN, as well as early (P24A80X) and late (P35A33X) maturing soybean varieties at Wanatah, IN. These were crossed with six fertility treatments: ammonium thiosulfate (ATS, 12-0-0-26S), potassium thiosulfate (KTS, 0-0-25-17S), K-Fuse (6-0-12-12S, NACHURS), 28% urea ammonium nitrate (UAN, 28-0-0), ammonium sulfate (AMS, 21-0-0-24S), and an untreated control. Starter S fertilizers were applied at 16.8 kg S ha^{-1} and 28% UAN was applied at a 7.9 kg Nitrogen (N) ha^{-1} rate, all in a single (0x5x1-cm) placement.

The earlier planting had greater stand and yield than the later planting. Starter fertilizers did not impact yield, protein or oil compared to untreated control. Earlier-planted soybean with KTS had higher S concentration in the leaves than UTC and other fertility treatments. Variety impacted leaf nutrient and seed protein concentration. Leaf nutrient concentrations was generally higher in the 3.5 variety compared to the 2.4 variety. Protein was higher in the 2.4 variety compared to the 3.5 variety. However, yield was not affected by variety, fertilizer, or a variety x fertilizer interaction. There was also no fertilizer effect on any essential nutrient concentration.

Soybean positive response to starter S fertilizer aligned with timely plantings rather than later plantings. Earlier plantings were cool and wet field conditions, which limited mineralization of soil organic matter and the supply of N and S. The highest yield was 4308 kg ha^{-1} with KTS in West Lafayette 2020, applied at a rate of 7.5 kg S ha^{-1}, followed by K-Fuse and ATS, respectively. Given the minimal response to different placements, it can be concluded that the difference between single and dual placements on soybean growth and yield is negligible.

CHAPTER 1: REVIEW OF LITERATURE

1.1 Soybean Production

1.1.1 History of Soybean Production

Soybean (*Glycine max* L.) center of origin was Asia, where it appeared on historical records as early as 4,500 years ago (Singh, 2010). Soybean was first cultivated in North America in 1765 when entrepreneur Samuel Bowen planted soybean near Savannah, Georgia (Hymowitz & Harlan, 1983). Soybean was initially used as a forage crop for cattle, but was also cooked and eaten as sprouts (Hymowitz, 1990). Later, some soybean production was for the purpose of making soy sauce, sago powder, and vermicelli as an American export. For the next 155 years, soybean was primarily used as a forage, until its usefulness as meal was studied in 1917. This resulted in its subsequent increase in popularity as a grain crop through the 1920's. 1941 was the first year a majority of soybean acres were planted as a grain crop.

1.1.2 Current Production in the Midwest

The USDA reported that over 36.4 million ha (90 million acres) of soybean was planted in 2017 (*USDA ERS - Soybeans & Oil Crops*, 2019). In 2018, more acres were planted in soybean than in corn (*Zea mays*) for the first time since 1983. In 2018, more than 81% of soybean acreage was located in the upper Midwest, with the top producing states being Illinois, Iowa, and Minnesota. The average price per bushel of soybean in 2018 was $8.60, and the value of production in the United States was $39,133,978 in 2018 (*USDA ERS - Soybeans & Oil Crops*, 2019). In Indiana in 2018, the average price per bushel was $8.75 and the value of production was $3.08 billion (Indiana State Department of Agriculture, 2019; *USDA ERS - Soybeans & Oil Crops*, 2019). In 2021, soybean acres in the US were estimated at 35.4 million ha (87.6 million ac) planted (USDA, 2021). The average soybean yield was 3380 kg ha^{-1} in 2020. In Indiana, weather was considered to be one of the most important factors limiting soybean net return, followed by weeds, marketing, and soil fertility (Conley & Santini, 2007).

Soybean production worldwide exceeds production of any other oilseed since the 1960s (Boerma et al., 2004). In 2002, 97% of soybean oil was used for food products such as cooking oils, shortenings, and margarine. Another important product is soybean meal. Soybean meal is high in protein and can be used in flour, baking products, or as a milk substitute (Hammond et al., 2005). Soybean meal is a major source of protein in animal diets, particularly that of the swine (*Sus scorfa domesticus*) and poultry (*Gallus gallus domesticus*) (Willis, 2003). Soybean meal is considered high in essential amino acids, making it very desirable worldwide as a feed source.

1.1.3 Growth of Indeterminant Soybean

The stem and leaves of indeterminant soybean continue grow as flowers, pods, and seeds develop (e.g., reproductive development) (Bernard, 1972). In indeterminant soybean varieties, terminal growth will continue and produce a tapered stem with little or no lateral growth near the top of the stem.

Vegetative Physiology

Soybean above-ground growth will typically begin within 4-7 days after planting, if planted in favorable conditions with adequate moisture and temperature (Hicks, 1978; McWilliams et al., 1999). Vegetative growth is slow initially as new trifoliate leaves emerge every 3-5 days during VC-V3 growth stages. Plant growth is rapid from V5-R4 (full pod, Fehr & Caviness, 1977; Hicks, 1978; McWilliams et al., 1999). In indeterminant cultivars, the average daily dry matter increased as much as 186 kg ha^{-1} between the development of nodes 5 to 9 (Hicks, 1978). Soybean height, nodal development, and leaf area is maximized between R5 to R6 (first to full seed, Fehr & Caviness, 1977; Hicks, 1978; McWilliams et al., 1999).

Reproductive Physiology

Flower development on indeterminant soybean typically begins at the fourth or a higher node, and continues up the stem and out on the lateral branches (Hicks, 1978). Thus, pods and seeds first develop on the lower portion of the main stem (Fehr et al., 1971). Soybean flowering is controlled by a number of factors including photoperiod, temperature, and genotype (Fehr et al., 1971; Major et al., 1975). Flowering is primarily affected by photoperiod followed by air temperatures. Any differences in flowering by genotype is a function of sensitivity to photoperiod. Cooler air temperatures delay flowering, while warmer air temperatures hasten it (Sinclair et al., 1991). Cooler air temperatures and longer daylengths were a common cause for delay of flowering in northern latitudes (Major et al., 1975).

Hicks (1978) described indeterminant soybean inflorescence as axillary racemes. The flowering period is greater in indeterminant than determinant soybean (Hicks, 1978). Indeterminate soybean reached 67% of their maximum height at flowering and produced 58% of above ground dry weight at flowering (Egli & Leggett, 1973). Progression into further reproductive stages, such as seed fill and drying, were a function of the number of degree days in the environment (Munier-Jolain et al., 1993). Yield of indeterminate soybean is a function of number of pods per plant, number of seed per pod, and seed size.

Root Development

Soybean develop primary and secondary lateral roots off of a primary taproot (Hicks, 1978). Under favorable conditions, the taproot reaches depths of 160 cm (Hicks, 1978). Approximately four sections of lateral roots develop around the taproot and grow horizontally about 35 cm then downward on eight varieties in 1971 (Mitchell & Russell, 1971). Soybean rooting depth was nearly 180 cm (Mayaki et al., 1976). Roots grew twice as fast as plant height during vegetative growth stages, but slow down as soybean enter reproductive development. Root depth can be greater than plant height during the growing season (Hicks, 1978).

Primary root growth emerge from the promeristem and primary meristem at the root tip, while secondary (lateral) root growth emerge from the pericycle tissue on the primary root (Hicks, 1978). Further root systems develop from the secondary roots. Root hairs develop on all root surfaces except the taproot. Root growth slow at the point of seed fill and cease prior to reaching maturity. In a study in Missouri in 1995, Turman (1995) determined that cultivar, maturity group, and growth pattern had a nominal impact on root growth (Turman et al., 1995). Allmaras (1975) found that root dry matter per unit of shoot dry matter in the field was generally greater in indeterminant soybean cultivars compared to determinant cultivars, but root depth between the two did not differ (Allmaras et al., 1975).

Leaf Development

Soybean develop four different leaf structures throughout the growing season: cotyledons, primary (unifoliate) leaves, trifoliate leaves, and prophylls (Fehr & Caviness, 1977). Cotyledon is a thick energy reserve that is part of the seed and emerges with the hypocotyl. The cotyledons drop from the plant early in vegetative development. The unifoliate leaf is the first leaf to develop immediately above the cotyledon node (Hicks, 1978). Trifoliate leaves develop and grow above the unifoliate in alternating pattern on the main stem. Prophylls are small simple leaves that develop around the base of flowers (Fehr & Caviness, 1977). Leaves of indeterminant soybean typically decrease in size toward the terminal node. Leaf pubescence was common and widespread on the leaf surface (Hicks, 1978).

Influences of Temperature and Photoperiod

Seed germinated fastest in soil temperature of 30°C (Hicks, 1978). Soybean seed can germinate in air temperatures from 5 to 40°C (Whigham & Minor, 1978). Seedling growth increase significantly when daytime air temperature went from 18°C to 27°C with no more advancement up to 33°C (Egli & Wardlaw, 1980). Soil temperature has also been shown to affect root growth. The elongation of taproot and lateral roots of indeterminate soybean increased as soil temperatures increased from 17, 21, 25 to 29°C (Stone & Taylor, 1983).

A general trend in indeterminant and determinant cultivars is that vegetative period increase with cooler air temperatures and longer photoperiods, but decrease with warmer temperatures and shorter photoperiods (Chen & Wiatrak, 2010). Trifoliate developed with the same number of degree-days whether soybean was grown at 12°C or 30°C (Whigham & Minor, 1978). Vegetative development of indeterminant and determinate cultivars ceased air temperature was less than or equal to 6°C. Optimal growth and development of soybean is 30°C (Hicks, 1978). Root growth is maximized between 27 to 32°C. However, root growth differed very little when air temperatures were greater than 12°C. Additionally, *Bradyrhizobium japonicum* growth (and nitrogen fixation) is limited in temperatures greater than 33°C (Whigham & Minor, 1978).

Leaf area of indeterminant and determinant soybean varieties increase as temperature increases (Ciha & Brun, 1975). Individual leaf area and total leaf area is maximized at the 26/18°C regime compared to 12/4, 19/11, 26/18, 33/25 and 40/32°C day/night temperatures (Patterson, 1992). Excessive temperatures of 40/32 °C limited individual leaf area and total canopy leaf area. Leaf area was least in the low temperature range of 12/4°C. Reproductive development was delayed and photosynthesis was decreased when indeterminant soybean was exposed to 8°C for 24 hours in the V4 and R1 growth stages (Wang et al., 1997). If the plant was in reproductive stages, cold exposure changed the partitioning from reproductive development to vegetative development. Leaf photosynthesis is optimized between 25 and 30°C (Whigham & Minor, 1978).

Air temperatures below 24°C delay flowering up to 3 days (Whigham & Minor, 1978). Flower initiation is inhibited at air temperatures below 10°C. Pods did not form at or below air temperatures of 18°C (Hesketh et al., 1973). Pod abscission occurred after exposure to heat stress above 40°C (Whigham & Minor, 1978). More recent studies have found that air temperatures above 23°C progressively decreased seed growth rate, seed size, and seed harvest index (Hatfield et al., 2011; Thomas, 2001). This occurred until a maximum air temperature of 39°C, at which point all yield development ceased.

Photoperiod is the length of time in darkness (night) and light (day). Garner and Allard (1930) characterized the effect of photoperiod on soybean flowering between varieties. Changes in photoperiod influences the time of nodal and trifoliate development as well as time of flowering (Johnson et al., 1960). Soybean exposed to long days/short nights (14.5 hr/9.5 hr) matured later than soybean grown in shorter days (13 hr day/11 hr night). Flowering was delayed 40 days in the 14.5 hr/9.5 hr regime.

Temperature and photoperiod can interact in soybean development. Cool growing conditions and long photoperiods (e.g., long days or short nights) delay flowering, while warm growing conditions and short photoperiods (e.g., short days or long nights) can expedite flowering (Garner, 1930). Early and late maturing soybean varieties grown in growth chambers flowered around the same time under optimal conditions (10-12 hr daylight, 28°C) (Cober et al., 2001).

Photoperiod influences pod production. More nodes and branches are produced when soybean are exposed to short days/long nights later in the season and thus, more pods are produced (Hicks, 1978). Other research has shown that extended photoperiods during reproductive development of indeterminate soybean resulted in greater number of nodes per plant, and subsequently more pods and seeds per plant (Kantolic & Slafer, 2005). Seed weight is also known to be reduced with increasing photoperiod.

Nodule Formation and Biological Nitrogen Fixation

Soybean inoculated with the bacterium *Bradyrhizobium japonicum* may form nodules on the root hair structures (Hicks, 1978). This is a symbiotic, mutualistic relationship facilitated by *Bradyrhizobium japonicum* after the soybean form root hairs. Soybean receive up to 60% of the nitrogen needs through biological nitrogen fixation (Salvagiotti et al., 2008).

The bacteria enter the root cell through either the root hair or the epidermal cell (Ott et al., 2005). The bacteria colony grow inward through the root tissue, into the cortex of the primary root. The bacteria colony undergo rapid division for about 2 weeks and mature to a nodule that is 3-6 mm in diameter. "Bacteriods" fill the nodule and fix atmospheric nitrogen

(Hicks, 1978). The nodule forms a continuous vascular system with the infected root for exchanging products.

A nodule is typically visible within 7-9 days after infection and is actively fixing N_2 near 12-18 days after infection (Boerma et al., 2004.). A healthy, mature nodule has a pink coloration, due to the presence of leghemoglobin (Hicks, 1978). Leghemoglobin is essential in oxygen transport and buffering during the N-fixing process (Ott et al., 2005). The nodule maximizes it size approximately 28 to 37 days after infection (Boerma et al., 2004). The nodule fixes N_2 until senescence occurs, which is between 50-60 days after infection. Younger portions of the root system can be reinfected, resulting in multiple ages and sizes of nodules throughout the root system.

Various abiotic factors can decrease biological N fixation (BNF). Water deficiency, low soil pH, and soil temperature stress all decrease BNF (King & Purcell, 2001; Salvagiotti et al., 2008). High concentration of N in the soil can reduce BNF (Salvagiotti et al., 2008). The development of N-fixing root nodules is suppressed when exposed to high concentrations of N (Saito et al., 2014). Additionally, the presence of nitrate (NO_3^-) strongly inhibited nodulation and BNF. Nitrate inhibition decreased nodule number, mass, and accelerated nodule senescence. There is also a negative exponential relationship between N fertilization rate and N fixation (Salvagiotti et al., 2008).

Practices That Influence Vegetative and Reproductive Development

Planting Date

Planting earlier in the growing season is a critical and simple management tool to increase soybean yield (Robinson et al., 2009). In 2007, two-thirds of farmers in Indiana planted soybean one to three weeks earlier than ten years before. Growers with more acres were more likely to plant earlier (Conley & Santini, 2007). Soybean yield increases in earlier plantings due to increased number of pods per plant and increased seeds per pod. Because of this, planting soybean in April or early May is considered an effective strategy to increase yield (Robinson et al., 2009). A study in Ohio in 2013 and 2014 using an Asgrow 3.2 relative maturity group indeterminant soybean variety demonstrated that yield decreased by an average of 39 kg ha^{-1} per day (0.58 bu ac^{-1}) compared to planting dates in May to June (Hankinson et al., 2015). Later planting dates decrease soybean yield because of shorter photoperiods and changes in temperature and precipitation, lessening the vegetative growth period. This reduces the number of branches and pods, plant height, and eventually yield (Chen & Wiatrak, 2010).

Field Conditions and Practices

The primary field conditions that affect soybean production are soil moisture, crop rotation, and cropping system. Soil moisture has the greatest impact on germination and plant growth. In order for soybean to germinate, soil moisture tension must be higher than -6.6 bars, with a seed moisture content of at least 50% (Whigham & Minor, 1978). Conversely, excessive soil moisture conditions inhibit germination and growth by limiting oxygen and respiration (Hicks, 1978). Soybean is best adapted to medium textured well-drained soils, as opposed to heavier clay and sandy soils (Tanner & Hume, 1978).

Moisture stress during the vegetative growth stages reduce the rate of plant growth and leaf enlargement (Whigham & Minor, 1978). Root to shoot ratio increase in moisture limited conditions, as root growth is prioritized in the search for water (Oqba, 2017). Moisture stress during reproductive growth stages reduce yield and yield components, including number of pods, number of seeds per pod, seed weight and total dry matter (Foroud, 1993; Oqba, 2017). Between R5-6, seed fill is the most sensitive reproductive stage to moisture stress (Meckel et al., 1984).

Crop rotation can impact yield through the introduction of previous crop residue, resulting in changes in soil characteristics. A 2-year maize-soybean rotation is the dominant cropping system in the Corn Belt (Conley & Santini, 2007). Meese (1991) found that a soybean monoculture resulted in yield decline over time, with yields about 15% less in a soybean monoculture than a maize-soybean annual rotation. Rotation improved yield through reduction of pest pressure and improved soil properties.

Tillage is another factor that impacts yield. Meese (1991) conducted a study in Wisconsin, USA using multiple determinant soybean cultivar. They found that under a fall tillage system, yield was increased 8-10% compared to the no-till system (Meese et al., 1991). This study suggested that cool, wet no-till conditions delayed emergence and thus, decreased yield. However, yield decreases in the no-till system were less likely in a maize-soybean rotation compared to a continuous soybean monoculture. Soybean yields are maximized under any conventional tillage system when compared to a no-till system, however the yield differences between treatments are very slight and do not result in differences in economic return (Vetsch et al., 2007). No-till yields are rarely more than 5% different from any conventional tillage system, including moldboard plowing, chisel plowing, reduced tillage, and field cultivation. Another study concluded that given the insignificant yield differences, economic returns favored a no-till system (Yin & Al-Kaisi, 2004).

Starter Fertilizer

Fertilizer can be applied to soybean in a variety of ways. Banding, or starter fertilizer, is a process of applying a small amount of fertilizer near the seed at planting (Tanner & Hume, 1978). Starter fertilizer is typically applied in a band 5 centimeters laterally from the seed. This band can be either placed on the soil surface or below the seed. Applying the fertilizer closer risks burning the seed. Applying fertilizer away from the seed reduces the risk of seed burn.

Modern Soybean Fertility and Plant Nutrition Practices

Nitrogen

Sources, Methods and Rates

Plants absorb nitrogen (N) as nitrate (NO_3^-) and ammonium (NH_4^+) (Havlin, 2005). Both forms move to the root through mass flow or diffusion and are primarily used in production of amino acids and proteins. Biological N fixation by N fixing rhizobia can be the main source of N for soybean plants (Salvagiotti et al., 2008). Sources vary on how much biological N fixation contribute to total N uptake by the soybean. Ciampitti and Salvagiotti (2018) reported that biological N fixation account for 44-72% of N uptake (Ciampitti & Salvagiotti, 2018). However, Salvagiotti (2008) reported biological fixation only provided 50-60% of the plant’s N needs (Salvagiotti et al., 2008). Córdova et al. (2019) found over two years in Iowa that biological N fixation contributed 23-65% of the total above-ground N content (Córdova et al., 2019). Mastrodomoenico and Purcell (2012) found that N fixation contributed at least 90% of the N content of the seed in multiple different maturity groups (Mastrodomenico & Purcell, 2012).

Historically, N fertilizer applications in soybean have not been recommended, as the presence of N fertilizer had resulted in decreased nodulation by *Bradyrhizobium japonicum,* and reduced N fixation and biologically fixed N supply (Streeter & Wong, 1988; Tanner & Hume, 1978). Traditionally it is understood that N fertilizer applications do not add to the natural symbiotic N additions, but instead replace it. Coupled with this, N fertilizer additions were typically less efficient in supplying N to the plant than N fixation. In a summary analysis of over 100 trials related to N uptake, fixation, and response to N fertilizer, Salvagiotti (2008) found that the proportion of plant N from biological N fixation decreased with increasing rates of N fertilizer (Salvagiotti et al., 2008). However, other studies have found exceptions to that trend. Gan (2003) found that N top-dressed at a rate of 50 kg ha^{-1} on soybean fields in China at either V2 or R1 resulted in more N accumulation, yield, and total N content in three different genotypes, compared to an untreated control (Gan et al., 2003).

Various studies continue to investigate N fertilizer applications in soybean to maximize yield while still preserving nodulation and nitrogen fixation. Sorensen and Penas (1978) found a positive linear yield response when they applied pre-plant ammonium nitrate in Nebraska fields at rates ranging from 0-224 kg N ha^{-1} in 8 of their 13 sites (Sorensen & Penas, 1978). Schmitt (2001) conducted a study in Minnesota analyzing different application times, placement methods and nitrogen sources (Schmitt et al., 2001). A mixed analysis concluded that polymer-coated urea or urea applied in August resulted in a significant yield increase, but generally less than 0.06 Mg ha^{-1}. Salvagiotti (2008) summarized the effects of 11 studies that analyzed foliar N fertilization as a method to reduce potential N_2 fixation inhibition while supplying additional N. They found that 5 of 11 studies had positive yield responses to foliar N fertilization, but that the agronomic efficiency (the change in yield as a proportion of N rate) of these applications were generally less than that of comparable soil applied applications after R3 (Salvagiotti et al., 2008). Foliar N fertilization was also limited by the N rate, as higher N rates caused leaf injury. For these reasons, Salvagiotti (2008) concluded that foliar N applications are unlikely to contribute to significant yield increases in soybean.

A summary analysis on N uptake, fixation and response to N fertilizer from 2008 found that in about half of the studies analyzed there was a positive response to N fertilizer broadcast applied or surface incorporated, and that the average yield increase with N fertilizer from 154 studies was 0.52 Mg ha^{-1} (Salvagiotti et al., 2008). However, the magnitude of response did not change across the different fertilizer N rate categories of 0-50, 50-100, and >100 kg N ha^{-1} applied. This summary also found that N applied after R3 can increase yield in high-yielding sites, but the mechanism of this was not clear. In terms of economic analysis, using fertilizer prices from 2002-2006, this summary concluded that N fertilization would still only be profitable in scenarios where N fixation was not able to meet N demand in high yielding soybean, and when the soybean to N price ratio was high (Salvagiotti et al., 2008).

More recently, Mourtzinis (2018) composited yield data from experiments in the US to evaluate the effect of N fertilization of soybean yield. They used hierarchical modeling and conditional inference tree analysis on the combined dataset to establish relationships between N management choices and soybean yield. This summary found that overall, fertilizer N effects on soybean were relatively small compared to other sources of variability, such as weather, soil and management decisions. They also concluded that the limited response to N as well as the associated costs of N application indicated that the slight positive effects of N fertilizer was unlikely to produce a positive economic return.

N Cycle

Approximately 78% of the earth's atmosphere is dinitrogen (N_2). Dinitrogen is not plant available and must be converted to be plant available (Havlin, 2005). This conversion can occur through fixation by micro-organisms, electrical discharge, or synthetic manufacturing. The N cycle is the process through which different forms of N move through the biosphere. Through the process of N inputs and outputs, N is mineralized, immobilized, and undergoes nitrification. Mineralization is the process through which soil micro-organisms transform organic N into ammonia (NH_2), then into ammonium (NH_4^+). Bacteria then converted much of NH_4^+ to NO_3^- through the process of nitrification. Both ions are plant available but could be converted back to organic N through the process of immobilization (Havlin, 2005).

Nitrogen in the N cycle can change from plant available to plant unavailable through various processes (Havlin, 2005). Denitrifying bacteria can convert NO_3^- to plant unavailable forms through denitrification. The resulting gaseous forms are then lost to the atmosphere. Nitrate can be lost through groundwater drainage below the root zone, making it no longer plant available. Lastly, volatilization, the process through which NH_4^+ is converted to NH_3, can result in N lost to the atmosphere. Volatilization can actually result in plant harm through exposing to the plant to toxic NH_3.

Biological Fixation

Soybean is well known for the symbiotic, naturally-occurring relationship with the bacteria *Bradyrhizobium japonicum.* The bacteria infects the soybean root, forming a nodule containing bacteroid and leghemoglobin (Hicks, 1978). The bacteroid in the nodules fixes and assimilates N_2 into NH_3. The bacteria provide the plant N in exchange for sugars, carbohydrates and ATP (Brun, 1978; Havlin, 2005). Many studies investigating biological N fixation have found that a large amount of the plant's N needs is met by biological nitrogen fixation, but they vary on exactly how much. Salvagiotti (2008) reported that on average 50-60% of the plants N demand was met by biological N fixation (Salvagiotti et al., 2008). Applying N fertilizer can depress nodule formation and decrease nitrogen fixation (Mourtzinis et al., 2018; Saito et al., 2014; Salvagiotti et al., 2008). Slow-release N sources, like polymer coated urea, may decrease the likelihood of biological N fixation depression, because they limit the NO_3^- supply in the nodulation zone while still providing additional N (Salvagiotti et al., 2008).

Nitrogen's Role in The Plant

Nitrogen is a macro-nutrient required in the most basic functions of the plant lifecycle (Havlin, 2005). Nitrogen is required for the synthesis of amino acids and proteins required for plant structures and is an important component in chlorophyll synthesis. Energy transferring compounds such as ATP and ADP also contain N. Soybean specifically requires a high amount of N because of its high protein and oil content (Mourtzinis et al., 2018).

Nitrogen Forms for Uptake, Translocation and Plant Processes

Nitrogen is plant available as either NH_4^+ or NO_3^-. Both forms are primarily transported to the root through mass flow or diffusion (Havlin, 2005). It is necessary to metabolize NO_3^- into NH_4^+ to make it available for amino acid and protein synthesis. When NO_3^- is taken up, it is either transported through the xylem to the leaves or stayed in the roots to be reduced. The enzyme nitrate reductase catalyzes the reduction process. NO_3^- reduction occurs in the soybean leaves (Brun, 1978). The first step in the reduction process is the reduction of NO_3^- to NO_2^- (Havlin, 2005). NO_2^- is toxic and needs to be quickly reduced again to prevent accumulation. The second step is the reduction of NO_2^- to NH_3. In this form, nitrogen can be assimilated into amino acids and proteins. NH_4+ requires one less step in the energy conversion process (Brun, 1978). In studying nutrient remobilization in soybean, Bender (2015) found that as the soybean plant moved into late reproductive development, nearly half of the N required for grain fill was remobilized from elsewhere in the plant (Bender et al., 2015). Nitrogen uptake to non-reproductive organs such as stems, petioles and leaves peaked between R4-R5, at which point N uptake of grain rapidly increases.

Nitrogen Availability Based on Field Conditions

Field conditions such as temperature, moisture, and aeration greatly impact N availability and plant uptake. Biological fixation of N is limited by cool, wet soil conditions, wherein rhizobia activity is restricted (Brun, 1978). Waterlogged soil conditions create anaerobic conditions, resulting in inhibited N fixation. Rhizobia initiation and development are also inhibited by soil nutrient deficiencies such as calcium (Ca), phosphorus (P), boron (B), iron (Fe), and copper (Cu) (Havlin, 2005). Soil pH < 5.5 also reduces rhizobia development.

Cold wet soils slow mineralization of organic matter into plant available N, which limit the availability of soil N (Mourtzinis et al., 2018). Water deficiency also decreases mineralization. The range at which mineralization rates are highest is when 80-90% of total soil pore space is saturated with water, or the soil is at field capacity (Stanford & Epstein, 1974). Increase temperature up to 25°C increase mineralization, depending on the source of nitrogen. (Agehara & Warncke, 2005).

Sulfur Fertility

Sources, Methods and Rates

Effective management strategies are the best way to produce a productive crop. To achieve high yields for soybean, large amounts of N, P and K are needed, with small amounts of S (McGrath et al., 2013). The 2000 Tri-State Fertilizer recommendations indicated that S sufficiency in soybean is between 0.21-0.40% S in the topmost matured leaf prior to flowering (Vitosh et al., 2000). Fertilizers such as blended ammonium sulfate ($(NH_4)_2SO_4$) with urea are cost effective ways to treat S deficiency (Camberato & Casteel, 2017). Fertilizer should be applied as soon as deficiency symptoms appear in order to reduce lost yield potential. Additionally, S fertilizer needs to be applied near where the plant will take it up, to prevent leaching loss. A meta-analysis conducted by Borja Reis (2021) of 72 S fertilization trials from the midwestern and southern United States found that yield and seed protein content were positively impacted by S fertilization, but this was primarily when S was applied at planting (Borja Reis et al., 2021). However, yield and protein content rarely responded when S was top-dressed in vegetative or reproductive stages. A high variance from environmental factors may have limited the scope of these conclusions. Soils in Indiana, Michigan and Ohio are thought to supply adequate S for crop production, but in cases where S deficiency was expected, growers should apply 11-22 kg S ha^{-1} every year to supply sufficient S for crop growth (Culman et al., 2020).

Sulfur Cycle

The S cycle is similar to the N cycle, as it has gaseous and mineral components, highly mobile forms, and is primarily driven by biological processes (Biederbeck, 1978; Havlin, 2005; Trudinger, 1979). The main difference is that S in the soil originates as metal sulfide (S^{2-})

minerals from the earth's crust (Havlin, 2005). Metal S^{2-} is not plant available and must be oxidized and weathered into plant available forms. More than 90% of the total S in surface soils is in an organic form, and is a component of soil organic matter (Biederbeck, 1978).

There are three major groups of organic S in the soil: HI-reducible S, carbon bonded S, and residual S (Havlin, 2005). HI-reducible S is most often the dominant form of soil organic carbon. Sulfur is also present in the atmosphere primarily as sulfate (SO_4^{2-}) in particles and gaseous SO_2 and H_2S (Junge, 1960). Atmospheric S, before industrialization, primarily came from oceanic or marshland release of SO_4^{2-} and S^{2-} (Biederbeck, 1978). Since industrialization, anthropogenic S emission has contributed to the majority of atmospheric S concentrations, through the burning of fossil fuels (Sievert et al., 2007).

Organic S is converted to plant available SO_4^{-2} through the process of mineralization, and converted back to organic S through immobilization (Trudinger, 1979). Soil micro-organisms digest organic matter, releasing SO_4^{-2}. Therefore, the plant's access to S is dependent on the SO_4^{-2} released from organic matter.

Biological Nitrogen Fixation

Sulfur fertilizer is known to have a direct impact on soybean nodule development and N fixation, especially in S deficient environments. Sulfur applications of 15% S gypsum ($CaSO_4$) applied before planting in soybean fields in Pakistan increased the amount of N fixed and the total N uptake from the soil (Hussain et al., 2011). In plots treated with 30 kg S ha^{-1}, the amount of N fixed increased by 60 kg N ha^{-1} and N uptake increased by 91 kg N ha^{-1} compared to untreated controls. This study saw this response in a sandy loam field with low (0.39%) organic carbon. This study also reported increased plant height, number of pods per plant, and overall dry matter yield when comparing S treatments to untreated control.

A meta-analysis from 2019 analyzed 92 studies for biological N fixation response to fertilizers (Santachiara et al., 2019). Four studies reported S fertilization had increased biological N fixation in soybean by about 6% relative to the untreated controls. Two studies reported this result at a rate <20 kg S ha^{-1}, and two studies at >20 kg S ha^{-1}, therefore no difference between S rates was observed. Increases in biological N fixation reported were likely secondary effects wherein increased enzyme synthesis allowed for increased N metabolism. In general, the instance of positive response was higher in other primary nutrient fertilizers analyzed. For example, 18 studies reported positive increases in N fixation from P fertilization, while only 4 studies reported positive increases from S fertilizer. Additionally, the amount of N derived from the atmosphere was typically 3 times higher in positive P response cases as opposed to S response cases.

Sulfur's Role in Soybean

Sulfur is a component in the plant-essential amino acids cystine, cysteine and methionine (Havlin, 2005). Sulfur is also a component of ferredoxin, a protein required for the synthesis of chloroplasts and N assimilation by N fixing bacteria. Sulfur deficiency can have a direct impact on protein composition, and can reduce nutritional value through lowered levels of cysteine or the concentration of the protein glycinin (Hitsuda et al., 2008a). Sufficient S levels are 0.21-0.40% S in the first trifoliate at early flowering (Dick et al., 2015). Sulfur levels between 0.15-0.20% are low, and deficient S levels are less than 0.15%.

Sulfur Forms of Uptake, Translocation, Plant Processes

Sulfur is absorbed in the roots primarily as sulfate (SO_4^{-2}), but thiosulfate ($S_2O_3^{-2}$) can also be absorbed (Havlin, 2005; Schoenau & Malhi, 2015). SO_4^{-2} forms of fertilizer were immediately available for plant uptake, while elemental S and S^{2-} must to be oxidized to SO_4^{2-} first (Schoenau & Malhi, 2015). Thiosulfate ($S_2O_3^{-2}$) fertilizers can be applied in combination with N fertilizers to supply additional N. Thiosulfate ($S_2O_3^{-2}$) is compatible with aqua ammonia, ammonium nitrate (NH_4NO_3) and urea (CH_4N_2O) (Dick et al., 2015). Schoenua and Malhi (2008) found that oxidation of $S_2O_3^{-2}$ was rapid compared to elemental S (Schoenau & Malhi, 2015). In general, 56-70% of $S_2O_3^{-2}$ was recovered as SO_4^{-2} after 25 days of incubation.

Sulfate (SO_4^{-2}) uptake can be inhibited by chromate (CrO_4^{2-}, CrO_7^{2-}) and selenate (SeO_4^{2}) anions (Havlin, 2005). High clay content and increasing soil organic matter have the potential of increasing SO_4^{-2} absorption, while high pH and other competing anions limit absorption. Sulfur dioxide (SO_2) is absorbed through plant leaves but can be toxic if absorbed in high quantities (Havlin, 2005).

Sulfur is transported to the above-ground portions of the plant through the xylem (Naeve & Shibles, 2005). Once absorbed, S is translocated through the phloem. During vegetative development, newly expanding leaves are the strongest driver in the transport and assimilation of S. Most S that enters mature leaves is rapidly transported to developing tissues and other sinks throughout the plant (Smith & Lang, 1988). Greater than 90% of the recently taken up SO_4^{-2} in a soybean plant is transported to the newest developing 1-2 trifoliate leaves. As the plant entered reproductive development, the leaves mobilizes S supplies and transports S to the developing pod and seed (Naeve & Shibles, 2005). Uptake of S to the leaves, stems and petioles peaked and began to decrease at R5, while S uptake to the developing grain rapidly increased (Bender et al., 2015). Bender (2015) found that S concentrations in the leaf tissues declined during seed fill, and about 20% of the seed's S needs were met by S remobilized from the leaf tissues. Pods and seeds were dependent on this S mobilized from other plant tissues.

Interaction with Nitrogen and Other Nutrients

Balance of Amounts in the Plant. Sulfur in the plant was closely linked with N. Sulfur is a component in cysteine and methionine, essential amino acids required for enzyme synthesis and particularly important in the synthesis of nitrate reductase and nitrogenase (Santachiara et al., 2019). Sulfur deficiency is primarily only notable when N is sufficient (Dick et al., 2015). For this reason, the N to S ratio (N:S) in plant tissues is considered a viable way of assessing S sufficiency (Franzen, 2008). With this method, a soil with a high N:S ratio can be considered more prone to S deficiency. Sulfur deficient plants accumulate non-protein N and decrease the N:S ratio (Havlin, 2005). Sulfur deficiency can inhibit NO_3^- reduction and therefore reduce the amount of N in plants in the form of proteins. NH_4^+ has also been shown to increase uptake of S, specifically in the form SO_4^{2-} (Duke & Reisenauer, 1986).

Ph. The Tri-State fertilizer recommend that soil pH needs to be between 6.0-6.8 pH for field crop production (Culman et al., 2020). This is to ensure in order to supply sufficient nutrients to the crop. In mineral soils with subsoil pH of > 6, the Tri-State fertilizer recommended a pH of 6.0 in surface soils for soybean. In mineral soils with subsoil pH of < 6, a surface soil pH of 6.5 is recommended. In organic soils, a pH of 5.3 is recommended for optimum soybean production. When the pH falls 0.2-0.3 pH units below the recommended levels, lime should be applied to correct pH levels. Liming rates to adjust pH vary based on soil factors as well as the liming material used. Highly acidic soils reduce the availability of key plant nutrients and limited plant growth because of deficiency symptoms (Havlin, 2005). The macro-nutrients N, P, K, as well as micronutrients such as Ca, Mg, and Mo become deficient in pH environments < 5.0. Aluminum (Al) and manganese (Mn) toxicity occur at pH < 5.5. Toxicity causes inhibition of root growth and development. Nutrient toxicity as a result of acidity induces nutrient deficiency and negatively impacts soybean growth (Ritchie, 1989).

Calcium and Mg deficiencies are most often a function of increased soluble Al in acid soils, and decreased nutrient uptake (Havlin, 2005). Increased Al levels in soybean grown in solution resulted in significantly less Ca, Mg, P, and Mn uptake (Noble & Sumner, 1988). This is because Al^{3+} replaced Ca^{2+} on the root cell exchange sites. Calcium and Mg^{2+} cations can also be lost through leaching due to the natural acidification of soils (Bache, 1978; Haynes & Swift, 1986).

Soil pH can affect certain strains of rhizobium species, including *Bradyrhizobium japonicum.* High pH reduces rhizobia activity and caused N deficiency in soybean (Coventry & Evans, 1989; Ritchie, 1989). Nodulation on soybean is specifically reduced at pH <5.9 (Bordeleau & Prevost, 1994). This is due in part because acidic soils have lower levels of P, Ca and Mo, which reduce nodule activity and N fixation. Excessive levels of Al and Mn in acidic soils are also toxic to rhizobia. Additionally, phosphate (PO_4^{3-}) immobilization in acid soils results in limited nodulation and rhizobia populations (Coventry & Evans, 1989).

Remaining Macro-Micronutrients. Phosphorus (P) is essential in energy production and transfer. It is a key component in ADP and ATP (Havlin, 2005). Crops store P as PO_4^{3-} compounds for energy use in vegetative and reproductive processes. Phosphorus is often applied in combination with N as either diammonium phosphate [$(NH_4)_2HPO_4$] or monoammonium phosphate [$(NH_4)H_2PO_4$] in the form P_2O_5. Phosphorus is one of the most important nutrients for early plant growth, especially in no-till conditions (Culman et al., 2020). Tri-state fertilizer recommendations for PO_4^{3-} application are based on soil test level and yield potential (Culman et al., 2020; Vitosh et al., 2000). Potassium (K) is absorbed by the plant root as K^+. Potassium regulates water pressure, osmotic pressure, and transport of photosynthates (Havlin, 2005). Potassium is applied as Potash in the form K_2O. Rates and recommendations vary based on local test level of yield potential (Culman et al., 2020; Vitosh et al., 2000).

The other micronutrients known to be essential for plant growth included calcium (Ca), iron (Fe), magnesium (Mg), boron (B), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo), nickel (Ni), and chloride (Cl) (Havlin, 2005). However, the tri-state fertilizer recommendations note that because these nutrients are required in such small supply, very few ever need to be considered for crop growth, as most soils in the Midwest adequately supply these nutrients (Culman et al., 2020).

The tri-state recommendations note that Mn, Zn and Mo are the micronutrients most often needed in soybean production (Culman et al., 2020; Vitosh et al., 2000). Manganese is absorbed as Mn^{2+} and is essential for photosynthesis, enzyme activity and root growth (Havlin, 2005). Manganese will most often became deficient in peat or muck soils with pH <5.3, or in black sand or lakebed soils with pH > 6.2 (Culman et al., 2020). Manganese can be applied to soybean using a foliar spray broadcast application (Shuman, 1998). Zinc is absorbed by the plant root as Zn^{2+} and is important in many enzymatic activities as well as chlorophyll synthesis and cell membrane activity (Havlin, 2005). Zinc is typically applied as inorganic metallic fertilizers. Zinc is most often deficient in peat, muck and mineral soils with pH > 6.5 (Culman et al., 2020). Molybdenum is absorbed as molybdate (MoO_4^{-2}) and is a component of nitrate reductase and nitrogenase, making it especially important in N fixation and metabolism (Havlin, 2005).

Molybdenum is most often deficient for soybean in acidic prairie soils (Culman et al., 2020). Most Mo deficiency is ameliorated with liming of soils of proper soil pH range.

1.2 Sulfur Fertility

1.2.1 Sulfur's Role in The Plant

Sulfur (S) is a macronutrient required for plant growth. Sulfur is required for the synthesis of amino acids and essential plant proteins (Havlin, 2005). Sulfur is also a key component in the chlorophyll molecule. Sulfate (SO_4^{2-}) within the plant is first "activated" where it is reduced, then incorporated into cysteine (Thompson et al., 1986). The activated forms of SO_4^{2-} are adenosine-5'-phosphate (APS) and 3'-phosphoadenosin-5'-phosphosulfate (PAPS). Once activated, S is transferred into essential compounds such as methionine. These compounds are then incorporated into the essential proteins required for regular plant function. Plants experiencing S deficiency have decreased S-containing amino acid content and inhibited synthesis of essential proteins (Dick et al., 2015). A corn and soybean rotation in the Midwest remove as much as 22.4 kg S ha^{-1} (20 lbs. of S ac^{-1}) from the soil over two growing seasons, with as much as 12.3 kg S ha^{-1} (11 lbs. S ac^{-1}) removed from soybean production with average yields around 4025 kg ha^{-1} (60 bu ac^{-1}) (Culman et al., 2020).

1.2.2 Clean Air and Water Act: Regulations of Sulfur in The United States

The Clean Air and Water Act of 1990 was signed into effect on November 15, 1990, by President George. W Bush. The legislation was designed to implement new requirements to control air pollution, acid rain, and depletion of the ozone layer (Waxman, 1991). This legislation included the registering of 189 chemicals listed as hazardous air pollutants, as well as the specific level of emissions permitted at every power plant in the nation. It also regulated emission standards for vehicles. The legislation called for tailpipe emission reductions in order to curb ozone layer depletion.

1.2.3 Sulfur Deposition in The United States

Sulfur dioxide (SO_2) is an air pollutant largely deposited in the air by power plants burning fossil fuels (US EPA, 2016). The regulation of power plant emissions detailed by the Clean Air and Water Act of 1990 resulted in 80% decreased S emissions, greatly decreasing S deposition to the soil (Elkin et al., 2016). The EPA has estimated a 91% decrease in national average parts per billion SO_2 in the atmosphere since 1980 (US EPA, 2016). In 2001, soils in Indiana received more than 14.6 kg ha^{-1} (13 lbs. S ac^{-1}) from the atmosphere, compared to less than 11.2 kg ha^{-1} (10 lbs. ac^{-1}) since 2015 (Camberato & Casteel, 2017). In January 2021, the EPA reported total S deposition in the Midwest to be as low as 3.4 kg ha^{-1} (3 lbs. ac^{-1}) (US EPA, 2021).

1.2.4 Sulfur Deficiency in Crops

A majority of the soil S supply is from organic matter mineralization and atmospheric deposition (Gaspar et al., 2018). Sulfur deposition has decreased markedly over the last 10-20 years, and crop yield continues to increase. An estimated 18-50% more S is removed from the soil by crops each season year, compared to past removal rates (Kost et al., 2008). Sulfur deficient crops have yellowed leaves, similar to nitrogen deficiency (Havlin, 2005). However, since S is not mobile, and deficiency is more severe in upper, newer leaves. Sulfur deficient crops also have had reduced growth rates and chlorotic tissue.

Corn

Sulfur deficient corn has similar diagnostic traits to N deficiency, but unlike N deficient corn, deficiency symptoms are more severe in the upper leaves (Camberato et al., 2012). Sulfur deficiency in corn also causes leaf striping deficiency symptoms. Sulfur deficiency in corn can be stunting and yield limiting (Havlin, 2005; Sawyer & Barker, 2002; Stecker et al., 1995).

Wheat

Sulfur deficient wheat is stunted and smaller, especially in earlier growth stages, with lighter color leaf, light green stripping, and chlorosis along the leaf vein (Haneklaus et al., 2008). Sulfur deficiency in wheat and other cereals can be easily misdiagnosed as N deficiency (Camberato & Casteel, 2010). Sulfur deficient wheat has reduced flowers per head, and subsequently less kernels per head, reducing yield (Haneklaus et al., 2008).

Soybean

Sulfur deficient soybean are smaller in size, with yellowed leaves (Hitsuda et al., 2008a). The leaves in the higher position of the soybean canopy slowly turn yellow together once S deficiency occurs. Sulfur deficient leaves eventually developed brown spots. Leaf yellowing is caused by decreased chlorophyll and protein levels due to the reduction in S-containing protein synthesis. Eventually, if deficiency is severe enough, the plant will stop producing new leaves and branches. If the plant is able to reach physiological maturity, it will have lower yields than S sufficient counterparts. Sulfur deficiency in reproductive stages of soybeans also leads to brown spots developing on pods.

1.2.5 Field Conditions That Are Conducive to Sulfur Deficiency

Soil Tests

Laboratory soil testing for S has been inconsistent when reporting extractable S in the soil, most likely due to the variability in analytical methods (Crosland et al., 2001). Different methods have produced different results, even if testing the same soil. Most methods measure the amount of S available for uptake using an extraction of soil with a weak salt solution (Fox et al., 1964). Various soil tests for S have been studied, including but not limited to heat-soluble procedures, KH_2PO_4 extraction, or water extraction. Hitsuda (2008) suggested that the Morgan reagent, $Ca(H_2PO_4)_2$, and KH_2PO_4 could potentially be suitable for determining available S content because of their correlation with relative yield, as well as the S uptake in control plants (Hitsuda et al., 2008a). However, S soil tests have been very laborious, and often cannot be utilized during the growing season. Overall, lab tests lack consideration of soil texture and organic matter in recommendations, and do not account for leaching post-testing, so confidence in lab soil tests alone remained low (Franzen, 2008). Furthermore, the Tri-State fertilizer recommendations (2020) note that there are no accurate soil test for S at this time, and that plant analysis is currently the best way to diagnosis S deficiency (Culman et al., 2020).

Soil Type

Certain soil characteristics are predictors for S deficiency, and certain soils are more prone to deficiency than others. Kost (2008) described S deficient soils as low in organic matter, with coarse texture, and subject to leaching (Chen et al., 2005; Kost et al., 2008). Soils low in organic matter release less S during mineralization, which contributed to S deficiency (Franzen, 2008). Texture, clay content, mineralogy, and pH of the soil all have a direct impact on S leaching (Tisdale, 1986). Sandier soils are susceptible to S deficiency as they tend to be low in organic matter and more susceptible to leaching (Franzen, 2008). However, increased clay content decreases leaching potential (Tisdale, 1986). Soils with more organic matter, as well as greater amounts of S deposition typically did not respond to S treatments (Chen et al., 2005). However, increased organic matter can result in increased S volatilization (Havlin, 2005). Overall, lower soil organic matter, contributing to less mineralization, and other factors like soil type, have the biggest effect on whether or not a soil will become deficient (Kost et al., 2008).

Soil Moisture

Precipitation and soil moisture conditions directly impact S availability. Sulfur is taken to the plant root through diffusion or mass flow, and both processes are inhibited by insufficient soil moisture (Dick et al., 2015; Kost et al., 2008). Biological breakdown of S through mineralization is also inhibited by deficient or excess water (Dick et al., 2015). Oxidation of S sources by microbes depend on soil moisture and is optimized near field capacity. Plant available S changes into unavailable pyrite in waterlogged soils. Along with this, S leaching was more common in saturated soils (Tisdale, 1986).

Soil Temperature

Soil temperature directly impacts S mineralization from organic matter, and therefore affects the amount of S in the soil (Kaur et al., 2018; Kost et al., 2008; Tisdale, 1986). Sulfur mineralization in soil occurs rapidly at soil temperatures between 20-30°C, but is reduced at temperatures below 10°C (Tisdale, 1986). Sulfur immobilization is greater than mineralization in colder soils (Castellano & Dick, 1991). Soil temperature also influences microbial activity, therefore affecting S oxidation (Dick et al., 2015). Sulfur oxidation rates increase with soil temperature, and is optimized between 25-40°C, yet limited at temperatures >60°C (Havlin, 2005).

Residue or Cover Crop

The amount of S available in the soil is heavily dependent on the organic matter content (Dick et al., 2015). Nitrogen mineralization is the process through which organic matter and previous crop residue is broken down, converting N into plant available forms (Eriksen, 2008). This process releases plant available S. For this reason, mineralization is an important source of plant available S. Sulfur mineralization consists of both biological and biochemical mineralization. Biological mineralization is driven by microbial oxidation of carbon-to-carbon dioxide, releasing S as a bi-product. Biochemical mineralization consists of the release of SO_4^{2-} from the sulfate-ester pool through the process of enzymatic hydrolysis. Incorporation of previous crop residue into the soil by tillage rapidly increases mineralization (Scherer, 2009).

Kaur (2018) calculated the total S content of different crop residues using an incubated sample of residue, digested with nitric acid (Kaur et al., 2018). They found that corn residue contained of 0.83 g S kg^{-1}, soybean residue contained 0.40 g S kg^{-1} and spring wheat contained 1.30 g S kg^{-1}. Sulfur mineralization from residue depends on the C/S ratio. Sulfur is mineralized at a C/S ratio < 200:1 and immobilized at a C/S ratio > 400:1. A C/S ratio between 200:1-400:1 results in no change in S concentrations or a small net increase or decrease (Dick et al., 2015). Heavy residue result in decreased mineralization by creating a high C/S ratio (Kaur et al., 2018).

Management Practices

To achieve high yields for soybean, large amounts of N, P and K are needed, with small amounts of S (McGrath et al., 2013). The 2000 tri-state fertilizer recommendations noted that sulfur sufficiency in soybean is 0.21-0.40% S in the topmost matured leaf prior to flowering (Vitosh et al., 2000). In-season soil and foliar applications in soybean are able to treat S deficiency (Camberato & Casteel, 2017).

1.2.6 Sulfur Fertilizer Forms and Availability

Elemental S (S^0) is often blended with N or P fertilizers, and must be applied and incorporated 2-5 months before planting in order to be oxidized to plant available SO_4^{-2} (Culman et al., 2020; Havlin, 2005). Due to the slow oxidation rate, elemental S may be insufficient for supplying crop S needs in a single season (Culman et al., 2020). Soybean response to elemental S is dependent on local nutritional needs and soil factors. Elemental S applied in rates ranging from 11.2-44.8 kg S ha^{-1} (10-40 lbs. S ac^{-1}) resulted in no significant difference in yield when compared to the control in Iowa soybean fields, but all rates resulted in a 0.2% improvement of leaf S concentrations (Sawyer & Barker, 2002). Elemental S applied with ammonium sulfate ($(NH_4)_2SO_4$) at various rates significantly increased yield when soil conditions were deficient, however elemental S oxidation was not significant enough for elemental S to directly contribute to increased yields (Casteel et al., 2019). Elemental S has also been useful in lowering pH of alkaline soils (Dick et al., 2015).

Ammonium sulfate (AMS, $(NH_4)_2SO_4$) is both a source of N and S and is applied shortly before planting (Havlin, 2005). Other blends include ammonium phosphate sulfate, and potassium sulfate (K_2SO_4). Granular AMS applied at various rates improved yield significantly more than other S treatments (Casteel et al., 2019). However, other studies have reported that AMS broadcasted at various rates and incorporated in the soil resulted in no significant difference in soybean yield (Sweeney & Granade, 1993).

Gypsum ($CaSO_4$) as a S source can be applied directly to the soil and is immediately available (Havlin, 2005). It is used as a soil health promoter, to help ameliorate sodic and acid subsoils, and has also been historically used as a S fertilizer (Casteel et al., 2019; Dick et al., 2015). Gypsum applications in a greenhouse improved soybean yield, but likely exclusively through the addition of exchangeable Ca (Fageria et al., 2014).

Thiosulfate (S_2O_3) can be blended with other fertilizers, but most commonly N, forming ammonium thiosulfate ($(NH_4)_2S_2O_3$) (Dick et al., 2015). It can be broadcast preplant, applied as a fluid starter, applied in irrigation water, or top-dressed at low rates. When applied to the soil, thiosulfate rapidly oxidizes to form tetrathionate, which quickly oxidizes to plant available sulfate (Goos & Johnson, 2001). Thiosulfate has also been blended with K or Ca, forming potassium thiosulfate (KTS, $K_2S_2O_3$) and calcium thiosulfate (CaO_3S_2) respectively (Dick et al., 2015; Havlin, 2005). KTS specifically has the added benefit of addressing K and S deficiencies at the same time (Quinn & Steinke, 2019).Goos and Johnson (2001) tested the oxidation rate of ammonium thiosulfate and potassium thiosulfate in loam and sandy loam soils at various soil temperatures, finding that the oxidation rate of the two were nearly identical at all soil temperatures (Goos & Johnson, 2001). They found that at soil temperatures of 25°C, oxidation was very rapid in all soils, completing within 1-2 weeks of application and with no apparent immobilization of S. At soil temperatures of 15°C, thiosulfate oxidation was slowed, but was still complete by 2-3 weeks in all three soils with some apparent immobilization of S. Lastly, at 5°C soil temperature, thiosulfate varied greatly between the soils tested, with some being totally oxidized by 2-6 weeks, while others had S immobilized for 12 weeks. ATS has inhibited germination and damaged plant roots, therefore it is generally not recommended for in-furrow placement (Camberato, 2019). The S in thiosulfate is considered mobile and can be lost to leaching.

Polysulfide blends often contain N or K, forming ammonium polysulfide (NH_4S_X) and potassium polysulfide (K_2S_X) (Havlin, 2005). Polysulfide cannot be blended with P containing fertilizers. Polysulfide has been used to acidify high pH soils.

1.2.7 Methods of Applying Sulfur Fertilizer

Prior To Growing Season

Gypsum ($CaSO_4$) has been historically used as a preplant sulfur fertilizer (Casteel et al., 2019; Havlin, 2005; Sawyer & Barker, 2002). In Iowa in 2008 and 2011-2013, gypsum affected soybean yield inconsistently (Sawyer, 2015). In two of thirteen sites in 2011-2013, preplant gypsum increased soybean yield, but in 2008 gypsum did not affect yield at either site.

In-Season

Foliar applications of elemental S at early vegetative stages or initial flowering stages increased yield (Hitsuda et al., 2008a). Spray elemental S was not absorbed through the leaf tissues, but instead fell to the soil and oxidized much faster than a course elemental S applied directly to the soil. Coarse elemental S has been useful as a slow-release fertilizer, releasing S to be available to the plant overtime, as the elemental S was oxidized (Eriksen, 2008). However, this process is dependent on temperature, moisture, and particle size. Foliar applications during seed fill have had mixed results, where some applications increased yield, but others caused foliar damage (Hitsuda et al., 2008a). Given the slow-release of elemental S, more soluble forms of S like ammonium thiosulfate ($(NH_4)_2S_2O_3$) are considered to be more applicable when applied in-season (Franzen, 2008).

Proactive Vs Reactive or Corrective

Proactive avoidance of S deficiency through inclusion of S in fertility programs is often more cost effective than reactive or corrective applications of S (Camberato & Casteel, 2017). If reactive S applications are needed, S should be applied in a liquid, readily available form, close to the plant (Camberato & Casteel, 2017; Hitsuda et al., 2008a; Kaiser & Kim, 2013).

1.2.8 Tissue Sampling for Sulfur

Proper Leaf Tissue to Sample

Since S deficiency looks similar to other nutrient deficiencies, tissue testing is necessary to confirm S deficiency (Franzen, 2008; Hitsuda et al., 2008a). However, tissue samples have rarely been useful in correcting S deficiency in that season, due to the timing and prep needed in processing samples. Leaf tissue from the third set of trifoliate leaf from the top of the plant, taken during flowering, is considered to be a prime sample for tissue S testing (Camberato & Casteel, 2017; Hitsuda et al., 2008a; Kaiser & Kim, 2013).

Sulfur Concentration as The Plant Grows, Develops, Reproduces

In general S concentration through the plant is driven by expanding new leaves (Hitsuda et al., 2008a). For this reason, newer, higher positioned leaves in the canopy have higher S concentrations than older, lower positioned leaves (Hitsuda et al., 2008a). Sulfur assimilation throughout the soybean plant profile is dependent on whole-plant dry weight growth (Naeve & Shibles, 2005). Sulfur is transported through the xylem, and large amounts are stored in expanding leaves. However, leaf tissue is unable to utilize stored SO_4^{2-}, so S in mature leaves is exported through the phloem into newer leaves. Naeve and Shibles (2005) found that 25% of S going to leaves came from S uptake in the roots, while the remaining majority was imported from older leaves. As the plant progressed into later reproductive stages, S was exported out of the leaves and into the developing pods and seeds (Hitsuda et al., 2008a). Pods and seeds were dependent on mobilized S from the leaves (Naeve & Shibles, 2005). Sulfate in the pod continued to accumulate until the pod reached 50% of its full length, at which point levels declined and SO_4^{2-} was incorporated into the seed as grain proteins (Sunarpi & Anderson, 1997).

1.2.9 Sulfur Accumulation Throughout the Growing Season

The total amount of S uptake in the plant is dependent on the S concentration in the soil and the plant dry weight (Hitsuda et al., 2005). All crops achieved optimum growth at 2.0 mg S L^{-1} in nutrient solution. Soybean has a relatively small amount of required S concentration for optimum growth compared to other crops. A synthesis analysis of soybean trials in Argentina and Kansas from 2009-2019 found that when yield averaged 3824 kg ha^{-1}, the total S uptake in the field was 15.6 kg S ha^{-1} (Salvagiotti et al., 2021). Across the wide environmental conditions, the average S concentration ranged from 1.26-5.47 g S kg^{-1} in the grain taken at harvest, and 0.40-2.67 g S kg^{-1} in vegetative tissues sampled at R7.

1.3 Starter Fertilization

1.3.1 Methodology of Starter Fertilizer Applications

Starter fertilizer is a method of applying a plant available source of nutrients at planting within the plant's initial root zone, making that nutrient available to the seedling (Kaiser & Kim, 2013; Touchton & Rickerl, 1986). Small amounts of fertilizer are typically placed in a concentrated area in a close proximity to the seed (Kaiser & Kim, 2013). Fertilizer can be placed over the seed, in the furrow with the seed, below the seed, to the side of the seed or both to the side and below the seed. Fertilizer must be placed at a low rate in order to reduce the risk of salt injury to the germinating seedling (Hergert et al., 2012; Mortvedt, 2001). Insufficient nutrient levels, cold or wet soils, high soil residue, root restricting barriers or slow mineralization are all reasons to consider applying starter fertilizer (Culman et al., 2020; Touchton & Rickerl, 1986).

In general, applying some or all of the fertilizer needed during the season at planting improves fertilizer efficiency (Culman et al., 2020). Applying starter fertilizer can also save fertilizer cost compared to broadcast fertilizer, and is an accessible way to apply custom blends of liquid fertilizers with multiple nutrients (Hergert et al., 2012; Kaiser & Kim, 2013). A common method of starter fertilizer placement is a two-by-two band of fertilizer. The fertilizer is placed two inches (5 cm) to the side of the seed, and two inches (5 cm) below it (Vitosh et al., 2000). Band placement to the side and below the seed are usually better than other placements, and reduce the chance of seedling injury (Culman et al., 2020). Starter fertilizer applications have also been an efficient method of applying micronutrients (Tanner & Hume, 1978). Micronutrients applied at planting in a band are generally taken up better and last longer into the season than foliar applied micronutrients (Culman et al., 2020).

Dry Versus Liquid

When applying a dry starter fertilizer, starter fertilizer should be applied as close to the time of planting as possible (Hergert et al., 2012). Some dry starter products have been incorporated into the soil through a surface disk or plow tillage. Touchton and Rickerl (1986) improved soybean yield using dry P and K starter fertilizer applied at rates of 19 and 44 kg ha^{-1} respectively (Touchton & Rickerl, 1986). Liquid starter fertilizers require specialized equipment, but can be used to easily create custom blends of multiple nutrients based on nutrition requirements (Kaiser & Kim, 2013). Kaiser and Kim (2013) saw mixed responses to liquid starter fertilizer combinations of N, P and S in Minnesota, only seeing yield increased in one site year. In contrast, Osborne and Riedell (2006) saw positive yield responses to liquid N starter fertilizer at 2 out of 3 site years in South Dakota (Osborne & Riedell, 2006).

1.3.2 Historic Usage in Soybean Production

Starter fertilizer applications on soybean has not been extensively considered until recently. In most cases, starter applications in soybean has not recommended, as the risk of seed burn or salt injury is too high (Hergert et al., 2012; Tanner & Hume, 1978). Soybean seedlings are very susceptible to salt damage, especially in drier conditions (Culman et al., 2020). Past considerations of starter fertilizer on soybean had produced limited and varied results. For example, Kamprath (1989) applied P, K and S as a starter fertilizer in North Carolina coastal plains at rates of 9 lbs. ac^{-1} P, 17 lbs. ac^{-1} K, and 20 lbs. ac^{-1} S using concentrated superphosphate, potassium chloride and calcium sulfate (Kamprath, 1989). This study found no response to fertilizer on plant nutrient concentrations or yield and concluded that soil levels of these nutrients were at sufficient plant levels. Borges and Mallarino (2000) applied starter P at rates of 14 and 28 kg ha^{-1} to no-till soybean fields in Iowa, but produced no yield benefit relative to a broadcast application (Borges & Mallarino, 2000). However, this same study found a slightly positive yield response to starter K applied at 33 and 66 kg ha^{-1} compared to a broadcast K application at the same rates. In another study, starter N fertilizer applied in South Dakota soybean fields to different tillage treatments, as either ammonium nitrate (NH_4NO_3) or urea, at rates of 8, 16, and 24 kg N ha^{-1} resulted in higher yields than plots that received no added N (Osborne & Riedell, 2006). As reduced tillage and no-till systems have become more common place, starter fertilization has become more popular (Hergert et al., 2012; Kaiser & Kim, 2013).

1.3.3 Yield Responses in Soybean

Osborne and Riedell (2006) reported that starter N fertilizers applied in South Dakota to different tillage treatments had a positive impact on early plant biomass production, N uptake, and plant vigor compared to plants that received no addition N in two out of three site years (Osborne & Riedell, 2006). Soybean yield in Alabama increased when using combinations of K, P, N-P, and N-K fertilizers, applied at 16 kg N ha^{-1}, 19 kg P ha^{-1} and 44 kg K ha^{-1} (Touchton & Rickerl, 1986). This study reported that starter fertilizer combinations of P, K, N-K and P-K had the greatest yield improvement compared to an untreated control. They also observed that N, N and P, and NPK starter fertilizers increased root and shoot dry weight when measured 21 and 28 days after emergence, theorizing that additional nutrients, primarily N, may have stimulated early season root growth (Touchton & Rickerl, 1986). Reduced tillage systems often have had colder, wet soil conditions at planting, limiting N fixation and mineralization. For this reason, starter fertilizer has been more responsive in these systems (Touchton & Rickerl, 1986). Starter fertilizer has also served as a way to increase yield in a situation where the growing season is shorter, by compensating for reduced light interception from the loss of canopy closure (Hankinson et al., 2015).

However, there are negative effects associated with starter fertilizer. Most notable is salt damage, where stunting or plant death can occur when high concentrations of fertilizers are placed near the seed (Havlin, 2005; Hergert et al., 2012; Mortvedt, 2001). Previous studies have shown starter fertilizer has a negligible effect on nodulation, biomass, canopy closure and grain quality in soybean in certain conditions (Hankinson et al., 2015). As soil temperatures increased because of delayed planting, likelihood of potential starter fertilizer yield increases declined

(Touchton & Rickerl, 1986). Thus, in general, a timely planting will result in a more positive response on soybean yield than applications of starter fertilizer.

Research in starter fertilizer on soybean has been limited. Kaiser and Kim (2013) conducted a study of four different starter fertilizer combinations in Minnesota using urea-NH_4NO_3 solution, ammonium polyphosphate, and ammonium thiosulfate ($(NH_4)_2S_2O_3$). Fertilizers were applied at rates of 22 kg ha^{-1} N, 10 kg ha^{-1} P, and 28 kg ha^{-1} S at different combinations of N, N-P, N-S, and a combination of the three. Yield response was dependent on site specific soil qualities such as soil organic matter, and there was no discernable yield effect from N or P (Kaiser & Kim, 2013). Osborne and Riedell (2006) had responses to two different N starter fertilizers applied at 4 different N rates, 0, 8, 16, and 24 kg N ha^{-1}. At two of the three site years, soybean yield and biomass increased from the 0 rate with increased N rate (Osborne & Riedell, 2006). Another study in Ohio applied 5 starter treatments of none, urea, applied at 30 lbs. N ac^{-1}, TSP (Triple Superphosphate) applied at 40 lbs. P_2O_5 ac^{-1}, TSP+ Urea, and DAP applied at 40 lbs. P_2O_5 ac^{-1}. This study did not find a starter fertilizer effect on yield at any site year (Hankinson et al., 2015).

1.3.4 Equipment Requirements and Placement Responses

Starter fertilizer traditionally has required specialized equipment to apply fertilizer at planting. Historically, it has been uncommon in soybean, due to the lack of soybean drills with starter fertilizer equipment (Hankinson et al., 2015). Metered equipment is necessary to ensure applications are at the correct rate and do not induce salt damage (Hergert et al., 2012). For this reason, thirty-inch row spacing are commonly required when using a starter system (Vitosh et al., 2000). Previous literature investigating the efficiency of fluid starter fertilizer placement configurations was lacking considerably, and further research is necessary to determine if starter placement is having an added effect on plant morphology.

1.3.5 Starter Sulfur Fertilizer Responses

Only one previous study considering fluid starter S fertilizer on soybean is known at this time. In this study, Kaiser, and Kim (2013) applied fluid starter S fertilizer at four different site years in Minnesota (Kaiser & Kim, 2013). The study was a field scale randomized complete block design, comparing a combination of fluid starter fertilizer response to an untreated control and a broadcast S treatment. The fluid starter fertilizer treatment combinations were N, N+P, N+S, and N+P+S. They found that S treatments increased early S concentrations and grain protein concentrations, while decreasing oil concentrations. Soybean yield responded to S at only one of the site years, but according to the researcher this was likely because “organic matter concentration was < 20 g kg^{-1}”. They further concluded that “extractable SO_4-S in the soil was negatively correlated to the yield response” observed at the one site year. At this time, there is no other known studies investigating the effect of fluid starter S fertilizer on soybean.

Kaiser and Kim did a similar study on corn in 2009 and 2009. In this study, with the same design as the previous study, treatments of a control, broadcast S (28 kg S ha^{-1}), and four fluid starter fertilizer combinations were compared (Kim et al., 2013). The fluid starter fertilizer combinations were N, N+P, N+S, and N+P+S. The rates of those fertilizers were 22 kg N ha^{-1}, 10 kg P ha^{-1}, and 28 kg S ha^{-1}. Starter S increased corn yield at two sites years, although this yield was not related to S soil tests and the effect decreased as soil organic matter increased. They found that the yield response was greatest when soil organic matter in the top 15 cm of soil was less than 20-40 g kg^{-1}, and yield plateaued from S treatments at soil organic matter > 40 g

kg^{-1}. They concluded that starter S fertilizer was “taken up by grain in greater quantities than needed for increasing or maintaining corn yield” and that “if enough S is available following mineralization from SOM, applied fertilizer S will not benefit yield.” (Kim et al., 2013). Other studies have seen similar responses to starter S fertilizer in corn, including a study from 2005 wherein 13.4 kg S ha^{-1} was applied as a starter fertilizer positively influenced yield when soil organic matter content was < 20 g ha^{-1} (Rehm & Clapp, 2008). This researcher concluded that S applied as a starter is “highly effective, and rates can be reduced compared to a broadcast application”.

CHAPTER 2: YIELD AND QUALITY RESPONSE OF SOYBEAN (*GLYCINE MAX* (L.) MERR.) TO STARTER SULFUR FERTILIZER, SOURCE, RATE AND PLACEMENT

2.1 Abstract

Over the past 10 years sulfur (S) needs of soybean (*Glycine max* (L.) Merr.) has increased as yield has increased with improved genetics and management, while atmospheric S deposition has decreased. Soybean growers have invested into agronomic practices to maximize production and alleviate potential S shortfalls, which includes the use of starter fertilizers. For this reason, the objectives of this study were to determine optimum source, rate, and placement of fluid starter S fertilizers for soybean yield and quality. A split-plot design of S source-rate and placement was used in the 2019 and 2020 growing seasons at West Lafayette and Wanatah, Indiana. Three starter S fertilizers were used: ammonium thiosulfate (ATS, 12-0-0-26S, Hydrite Chemical), potassium thiosulfate (KTS, 0-0-25-17S, Hydrite Chemical), and K-Fuse (derived from potassium acetate, ammonium thiosulfate and urea, 6-0-12-12S, NACHURS). These products were applied at four S rates: 5.6, 11.2, 16.8, and 22.4 kg S ha^{-1} to determine optimal S rate and in two placements (single: 0 x 5 cm x 1; dual: 0 x 5 cm x 2).

Placement had minimal impact, except early season plant stands were reduced with dual placement with all three fertilizer sources in West Lafayette 2019 (late planting). Leaf concentrations of S, nitrogen (N), and potassium (K) were above critical levels and were not influenced by starter fertilizer that supplied these nutrients. Some micronutrient concentrations changed by source-rate combinations in different years, with increases in manganese (Mn) from ATS in 2019 and 2020 and Wanatah. In West Lafayette 2020 (timely planting), all three starter sulfur fertilizers increased yield and protein even though broadcast AMS did not improve yield

or protein. Yield plateau with ATS was 4138 kg ha^{-1} at a rate of 4.7 kg S ha^{-1}. Yield plateau with KTS was 4308 kg ha^{-1} at a rate of 7.5 kg S ha^{-1}. Yield plateau with K-Fuse was 4198 kg ha^{-1} at a rate of 11 kg S ha^{-1}. Yield and protein did not change with starter S fertilizer treatments in the remaining site-years, which was likely due to plantings that were later than recommended.

2.2 Introduction

Soybean production worldwide continues to increase with the development of improved cultivars. The USDA reported that in 2019 over 30 million ha (76 million ac) of soybeans were planted, with the production value around 31 billion US dollars (USDA-FAS, 2019). As production increases, more nutrients are removed from the field each season, and therefore, nutrients not traditionally managed can quickly become deficient for the crop, including sulfur (S) (Gaspar et al., 2018; Hitsuda et al., 2008a; Kaiser & Kim, 2013). Sulfur is a secondary nutrient required for plant growth and needed for the synthesis of amino acids. Sulfur is also required for the synthesis of chlorophyll and plays an essential part in the assimilation of nitrogen (N) by N-fixing bacteria (Havlin, 2005).

The Clean Air and Water Act of 1990 introduced regulation on air pollution, particularly that of power plant emissions (Waxman, 1991). After this legislation passed, notable downward trends in atmospheric S deposition occurred across the Midwest (Camberato & Casteel, 2017; Elkin et al., 2016; Kost et al., 2008). In some instances, S removal from crops has become equal to or greater than S deposition. In 2013, 26% of fields over 300 cropland fields sampled in Pennsylvania had soil SO_4-S levels below the agronomic optimum level (Elkin et al., 2016). In 2001, most soils in Indiana received more than 14 kg of S ha^{-1} (13 lbs ac^{-1}) from the atmosphere; however, since 2015 most S deposition has been less than 11 kg of S ha^{-1} (10 lbs ac^{-1}) (Camberato & Casteel, 2017). Additionally, annual SO_4 deposition in Ohio has decreased from

11.6 kg ha^{-1} to 7.3 kg ha^{-1} between 1979 and 2005 (Kost et al., 2008). Low soil organic matter, no-till management, early planting, and heavy residue are also possible causes for S deficiencies, which can be attributed to the reduction of mineralization (Chen et al., 2005; Kost et al., 2008; Tisdale, 1986).

Preventative and corrective S applications have been studied in the past. Studies in soybean have found that corrective applications of S should take place as soon as S deficiency symptoms (e.g., general yellowing of leaves), are observed (Camberato & Casteel, 2017). Foliar and soil applications of S have been shown to increase soybean yield in S deficient scenarios (Hitsuda et al., 2005).

Along with the previously mentioned S management practices, various methods of S applications have been explored, including a starter fertilizer application. Kaiser and Kim (2013) evaluated the effect of ammonium thiosulfate (ATS, 12-0-0-26S) in a fluid starter application on early soybean growth patterns, S uptake, grain yield, S removal, and protein in loamy fine sand, clay loam and silt loam strip plots in Minnesota in 2013 (Kaiser & Kim, 2013). Both ATS and broadcast K_2SO_4 (0-0-42-18S) increased soybean yield at one out of four locations where soil organic matter was < 20 g kg^{-1}. In response to S fertilizer at all four sites, there were increases of S plant concentrations, S uptake, S grain removal, and grain protein, while grain oil decreased. Since the optimum combination of source, rate, and placement of starter S fertilizer has yet to sufficiently explored in soybean, the objectives of this study were to determine these factors with starter S fertilizer in order to maximize yield and correct potential S deficiency.

2.3 Materials and Methods

2.3.1 Site Characterization

Field experiments were conducted in 2019 and 2020 at the Purdue Agronomy Center for Research and Education (ACRE, 40°28'18.8"N, 86°59'28.3"W) in West Lafayette, IN and the Pinney Purdue Agricultural Center (PPAC, 41°26'42.1"N, 86°55'48.5"W) in Wanatah, IN.

The West Lafayette trial in 2019 was conducted on a field containing several soils; 33% of the area was Chalmers silty clay loam, 0-2% slope (Fine-silty, mixed, superactive, mesic Typic Endoaquolls), 22% was Drummer silty clay loam, 0-2% slope (Fine-silty, mixed, superactive, mesic Typic Endoaquolls), and 45% was Raub-Brenton complex silt loam, 0 -1% slope (Fine-silty, mixed, superactive, mesic Aquic Argiudolls) (USDA-NRCS Official Soil Series Description). The West Lafayette trial in 2020 was on a Chalmers silty clay loam. The Wanatah trial in 2019 and 2020 was on a Sebewa loam, shaley sand substratum (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Argiaquolls) (USDA-NRCS Official Soil Series Description).

The previous crop was corn (*Zea mays* L.) in both locations and years. The 2019 trials were not tilled, whereas the 2020 trials were conventionally tilled. Thus, these fields were chisel plowed in fall of 2019 and field cultivated in the spring of 2020 prior to planting. Soil fertility levels before planting and fertilizer applications can be seen in Table 2-1.

2.3.2 Experimental Design

A split-plot design was used to determine the optimum source, rate, and placement of starter S fertilizer. The main plot factor was source-rate, and the sub-plot factor was placement, creating a 12 x 2 + 1 + 1 factorial (12 source-rate treatments x 2 placements + 1 standard + 1 untreated control, Table 2-2). Twelve source-rate treatments were designed from three sources applied at four S rates. Three fertilizer sources used were ammonium thiosulfate (ATS, $(NH_4)_2S_2O_3$, 12-0-0-26S, sourced from Hydrite Chemical), potassium thiosulfate (KTS, $K_2S_2O_3$, 0-0-25-17S, sourced from Hydrite Chemical), and K-Fuse (derived from potassium acetate, ammonium thiosulfate and urea, 6-0-12-12S, sourced from NACHURS). Starter fertilizer sources were applied at four S rates: 5.6, 11.2, 16.8, and 22.4 kg S ha^{-1} in single or dual starter stream placement. In a single placement (5x0x1), the band of fertilizer was applied 5 cm laterally from the seed on the soil surface and on one side of the seed. In a dual placement (5x0x2), the band of fertilizer was applied 5 cm laterally from the seed on the soil surface, and on both sides of the seed. The standard application was ammonium sulfate (AMS; 21-0-0-24S) broadcasted at 22.4 kg S ha^{-1}. Untreated control (UTC) was used as the zero rate for each of the starter fertilizer rates, with two UTC per replicate. Liquid application rates of the starter fertilizers were 15.8 to 63.0 L ha^{-1} for ATS, 35 to 139.9 L ha^{-1} for K-Fuse, and 21.6 to 86.9 L ha^{-1} for KTS (Table 2-2). Resulting additional nutrients in starter treatments can be seen in Table 2-2.

2.3.3 Field Design

The 26 treatments (12 source-rates x 2 placements + 1 standard + 1 untreated control, Table 2-2) were replicated five times. Individual plots were approximately 3-m wide (four 76-cm rows) by 9-m in length. This study utilized the Pioneer soybean variety P31A22X, which is a 3.1 maturity group, in both years and locations.

Due to a wet spring with excessive rain in the 2019 season, both the West Lafayette and Wanatah sites were planted later than ideal. The Wanatah trial was planted June 7, 2019, at 340,000 seeds ha^{-1} in 76-cm rows. The West Lafayette trial was planted June 12, 2019, at 370,000 seeds ha^{-1} in 76-cm rows. The increase in seed rate at West Lafayette was to account for the delayed planting. In 2020, the West Lafayette trial was planted May 13, and the Wanatah trial was planted on June 2. Both locations were planted at 340,000 seeds ha^{-1} in 76-cm rows. Row cleaners were used at planting in both years to remove surface residue and create direct soil to fertilizer contact.

A Kincaid Voltra was used to plant the seed and apply the starter fertilizers. Two starter systems allowed two starter fertilizers to be applied in a single planting pass. Each starter system contained the supply tank, which controlled the liquid fertilizer rate by John Blue 12V DC speed control that adjusted a Shurflow diaphragm pump. Starter fertilizer flow was equally distributed and monitored with Visagage II. Liquid starter fertilizer was surface applied at planting 5 cm to the side of the seed furrow. Between each replicate, an exchange plot was used to flush and prime the hoses and prepare the next fertilizer treatment. The exchange plots were the same length and width as the test plots. Switch-valve gauges were used at the individual row unit to switch between a single (5x0x1) or a dual (5x0x2) placement. The starter system and tanks were flushed with water before adding the next product.

2.3.4 Data Collection

Several in-season measurements were conducted to evaluate treatment effects (Table 2-3). Plant stands were taken near V2 to V3 (early season) and prior to harvest. Plants were counted within 1-m of the interior row in the front, middle, and back, for a total of three counts per plot.

Most recent mature leaf samples were collected from the uppermost, fully expanded trifoliate leaf (usually 3rd to 4th leaf from the terminal bud) at R3 (First Pod) to assess leaf nutrition. Leaf samples were collected from all four rates of the three starters in the single placement, AMS standard, and UTC to total 14 treatments. Leaf samples were only taken from two rates (5.6 and 16.8 kg S ha^{-1}) of the dual placement for all three starter sources to reduce sampling complexity. For this reason, the dual placement is not presented in the data analysis. Approximately 15 to 20 trifoliate samples were collected between rows two and three throughout the plot. Samples were dried at 60°C for 3-5 days, ground to a 0.5 mm powder, then sent to A&L Great Lakes Laboratories (3505 Conestoga Dr, Fort Wayne, IN 46808) to assess leaf nutrient concentrations. Plant heights from each plot were measured near R5 (First Seed). The average height of six plants were measured to the apical meristem of the main stem.

All plots were end-trimmed prior to harvest to remove border effects. The two center rows were harvested from each plot using a Kincaid 8-XP plot combine. Plot grain weight and grain moisture were collected at harvest, and a grain subsample from each plot was also collected. The West Lafayette trials were harvested on October 15, 2019, and October 7, 2020. The Wanatah trials were harvested on October 10th, 2019, and October 8th, 2020. Grain yield was adjusted to 13% moisture. Grain subsamples were used to determine protein and oil via NIR (Infratec 1229 grain analyzer in 2019 and Perten Model DA7350 NIR in 2020). Seed weight was determined by weighing two 100-seed subsamples from each plot and adjusting to 13% moisture.

2.3.5 Field Management and Trial Assessment

Dates for in-season field activity are in Table 2-3. In-season weed control, foliar manganese (Mn), and insecticide applications were needed throughout the growing seasons in both years. In 2019, the West Lafayette site received a spray application of 0.91 L ha^{-1} diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.58 L ha^{-1} fluazifop-P-butyl, 2.19 L ha^{-1} glyphosate, and 1.05 L ha^{-1} Ridion (a surfactant) on July 19. In 2020, the site received 2.1 L ha^{-1} of Monty’s Mn at a rate of 165.3 g Mn ha^{-1} on July 15 in response to suspected Mn deficiency.

In 2019, Wanatah received an early post herbicide application of 0.04 L ha^{-1} cloransulam-methy and 1.72 L ha^{-1} glyphosate on July 3. Foliar Mn was applied with Eezy Man (2% S, 5% Mn) at 142.9 g Mn ha^{-1} (2.3 L product ha^{-1}) on July 9 and at 285.8 g Mn ha^{-1} (4.7 L ha^{-1}) mixed with 0.23 L ha^{-1} alpha-cypermethrin on August 16. The latter application was in response to suspected Mn deficiency and a mild aphid infestation. In 2020, Wanatah had two post-emergence applications, one on June 18 of 0.28 L ha^{-1} glyphosate, 0.007 L ha^{-1} cloransulam-methyl, and 0.76 L ha^{-1} of glyphosate activator adjuvant. The second application on July 6 was 0.38 L ha^{-1} glyphosate, 142.9 g Mn ha^{-1} (2.3 L ha^{-1} Eezy Man), and 0.76 L ha^{-1} of glyphosate activator adjuvant.

Planting at Wanatah in 2020 was on June 2. During a scouting visit on June 16 of that year, large areas of stand and emergence issues were noted. Excessive down pressure applied by the planter coupled with wet conditions and a sandier soil resulted in deep seed placement and soil crusting. Plots with less than 160,000 plants ha^{-1} at harvest were omitted, which resulted in 92 of 140 plots remaining for data analyses. Treatment replicates were unbalanced (Table A-1). Poor stands were random across all treatments and thus, individual treatments (e.g., starter fertilizer, rate, or placement) were not the source of stand loss.

2.3.6 Data Analysis

Analysis of variance (ANOVA) using PROC GLM in SAS (SAS Institute, version 9.4) was used to test the interaction effect of source-rate as the main plot factor, and placement as a subplot factor over years and locations. Years and locations were treated as random and specific error terms and were defined to test main effects and interactions. Years and locations were reported separately due to interactions with source-rate and placement, which was likely due to planting date and weather differences in 2019 and 2020. Overall ANOVA results by site-year can be viewed in Table 2-4 and Table 2-5. Sulfur rate responses for each source and placement (per ANOVA determination to pool or separate) were determined using PROC REG and NLIN in SAS (SAS Institute, version 9.4) Thus, the best fit for these data were selected among linear, quadratic, and quadratic plateau regression models. Additionally, within each site year, a single degree of freedom contrast was used to compare UTC and AMS standard to ATS, KTS, and K-fuse individually pooled over rate and placement as well as collectively pooled over source, rates, and placement.

2.4 Results and Discussion

2.4.1 Weather

Weather data from the 2019 and 2020 growing seasons from West Lafayette and Wanatah are displayed in Figure 2-1, Figure 2-2, Figure 2-3, and, Figure 2-4 and past growing season averages from 2002-2018 were compiled and calculated and presented in Table 2-6 and Table 2-7. The past averages presented were the weather data available for the two locations. Weather data from West Lafayette from June 13 to July 3, 2019, were lost due to instrument maintenance. Therefore, weather data from the Throckmorton-Purdue Agricultural Center (40°17'48.7"N 86°54'07.1"W), approximately 20.6 km southeast of the West Lafayette site, was used as a proxy. Daily weather data from all site-years 14 days after planting are presented in Table 2-8.

West Lafayette. The average maximum and minimum air temperatures at West Lafayette were similar for both years when compared to the past averages. Minimum air temperatures in May, June, August, and September were slightly warmer in 2019 than 2020 and past averages. The minimum air temperature in September 2019 was 15.4°C, compared to 11.9°C in 2020 and

12.0°C from the past. The increased average air temperature in September 2019 could have hastened maturation and increased seed fill.

Large amounts of precipitation in May 2019 delayed planting until the middle of June. Fields at West Lafayette in May 2019 received 23.7 mm more rain than 2020 and 32 mm more rain than the past averages (Table 2-6). The abundant precipitation early in 2019 was followed by a drier July and August, and warmer July compared to the past averages. This deficiency of water later in the growing season, combined with the late planting likely contributed to shorter plants and lower yields in the 2019 season compared to the 2020 season.

In contrast, July 2020 was slightly wetter than the past average, with 17 mm more rain. August and September 2019 and 2020 were drier than the past. Precipitation in August was the lowest compared to the past, with 67 and 88 mm in 2019 and 2020 respectively, compared to 102 mm in the past. Although October 2019 received more rain than 2020, harvest was only delayed by one week in 2019 compared to 2020, but the extra rain could have contributed to better seed fill.

Wanatah. The average temperatures at Wanatah in 2019 and 2020 varied at planting in June, with maximum temperatures in 2019 being slightly colder (24.3°C) than 2020 (27°C) and the past averages (26.8°C) (Table 2-7). In July 2019 and 2020, the average minimum temperature was slightly warmer than the past averages, at ~17°C compared to 15°C. September was approximately 4°C warmer in 2019 than in 2020. This warmth could have contributed to the faster maturing plants leading to greater seed fill.

May 2020 had 127 mm of precipitation, compared to 90 mm in 2019. This delayed planting significantly. Fields in 2019 received about 50 mm less rain in July and about 60 mm less in August than past averages. This shortfall in water likely contributed to limited pod retention and/or seed fill during this critical growing period and therefore lowered yields. Following this, September 2019 received about 40 mm more rain than the 2020 and past averages, further contributing to increased seed weight. August precipitation in 2020 was about 50 mm less than the past averages. Both years received less rain in October than past seasons, at 66, 58 and 84 mm in 2019, 2020 and past seasons, respectively.

2.4.2 Early and Harvest Stands

West Lafayette. Plant stands results were separated by year. Plant stands of UTC and AMS did not differ during the season or at harvest in 2019 and 2020 at West Lafayette (Table 2-9). Early-season stands were 290,683 plants ha^{-1} for UTC and 288,714 plants ha^{-1} for AMS in 2019 and 309,712 plants ha^{-1} for UTC and 315,690 plants ha^{-1} for AMS in 2020. Plant stands of UTC and AMS did not differ from ATS, KTS, K-Fuse, and all starters when averaged over all four rates and both placements during the season or at harvest in 2019 and 2020 (Table 2-9) as evaluated by a single-degree-of-freedom contrasts.

Plant stand was influenced by increasing starter rate within source in 2019, but not in 2020 (Table 2-10). There was a placement interaction on starter source-rate in 2019. Early-season stand was unaffected by increasing rates of ATS-single and KTS-single. Early-season stand increased linearly with increasing rates of K-Fuse-single (891 plants ha^{-1} for each additional 1 kg of S ha^{1}) and at harvest increased quadratically with the K-Fuse-single rate (Table 2-10). In the dual placement, all three fluid starter sources decreased early season stand as rates increased to ~5 kg S ha^{-1}. Plant stands were reduced by nearly 9000 plants ha^{-1} from the y-int to a minimum of ~280,000 plants ha^{-1} (Table 2-10).

Stands at harvest for ATS-dual and KTS-dual increased linearly with rate by ~1100 plants ha^{-1} and ~1000 plants ha^{-1}, respectively, per 1 kg of S ha^{-1} (Table 2-10). Plant stands were still within acceptable agronomic levels and would likely not influence yield based on population alone.

Wanatah. Early-season plant stands of UTC and AMS at Wanatah did not differ in 2019 and 2020, ranging from 260,000 to 289,000 plants ha^{-1} (Table 2-9). Early season plant stand counts with AMS was 18,865 plants ha^{-1} greater than KTS in 2019 (Table 2-9). Otherwise, early-season plant stands of starter sources did not differ from stands with UTC or AMS in 2019 and 2020. Harvest plant stands of AMS (~320,000 plants ha^{-1}) was higher than UTC and all the liquid starter sources (~300,000 plants ha^{-1}) (Table 2-9). In 2020, UTC had greater stands at harvest than K-Fuse-all (the average of all rates within source).

Plant stands in 2019 at Wanatah were influenced by one liquid S starter source-rate interaction, but not placement. K-Fuse applied at Wanatah in 2019 linearly increased plant stands from the y-int at a rate of ~800 plants ha^{-1} for each addition 1 kg S ha^{-1} (Table 2-10). This occurred both in early-season stands and at harvest.

Early-season plant stand was unaffected by placement at Wanatah in 2020, but there was one liquid S starter source-rate interaction. K-Fuse decreased plant stands at harvest (at a rate of nearly 7000 plants ha^{-1}, to a minimum of 239,000 plants ha^{-1} at 5.2 kg of S ha^{-1} applied), and stand with K-Fuse-all was also less than that with UTC (Table 2-9, Table 2-12). Plots at this site encountered emergence issues discussed previously, which reduced the number of viable plots for data analysis.

Discussion. Starter S fertilizers did not improve early or harvest plant stands compared to UTC and AMS. Placement of starter fertilizer only affected early and harvest plant stands at West Lafayette in 2019, with K-Fuse in a single placement, increasing stand with increasing rate, while all other starter sources in the dual placements reduced stand with increasing rates.

The early season stand reduction in a dual placement in 2019 at West Lafayette was likely related to slightly warmer and drier conditions in June compared to trials in 2019 and 2020 at Wanatah (Table 2-6, Table 2-7). Starter fertilizer movement through the soil was likely limited due to dry conditions and thus, high concentrations of fertilizer were present near the seeds and seedlings. In a summary of starter fertilizer use, Hergert (2012) described how a concentrated band of fertilizer may cause salt injury by increasing ionic strength near the seed; thereby, interfering with germination, root growth, or emergence. In West Lafayette 2019, the dual placement of fertilizer could result in greater exposure of starter fertilizer by placing it on both sides of the seed. Although the concentration of the fertilizer on either side of the seed was reduced compared to the single placement, it is possible that surrounding the seed with fertilizer as opposed to placement on one side increased the chance of salt injury.

Another possible source of the early season stand decrease is related to the ATS treatment. In their summery of starter fertilizer usage, Hergert (2012) noted that liquid ATS can produce free ammonia (NH_3), which can reduce germination or increase seedling death (Hergert et al., 2012). Dry soil conditions, increased surface residue, and increasing temperatures, all of which were experienced at West Lafayette in 2019, can favor free NH_3 injury (Havlin, 2005; *Salt Index, Starter and Band Applications of Fertilizer*, 2021). Changes in pH in the seedling root zone from the starter fertilizer applications could also be a source of early season stand decreases. ATS and KTS are both slightly acidic, and it can be inferred that K-Fuse is also slightly acidic, as it contains ATS (Havlin, 2005). However, most plant growth issues with increased acidity are related to toxicity effects in roots and decreased nutrient availability, not necessarily decreased germination. Also, given the small area in which the fertilizer was applied, it is unlikely that the changes in pH were so drastic to induce these issues. Ultimately, based on the results in this study, single placement is likely the best option for fluid fertilizer placement, simply through the ease and accessibility of a single placement system compared to the added cost of installing a dual placement system with no perceivable benefit.

Early stand increased within increasing K-Fuse rates at West Lafayette and Wanatah in 2019. Both site years were planted in later (mid-June), warmer, and drier conditions. It is possible that the addition of both N, K and S in one fluid fertilizer application of K-Fuse produced the positive early stand response compared to ATS and KTS. Touchton and Rickerl (1986) documented greater root and shoot dry weight of soybean 21 and 28 days after emergence when N, N+P, and N+P+K starter fertilizers were used in the coastal plains of Alabama (Touchton & Rickerl, 1986). They theorized that additional nutrients (primarily N) may have stimulated early season root growth. Although no root biomass data was collected in this study, a possible explanation for the increasing early season stand was that increased nutrition in the root zone may have stimulated greater root growth early in the season, similar to what Touchton and Rickerl observed, and allowed for more access to water later in the season, with much drier conditions (Table 2-6).

One unexpected difference noted was the increase of harvest plant stand from the AMS treatment compared to UTC and all liquid starter fertilizers at Wanatah in 2019. This was another June planted site year, with conditions slightly cooler than other site years. As the season progressed, August was much drier, while September was warmer and wetter. If liquid starter fertilizer alone had reduced harvest stand compared to AMS, it could be said that liquid starter fertilizers were the primary reduction agent. However, harvest stand in the UTC was also less than AMS, and liquid starter fertilizer did not differ than UTC. One possible explanation is that the slower incorporation of granular AMS, compared to the rapid breakdown of thiosulfates in the liquid starter fertilizers, may have provided soil nutrition to stimulate root growth later into the early-season, similar to what Touchton and Rickerl (1986) saw in their study of starter fertilizer. Increased root mass would have been advantageous during the drier August, and could have allowed for more plants to survive to maturity.

Overall, the effect on early and harvest stand from liquid starter fertilizers appeared to be dependent on precipitation and subsequent movement of starter fertilizer in the soil profile. In warmer and drier conditions, like in West Lafayette (2019), movement of starter fertilizer was reduced, and fewer plants were counted. Liquid starter fertilizers never increased stand early in the season or at harvest compared to AMS and UTC. However, increasing rates of starter fertilizer did increase stand within starter comparisons, suggesting that increased nutrient concentrations in the root zone may stimulate root growth and benefit stand persistence both early in the season and at harvest. With regards to placement, a dual placement may increase the likelihood of stand reductions compared to a single placement and does not seem to be beneficial. This was likely because the dual placement increased the risk of salt injury by surrounding the developing seedling in fertilizer compared to a single placement on one side.

2.4.3 Plant Nutrient Concentration: N, P, K, S, and N:S

West Lafayette. Leaf nutrient concentrations were separated by year. Leaf nutrient concentrations reported at all site-years were from the single placement only. The dual placement was not sampled in its entirety and therefore not presented.

Leaf N, P, K, and S did not differ between UTC and AMS in 2019 and 2020 (Table 2-13). Liquid S starter fertilizers did not affect leaf N, P, or K in either year compared to UTC and AMS (Table 2-13). Leaf S was greater in AMS than KTS-all in 2019, and greater than K-Fuse-all in 2019 and 2020 (Table 2-13). Additionally, all starter fertilizer averages had less leaf S than AMS in 2020. The N:S ratio did not differ between UTC (15.5), AMS (15.2), and the starter fertilizer averages (~16.0) in 2019. However, N:S was higher with UTC (18.6) compared to AMS (17.3), ATS-all (17.9), and all starter fertilizer averages (18.0) in 2020 (Table 2-13). Both KTS-all and K-Fuse-all had N:S ratios greater than AMS in 2020.

Within individual starter sources and rate interactions, there was no impact on leaf N, P, K, and S in 2019 and 2020. Leaf N:S was only affected by starter source-rate in 2020, where the leaf N:S ratio decreased quadratically as more ATS was applied (Table 2-15, Figure 2-6).

Wanatah. Leaf N and N:S was higher in UTC than AMS, but leaf P, K, and S did not differ between UTC and AMS in 2019 (Table 2-13). In 2019, leaf N in KTS-all was lower than UTC, while leaf N in K-Fuse-all was greater than AMS. K-Fuse-all had greater P than AMS in 2019, while AMS had greater leaf K than ATS-all and all starter fertilizer averages. Along with AMS, the UTC had a higher N:S than ATS-all, KTS-all and all starters fertilizer averages in 2019 (Table 2-13).

Leaf N, K, S, and N:S did not differ between UTC and AMS in 2020, but leaf P was higher in UTC than AMS. Additionally, leaf P of KTS-all, K-Fuse-all and All starter fertilizer averages were greater than AMS in 2020. Leaf P in ATS-all was lower than UTC. Leaf N:S in 2020 was higher in UTC compared to ATS-all, K-Fuse-all, and All starter fertilizer averages (Table 2-13). Leaf S was not impacted by starters in either year.

KTS was the only starter to influence leaf N and P by rate at Wanatah, where in 2019 leaf concentrations of N and P decreased linearly as higher KTS rates were applied (Table 2-13). Otherwise, starter source- rate did not impact leaf N, P, K, S, and N:S in 2019 and 2020.

Discussion. Sulfur from AMS and starter fertilizers did not improve leaf S compared to UTC at R3 in any site-year, but all leaf concentrations were above the critical level of 2.5 g S kg^{-}

[1]. Leaf N concentrations did not improve even when fertilizers that supplied N were applied (i.e., AMS, ATS, and K-Fuse). However, the N:S ratio was decreased in two of four site-years with AMS and all of the starter fertilizers. The N:S ratio of UTC was 18.6 in 2020 at West Lafayette, which was reduced to 18.0 as more S was supplied by the starter fertilizers. Soil K was not limiting in any site-year, so the additional K from KTS and K-Fuse was not needed, and starter fertilizers containing K did not improve leaf K concentrations.

Soil organic matter was moderate to high (35 to 49 g kg^{-1}) at the four-site years, which according to studies by Franzen (2008) and Kost (2008), would typically indicate limited need or probability of S responsiveness (Franzen, 2008; Kost et al., 2008). Our trials were planted late in 2019 (June 7 and June 12) which coincided with warmer soil conditions and thus, greater potential for mineralization of soil organic matter to supply N and S. Conversely, the May 13 planting in 2020 at West Lafayette was much more timely, and mineralization of soil organic matter was likely slower, leading to the opportunity for an S response. Starter fertilizers supplied S and decreased the ratio of N:S at West Lafayette in 2020 indicating that the limited mineralization of S may have occurred. The reduction of N:S ratio at West Lafayette in 2020 coincided with the positive yield response from starter S applications. Additional S from AMS and starter fertilizers decreased the N:S ratio compared to UTC in 2019 at Wanatah. Multiple leaf samplings throughout the growing season would have helped determine if there were early differences that were transient or if differences in growth occurred later in the season.

2.4.4 Plant Nutrient Concentration: Ca, Mg, Zn, Mn, Fe, Cu, and B

West Lafayette. Leaf concentrations of calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), and iron (Fe) did not differ between UTC and AMS in 2019 and 2020. Leaf boron (B) was lower with AMS than UTC in 2019 and 2020 (Table 2-14). Leaf copper (Cu) did not differ between AMS and UTC in 2019 but was lower in the AMS than UTC in 2020.

Some starters treatments had lower leaf concentrations of Cu than UTC in 2019 (KTS-all, K-Fuse-all) and 2020 (ATS-all, KTS-all, and K-Fuse-all, Table 2-14). Leaf B was lower with K-Fuse-all than with UTC in 2019. Other nutrient differences were minimal across the starters. (Table 2-14).

Leaf Ca, Mg, and Zn concentrations were not impacted by source-rate in 2019 and 2020. Leaf Mn, Fe, Cu, and B concentrations were influenced only by KTS in 2019 (Table 2-14). Mn increased linearly at a rate of 0.80 mg kg^{-1} with each additional 1 kg S ha^{-1} of KTS applied (Table 2-15, Figure 2-5), while leaf B decreased linearly. Additionally, leaf Fe and Cu concentrations decreased quadratically in response to increasing KTS rates (Table 2-15). There was only one liquid starter source-rate interaction in 2020, wherein leaf Cu concentration declined slightly with increasing rates of K-Fuse (Table 2-15).

Wanatah. Leaf concentrations of Ca, Mg, Zn, Fe, and B did not differ between UTC and AMS in 2019 and 2020. Leaf Mn was greater in AMS than UTC in 2019 and 2020 (Table 2-14). Additionally, leaf Mn with AMS was higher than leaf Mn with ATS-all, K-Fuse-all, and all starter fertilizer averages in 2019 and greater than ATS-all in 2020 (Table 2-14). KTS-all and K-Fuse-all had higher leaf Mn than the UTC in 2020 (Table 2-14). The UTC had a greater leaf Cu concentration than KTS-all in 2019, while UTC and K-Fuse-all had greater leaf Cu concentration than AMS in 2020 (Table 2-14).

Starter source-rate affected leaf Mn, Fe, and Cu in 2019 and 2020. The only starter to increase leaf Mn with increasing rate of S was ATS. Leaf Mn increased quadratically until about 10 kg S ha^{-1} applied for ATS in 2019, and increased linearly in 2020 with ATS (Figure 2-5,

Table 2-16). All three starter treatments decreased leaf B concentrations as S rate increased in 2019 (Table 2-16). Leaf Fe increased quadratically with increasing KTS rates and linearly with increasing K-Fuse rates (Table 2-16). Lastly, Leaf Cu decreased to 8.1 mg kg^{-1} with a 5.7 kg S ha^{-1} KTS rate (Table 2-16).

Discussion. Generally, leaf Mn was increased with AMS and starter fertilizers at West Lafayette in 2020, and Wanatah in 2019 and 2020. The response in leaf Mn to fertilizer treatment was notable because it occurred in the site years which received foliar Mn applications in response to suspected Mn deficiency. Plants that received additional N, K or S had notable differences in Mn concentration when faced with Mn deficiency compared to plants which received no additional fertilizer. In an experiment testing microbial reduction of Mn interaction with S, Myer and Nealson (1988) found that thiosulfate can increase Mn reduction from the soil into plant available forms by lowering pH, as well as increasing rates of Mn reduction by soil microbes from plant unavailable MnO_2 into plant available Mn^{+2} (Myers & Nealson, 1988; Vavra & Frederick, 1952). In the presence of thiosulfate, soil microbes can use thiosulfate reductase to reduce thiosulfate into sulfides, which will reduce MnO_2 into plant available Mn^{2+}, resulting in more Mn^{2+} (Myers & Nealson, 1988). This could be a reason why Mn uptake increased in response to starter fertilizers. These fertilizers could have also improved root and shoot growth early in the season, as described by Touchton and Rickerl (1986), allowing the roots to take up more Mn from the soil, allowing for more absorption of the foliar applied Mn (Touchton & Rickerl, 1986). Additionally, the acidity of the liquid starter fertilizers may be decreasing the pH in the root zone, thereby increasing Mn availability (Havlin, 2005). Leaf Mn concentrations were also improved with AMS applications at West Lafayette in 2019. This could be a result of pH changes from a broadcast application of AMS increasing Mn availability, or an increase in uptake from increased biomass development as described by Touchton & Rickerl (1986).

Leaf Fe concentrations responded to fertilizer treatments at one site-year, West Lafayette in 2019. At this site, additional K and S from KTS fertilizer may have resulted in greater root biomass earlier in the season, and therefore subsequent greater uptake and transportation of Fe in the soil (Touchton & Rickerl, 1986).

Copper and B concentrations in the leaves changed with fertilizer treatment at every site-year, where the trend was that Cu and B concentrations were lower in the leaves of starter fertilizers compared to UTC. Leaf Cu decreased with increasing rates of liquid starter fertilizer in all four site-years. Likewise, leaf B decreased with increasing rates of liquid starter fertilizer at West Lafayette and Wanatah in 2019. The dry weather patterns at Wanatah in August of 2019 and 2020 could have decreased uptake of the Cu and B. Salt injury could have occurred from increasing rates of starter fertilizer in droughty conditions, and thus restricted root mass would limit uptake of Cu and B from the soil, reducing ability to take up Cu and B. The increased Mn uptake as a result of thiosulfate oxidation could have also increased competition for cation transport across plant membranes. Greater amounts of reduced Mn^{2+}, via the microbial interaction with thiosulfate discussed earlier, would be more available and more readily taken up compared to Cu cations (Myers & Nealson, 1988). With regards to B, Havlin (2005) described how K containing fertilizers could decrease B uptake. Potassium fertilization has been known to interfere with B availability, as K fertilizer can alter the CEC, displacing Ca cations (Havlin, 2005). Thus, the Ca cations will then interfere with B absorption by the plant. At Wanatah in 2019, all three starter treatments reduced B uptake from the UTC treatment with increasing starter rate, suggesting that a combination of droughty conditions, injury, and Ca displacement had reduced B uptake.

2.4.5 Plant Height

Plant heights were collected at R5 at 3 of the 4 site years and separated by year. Additionally, plant height was influenced by liquid starter source-rate, but not placement, and was thus pooled across placement. UTC was taller than AMS (84.2 cm vs. 78.8 cm) at West Lafayette in 2019, but not in 2020. There were no differences in UTC or AMS to any of the S starter fertilizers at West Lafayette in 2019 or 2020 (Table 2-9). Plant height decreased linearly with increasing KTS rates (-0.26 cm for each additional 1 kg S ha^{-1}) in 2019 at West Lafayette (Table 2-18). At West Lafayette in 2020, soybean increased plant height as more K-Fuse was applied until 20.2 kg S ha^{-1} with a height of 102.3 cm (Table 2-19).

At Wanatah in 2019, AMS (73.3 cm) was taller than both KTS-all (71.0 cm) and K-Fuse-all (70.6 cm) (Table 2-9). There was not a plant height interaction from liquid S starter source-rate at Wanatah in 2019. Plant height data was not collected at Wanatah in 2020 due to the lack of quality plots.

Discussion. Plant height was inconsistently affected by fertilizer treatments. Due to the inconsistency between years, the observed responses reflect more of the environmental conditions of the site year, rather than an effect of the starter fertilizers. The drier conditions at West Lafayette in July and August of 2019 likely decreased root and above ground biomass growth, compared to the earlier planting in 2020, which experienced more regular precipitation in July and August. Plant height did increase with increasing K-Fuse rates at West Lafayette in 2020, but this height increase with rate did not correlate to plants taller than those of the UTC or AMS treatments.

One explanation for the height reduction is that the thiosulfate in K-Fuse and KTS may have stunted early season growth, similar to the observation with the reduced plant stands, and impacted the overall plant height later in the season (Hergert et al., 2012). If thiosulfate caused reductions in height, one would expect a similar response with ATS. However, an ATS effect was not observed. This means the only common factor between the starter fertilizers that reduced height was K. The K in K-Fuse and KTS could be causing salt injury in the drier conditions, delaying germination of plants, or stunting seedlings, causing the reduced height observed later in the season. Greater volume of KTS and K-Fuse had to added compared to ATS in order to achieve the desired S rate (Table 2-2). If K soluble salt concentrations, particularly in the form of KCl, were higher in the soil than the in developing seed, water would have diffused out of the plant cells, causing plant cells to collapse. In a summary of salt injury and its mechanisms, Mortvedt (2001) listed the salt index for KTS is 68.0 per equal weights of materials (Mortvedt, 2001). Porter (2016) listed the salt index for potassium acetate, which is the main K component of K-Fuse, is 43.8 per equal weights of materials (Porter, 2016). KTS, with a higher salt index, would be more prone to salt injury than K-Fuse. However, K-Fuse also contains ATS and Urea. Havlin (2005) lists the salt indexes of ATS and Urea as 74.4 and 90.4 respectively, potentially explaining why the combination in K-Fuse reduced height the most (Havlin, 2005).

2.4.6 Yield

West Lafayette. Year was separated. Yield of AMS (3225 kg ha^{-1}) was less than UTC (3752 kg ha^{-1}) in 2019, but did not differ in 2020 (4001 and 3925 kg ha^{-1}, respectively) (Table 2-17). Yield of AMS was 421 kg ha^{-1} less than ATS-all and 357 kg ha^{-1} less than all starter fertilizer averages in 2019. Similarly in 2020, yield of AMS was also 300 kg ha^{-1} less than KTS-all and 206 kg ha^{-1} less than all starter fertilizer averages. The yield of UTC did not differ from

All-starters in 2019 (3520 to 3752 kg ha^{-1}), but yield of UTC was 212 to 376 kg ha^{-1} less than all starter products in 2020 (Table 2-17).

Yield responses from starter source-rate combinations varied by site year. Placement did not affect yield in either year, so yield was pooled over placement. Yield did not change as more S was applied from ATS and K-fuse in 2019. However, applications of KTS above 5.5 kg S ha^{-1} reduced yield (Table 2-18, Figure 2-7) in a similar fashion as the plant height reductions (Table 2-18). In 2020, the quadratic plateau model best described the yield response of all three starter sources (Table 2-19). The optimal rate of S applied was 4.7 kg S ha^{-1} for ATS (yield of 4137 kg ha^{-1}), 7.5 kg S ha^{-1} for KTS (yield of 4308 kg ha^{-1}), and 11.0 kg S ha^{-1} for K-Fuse (yield of 4198 kg ha^{-1}) (Table 2-19, Figure 2-7).

Wanatah. The yields of UTC and AMS did not differ in 2019 and 2020 (Table 2-17). None of the starters differed in yield from UTC and AMS in 2019 and 2020, except for K-fuse-all in 2019. K-Fuse-all yielded 213 kg ha^{-1} less than AMS in 2019 (Table 2-17). Yield also decreased in a quadratic function until ~11 kg S ha^{-1} of K-Fuse was applied, at which point yield increased (Table 2-11, Figure 2-8).

Discussion. The yield of AMS was less than UTC at West Lafayette in 2019, whereas there was no difference between AMS and UTC in the other three site-years. The broadcast application of AMS was 17 days after planting (after soybean emerged) in 2019 at West Lafayette. Casteel (2018) described how foliar applied AMS can cause leaf burn on soybean. AMS applied after emergence could have caused direct damage to the plants through AMS contact burning leaves, effectively stunting plants, and potentially decreasing overall plant height (Casteel, 2018). Additionally, plant height decreased from UTC at West Lafayette in 2019, which is further evidence that crop injury and stunted growth could have been the source of lower yields observed.

Liquid S starter fertilizers only improved yield more than the UTC at one of the four site-years (2020 at West Lafayette). There were a few potential explanations for the increased yield at this site-year. In the earlier planting date in 2020, limited mineralization of soil organic matter could have occurred and thereby, limited N and S supply for the developing seedling. With the addition of starter fertilizers, the plant was supplied the necessary N and S earlier in the season to increase early root and shoot growth, as the developing seedlings grew to access the fertilizer (Touchton & Rickerl, 1986). In a summary of starter fertilizer usage, Hergert (2012) explained how increased biomass growth may have contributed to the increase in yield later in the season, through the increased ability to uptake nutrients and photosynthesize, leading to earlier flowering (Hergert et al., 2012). Additionally, S supplied as a starter in S limited conditions could benefit nodulation and N_2 fixation, as S is a key component in nitrogenase, the enzyme necessary for catalyzing nitrogen fixation (Havlin, 2005; Hitsuda et al., 2005). The yield plateau and coinciding S rate differed among the starter fertilizers sources. The highest yield plateau was with KTS followed by K-Fuse then ATS. The two highest yield plateaus also coincided with starters providing K.

In 2019 at West Lafayette, yield slightly declined with an increasing KTS rate. As discussed earlier, as KTS rates increased, the likelihood of incurring salt injury to the roots increased, limiting the potential yield benefits of KTS at higher rates (Mortvedt, 2001).

Therefore, in situations where mineralization is limited, such as cold, wet soils, an earlier planting, or increased residue, it is likely this yield increase with starter S fertilizer could be repeated. Other studies, like that of Miller (2020) have found similar results, indicating that an early application of S can be beneficial to the developing soybean crop, and can increase yield later in the season (Miller, 2020). The lack of response in the other site-years may be related to adequate mineralization of soil organic matter within warmer conditions and thus, supplied more N and S from the soil, resulting in less need for starter S fertilizers.

2.4.7 Seed Weight

West Lafayette. Seed weight was separated by year. In 2019, there was a seed weight response to a starter source-rate, but not placement or an interaction between rate and placement. In 2020, there was a source-rate x placement interaction, so seed weight was separated by placement. The seed weights of the UTC and AMS did not differ in 2019 or 2020 (Table 2-17). The seed weight of UTC and AMS did not differ from starter sources, except in 2020 where seeds of UTC were smaller than K-Fuse-all (17.0 vs. 17.4 g 100 seeds^{-1}).

Seed weight decreased as S rate increased with ATS in 2019 (pooled across placement) and 2020 (within single placement) (Table 2-19). Seed weight increased linearly with increasing KTS rates in dual placement in 2020. Seed weight was not affected by any other starter sources (Table 2-19).

Wanatah. Placement did not influence seed weight and was pooled within each year and source. Seeds were larger with AMS (18.0 g 100 seeds^{-1}) than UTC (17.1 g 100 seeds^{-1}) in 2019 but did not differ in 2020 (Table 2-17). ATS (17.8 g 100 seeds^{-1}) and KTS (17.8 g 100 seeds^{-1}) produced larger seeds than UTC (17.1 g 100 seeds^{-1}) in 2019. No starter S fertilizers altered seed size in 2020 (Table 2-17).

Starter source and rates influenced seed weight in both years at this location. Seed weight plateaued at 17.8 g 100 seeds^{-1} at 9.9 kg S ha^{-1} from ATS in 2019 (Table 2-11). Additionally, seed weight increased quadratically with KTS to 17.8 g 100 seeds^{-1} with 10 kg S ha^{-1} in 2019

(Table 2-11). Seed weight of K-Fuse increased with rate in 2019 and 2020. The seed size plateaued at 15.1 g 100 seeds^{-1} with 5.0 kg S ha^{-1} in 2020 (Table 2-11).

Discussion. Seed weight increased by fertilizer at two site-years, West Lafayette in 2020 and Wanatah in 2019. All three starter sources, increasing in rate, changed seed weight, but the changes were inconsistent. Seed weight was also impacted by one of the placement interactions (West Lafayette in 2020).

With regards to the observed placement effect, the longer growing season and higher yield (e.g., more pods likely) at West Lafayette in 2020 likely allowed for more differentiation of source-rate and placement interactions. Ultimately, neither of the products which had different seed weights by placement had higher seed weights compared to the UTC and AMS treatments.

With regards to the specific differences in seed weight at Wanatah in 2019, it is possible that N and S supplied at higher rates potentially increased time to maturity and thus, lengthen the seed fill period (Hergert et al., 2012). In the adverse conditions of Wanatah in 2019, added K could have contributed to greater transport of photosynthates to the seed late in the season, increasing seed weight (Havlin, 2005). Conversely, in the other site years, the shorter growing season for a late planting hastened seed fill through reduced number of days in the season, contributing to the lack of a seed weight increase. Conditions during seed fill were also droughty at Wanatah in 2020, further decreasing the likelihood of a similar response.

Improvements in seed weight at West Lafayette in 2020 (ATS-single, KTS-Dual) contributed to yield increases with ATS and KTS; however, another yield component, the number of pods, was likely the main source of yield improvement from ATS, KTS, and K-fuse (data not collected).

2.4.8 Protein and Oil Content

West Lafayette. Analysis was separated by year. Placement did not affect protein or oil for any site-year and thus, was pooled within site-year. In 2019, UTC and AMS did not differ in protein or oil content (Table 2-17). The protein and oil of UTC and AMS did not differ with any starter fertilizers, with the exception of oil being lower in KTS-all compared to AMS (Table 2-17). Protein and oil were not affected by source-rate interactions in 2019 (Table 2-18).

In contrast, after an earlier planting in 2020, protein effects were noted. Every starter fertilizer resulted in 0.9-1.2% higher protein content than UTC (Table 2-17) but did not differ from AMS. Protein also increased with increasing rates of ATS (41.5%) and K-Fuse (41.8%). This coincided with the increases in yield from every starter fertilizer treatment, as discussed previously. Oil content for ATS-all, K-Fuse-all, and all starter fertilizer averages was nearly 0.7% lower than AMS (Table 2-17).

Wanatah. In 2019, protein and oil content of UTC and AMS differed. Oil content in UTC (20.8%) was higher than AMS (20.2%). Conversely, the protein content was lower in UTC (35.5%) than AMS (36.0%) (Table 2-17). Starter fertilizers did not differ from UTC in oil, but ATS-all, K-fuse-all, and all starter fertilizer averages were higher in protein than UTC (Table 2-17). Starters were 0.4-0.5% higher in oil compared to AMS, but the starters and AMS did not differ in protein in 2019. Increasing ATS rate caused a quadratic increase of % oil content from the y-int at a rate of 0.01% with a slope of -0.001 (Table 2-11).

The oil and protein content in UTC and AMS did not differ in 2020. KTS-all and all starter fertilizer averages were lower in oil than UTC (Table 2-17). Otherwise, oil and protein did not differ among the starter treatments, UTC, and AMS. Increasing the KTS rate positively impacted protein, while decreasing oil (Table 2-12). Protein increased from the y-int at the rate of 0.2%, to a maximum plateau of 42.5% at the KTS rate 21.7 kg S ha^{-1}. Conversely, oil decreased at a rate of 0.2% with the addition of 1 kg S ha^{-1} of KTS, which sloped at a rate of 0.004 (Table 2-12).

Discussion. Fertilizer treatments changed the protein and oil content in the seed differently between site-years. Ultimately, the different weather conditions and growth patterns between site-years likely played the biggest role in the inconsistent response. For example, the droughty conditions later in the season at West Lafayette in 2019 likely decreased oil and protein content. Hammond (2005) described how late season drought can reduce oil and protein biosynthesis. This may explain the lack of response observed at that site year.

Protein was generally greater from starter fertilizers compared to UTC. Protein also increased and oil decreased as more S was applied (e.g., KTS). Protein and oil contents have a direct inverse relationship (Hammond et al., 2005). An increase in protein content with starter source-rate was observed with all three liquid starter sources in West Lafayette in 2020. Additionally, yield was improved at West Lafayette in 2020 by starter fertilizers, meaning that the ability to improve both protein content and yield is a potential benefit of starter S fertilizers. In their summary of S management in soybean, Hitsuda (2008) noted that S is a critical component in amino acids, such as cysteine and methionine, and it is possible that increased S applications lead to more amino acid generation and more protein biosynthesis (Hitsuda et al., 2008a). Sulfur from starter fertilizers could also benefit nodulation and nitrogen fixation, increasing N supply to be used for amino acid production (Hitsuda et al., 2005, 2008a). Applications of ATS and KTS resulted in highest increases in protein production, coupled with the lowest oil production. It is likely that additional S was more likely the source of the responses as opposed to the addition of N or K, as responses were observed in both products. Kaiser and Kim (2013) observed a similar response in grain protein in areas more susceptible to S fertilizer responses in Minnesota (Kaiser & Kim, 2013). They connected this response to a theory that Hitsuda (2004) proposed, wherein may be functionally deficient, even in the absence of a physical yield response, if grain protein was improved by S fertilizer (Hitsuda et al., 2004; Kaiser & Kim, 2013). This could mean that in sites like Wanatah in 2019 and 2020, S may have still been deficient, despite lack of yield response with the corresponding protein responses, and that starter S may have provided much needed S. Additionally, K plays a key role in the movement of carbohydrates to storage organs and subsequent conversion into other plant products, so additional K in KTS may have contributed to greater protein synthesis (Havlin, 2005).

2.5 Conclusions

Since the effects of fertilizer placement were minimal, either single (0x5x1) or dual (0x5x2) placement would be acceptable. The results of this study indicated that there was little evidence a grower would need to invest in a dual system over the single placement. This study did not assess starter placement below the seed, which may have provided for increased stands and improved yields as the seedling was able to access the fertilizer later in the season.

Improvements in plant nutrition, growth, yield, and quality were variable based on the site-years. Delayed plantings (e.g., early-mid June) limited yield responses to starter fertilizer, as well as increased the likelihood of negative deceased stand (e.g., West Lafayette in 2019). For this reason, liquid starter S fertilizers did not appear to be effective when planting in June. Mineralization was likely active in the warm soils and thus, decreased the need and response to additional S. Furthermore, drier conditions, like what can be experienced in June, were more conducive to salt injury through reduced movement of fertilizer out of the root zone.

At a timely planting, starter S rate was optimized between 3 to 10 kg S ha^{-1} with yield increases between 212 to 383 kg ha^{-1} at West Lafayette in 2020. Protein also increased as starter

S was applied during this time. The uptake of Mn was improved with lower rates (3 to 5 kg S ha^{-1}). Cool and wet conditions typically associated with early plantings may require S fertilizer applications due to limited mineralization of soil organic matter (e.g., supply of N and S). During early planting dates, when soils were wet and cool, like in West Lafayette in 2020, liquid starter S fertilizer has the capacity to both increase yield and improve protein content over UTC. The improved N:S ratio at West Lafayette in 2020 indicated that mineralization was limited in the cooler, wetter conditions, and that starter S provide necessary S to improve the N:S ratio.

Leaf S concentrations were above the critical level (2.5 S g kg^{-1}) in the UTC of all site-years. Soybean response to S was limited in these site-years, and leaf S concentrations did not improve with starter S fertilizer. The full potential of starter S fertilizers to benefit soybean should be evaluated in more early plantings (e.g., cool and/or wet soils), low organic matter soils, and any other condition that limit S availability (e.g., high residue).

Table 2-1. Soil fertility in 2019 and 2020 near West Lafayette and Wanatah, Indiana. Samples were taken prior to fertilizer applications and planting in each year. The mean values are averaged over 5 replications. (± standard deviation).

	West Lafayette		**Wanatah**	
Soil Analyses	**2019**	**2020**	**2019**	**2020**
OM (g kg^{-1})	35 ± 2	44 ± 4	49 ± 9	42 ± 4
pH	6.1 ± 0.2	6.6 ± 0.2	6.2 ± 0.1	5.9 ± 0.3
CEC (cmol kg^{-1})	22 ± 1	24 ± 3	20 ± 1	19 ± 2
P (mg kg^{-1})	21 ± 3	43 ± 14	24 ± 4	40 ± 11
K (mg kg^{-1})	145 ± 9	136 ± 16	115 ± 11	155 ± 27
Mg (mg kg^{-1})	626 ± 42	823 ± 62	605 ± 38	52 ± 42
Ca (mg kg^{-1})	2539 ± 155	3041 ± 319	2328 ± 132	2054 ± 139
S (mg kg^{-1})	10 ± 1	19 ± 7	7 ± 1	7 ± 1
Zn (mg kg^{-1})	1.5 ± 0.2	2.1 ± 0.3	3.0 ± 0.2	2.6 ± 0.3
Mn (mg kg^{-1})	30 ± 5	13 ± 5	5 ± 0	12 ± 4
Fe (mg kg^{-1})	168 ± 6	159 ± 49	225 ± 9	194 ± 24
Cu (mg kg^{-1})	2.3 ± 0.2	2.8 ± 0.5	2.1 ± 0.3	2.2 ± 0.3
B (mg kg^{-1})	0.5 ± 0.1	0.6 ± 0.1	0.2 ± 0.1	0.4 ± 0.1

Table 2-2. Starter Sulfur Fertilizer by Placement treatments with addition nutrients provided.

TREATMENT		**Product Rate**	**S Rate**	**Placement**	**N**	**K_2O**
		L ha^{-1}	kg ha^{-1}	Side of Seed	kg ha^{-1}	kg ha^{-1}
1	ATS_5.6_one	15.8	5.6	Single	2.6	0
2	ATS_11.2_one	31.5	11.2	Single	5.2	0
3	ATS_16.8_one	47.3	16.8	Single	7.8	0
4	ATS_22.4_one	63.0	22.4	Single	10.3	0
5	Fuse_5.6_one	35.0	5.6	Single	2.8	5.6
6	Fuse_11.2_one	69.9	11.2	Single	5.6	11.2
7	Fuse_16.8_one	104.9	16.8	Single	8.4	16.8
8	Fuse_22.4_one	139.9	22.4	Single	11.2	22.4
9	KTS_5.6_one	21.6	5.6	Single	0	8.2
10	KTS_11.2_one	43.3	11.2	Single	0	16.5
11	KTS_16.8_one	64.9	16.8	Single	0	24.7
12	KTS_22.4_one	86.9	22.4	Single	0	32.9
13	ATS_5.6_TWO	15.8	5.6	Dual	2.6	0
14	ATS_11.2_TWO	31.5	11.2	Dual	5.2	0
15	ATS_16.8_TWO	47.3	16.8	Dual	7.8	0
16	ATS_22.4_TWO	63.0	22.4	Dual	10.3	0
17	Fuse_5.6_TWO	35.0	5.6	Dual	2.8	5.6
18	Fuse_11.2_TWO	69.9	11.2	Dual	5.6	11.2
19	Fuse_16.8_TWO	104.9	16.8	Dual	8.4	16.8
20	Fuse_22.4_TWO	139.9	22.4	Dual	11.2	22.4
21	KTS_5.6_TWO	21.6	5.6	Dual	0	8.2
22	KTS_11.2_TWO	43.3	11.2	Dual	0	16.5
23	KTS_16.8_TWO	64.9	16.8	Dual	0	24.7
24	KTS_22.4_TWO	86.9	22.4	Dual	0	32.9
25	Broadcast_AMS	93.3 kg ha^{-1}	22.4	Broadcast	19.6	0
26	UTC	0	0	NA	0	0

Table 2-3. Planting, sampling dates and harvest at West Lafayette and Wanatah in 2019 and 2020.

West Lafayette, IN	
Date	**Field Activity**
06/12/2019	Planted
08/05/2019	MRML Samples Collected (~R3)
09/03/2019	Biomass Samples Collected and Plant Heights Taken (~R5-6)
10/15/2019	Harvested
05/13/2020	Planted
07/10/2020	MRML Samples Collected (~R3)
08/06/2020	Biomass Samples Collected and Plant Heights Taken (~R5-6)
10/07/2020	Harvested
Wanatah, IN	
Date	**Field Activity**
06/07/2019	Planted
08/07/2019	MRML Samples Collected (~R3)
09/11/2019	Plant Heights Taken (~R5-6)
10/08/2019	Harvested
06/02/2020	Planted
07/23/2020	MRML Samples Collected (~R3)
10/08/2020	Harvested

Table 2-4. Summary of ANOVA for Sulfur Source-Rate x Placement Study at West Lafayette and Wanatah in 2019 and 2020 for plant stands, plant height, yield, grain moisture, seed weight, protein, and oil.

Main Effects †	ANOVA Results															
	2019								2020							
	Early Stand	**Harvest Stand**	**Plant Height**	**Yield**	**Moisture**	**Seed Weight**	**Oil**	**Protein**	**Early Stand**	**Harvest Stand**	**Plant Height**	**Yield**	**Moisture**	**Seed Weight**	**Oil**	**Protein**
	West Lafayette															
CV%	4.9	7.5	3.8	8.4	3.5	2.1	1.1	0.8	7.1	9.0	5.0	5.9	2.0	2.7	3.7	2.7
Source-Rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	X	ns	ns	X	**
Placement	X	**	ns	ns	**	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
Source-Rate* Placement	**	X	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	X	ns	ns
	Wanatah															
CV%	6.6	6.5	3.9	8.0	2.2	2.8	1.2	1.1	13.0	10.5	††	2.8	5.9	4.6	4.4	2.7
Source-Rate	ns	ns	ns	ns	ns	***	ns	ns	ns	ns	.	ns	ns	ns	*	**
Placement	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	.	ns	ns	ns	ns	ns
Source Rate* Placement	ns	ns	ns	ns	ns	ns	ns	ns	ns	X	.	ns	ns	ns	ns	ns

† Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively, ns, not significant.
†† Plant heights at Wanatah in 2020 were not collected

Table 2-5. Summary of ANOVA for Sulfur Source-Rate x Placement Study at West Lafayette and Wanatah in 2019 and 2020 for nutrient concentrations in the most recently matured leaf sampled ~R3. ANOVA uses the single placement within each fertilizer source and rate since not all rates were sampled from dual placement.

Site Year	Interaction Main Effects	ANOVA Results											
		N	**P**	**K**	**S**	**N:S**	**Ca**	**Mg**	**Zn**	**Mn**	**Fe**	**Cu**	**B**
West Lafayette 2019	**CV%**	4.4	5.5	8.5	5.2	4.4	4.9	6.2	9.3	12.8	12.9	6.8	3.7
	Source-rate †	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	X	ns
West Lafayette 2020	**CV%**	3.7	7.9	11.6	4.5	3.9	6.1	5.0	5.2	13.6	9.8	5.1	4.5
	Source-rate †	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
Wanatah 2019	**CV%**	3.6	4.4	7.1	4.0	4.6	3.5	4.8	7.1	12.2	8.4	9.6	7.4
	Source-rate †	*	**	ns	ns	ns	ns	ns	ns	ns	ns	**	**
Wanatah 2020	**CV%**	4.5	4.4	6.3	4.4	4.1	6.8	5.2	4.7	8.5	4.5	6.1	4.2
	Source-rate †	X	*	ns	ns	ns	ns	ns	ns	ns	ns	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 2-6. West Lafayette average weather data, including maximum and minimum Air temperatures, and total rainfall by month from 2019, 2020 and past average (2002-2018).

West Lafayette, IN	**2019**	**2020**	**Past Average**
Maximum Air Temperature		°C	
May	22.6	21.8	23.9
June	26.6*	29.6	28.0
July	30.0	31.0	28.9
August	28.0	29.1	28.5
September	27.2	25.6	26.0
October	18.2	18.3	18.6
Minimum Air Temperature		°C	
May	11.3	10.2	10.8
June	16.7*	15.7	16.0
July	18.8	18.2	17.0
August	16.2	15.4	15.9
September	15.4	11.9	12.0
October	6.2	5.2	6.0
Precipitation		mm	
May	116.1	88.7	83.6
June	85.1*	67.6	116.8
July	47.2	105.2	88.4
August	67.6	87.9	102.0
September	67.8	61.2	74.9
October	91.2	65.5	70.5

* Missing weather data from 6/13/2019-7/3/2019 from West Lafayette was substituted with data from Throckmorton Purdue Agriculture Center in 2019.

Table 2-7. Wanatah average weather data, including maximum and minimum Air temperatures, and total rainfall by month from 2019, 2020 and past average (2002-2018).

Wanatah, IN	2019	2020	Past Average
Maximum Air Temperature		°C	
May	19.4	23.1	21.8
June	24.3	27.7	26.8
July	28.5	28.7	28.2
August	26.2	27.4	27.2
September	25.0	23.5	23.9
October	15.5	15.6	16.6
Minimum Air Temperature		°C	
May	8.7	13.1	8.7
June	14.4	14.9	14.5
July	17.8	17.5	15.4
August	14.9	14.0	14.5
September	14.3	10.6	10.5
October	5.4	3.7	5.0
Precipitation		mm	
May	89.7	127.0	84.1
June	69.5	60.2	66.9
July	25.3	77.7	74.4
August	33.4	39.9	93.8
September	90.0	42.9	56.4
October	65.8	57.9	84.3

Table 2-8. Average weather data from both locations 14 days after planting, including maximum air temperature, minimum air temperature, and total precipitation.

Day	Maximum Air Temperature (°C)	Minimum Air Temperature (°C)	Total Precipitation (mm)	Day	Maximum Air Temperature (°C)	Minimum Air Temperature (°C)	Total Precipitation (mm)
West Lafayette, 2019				**Wanatah, 2019**			
6/13/2019	18.3	8.9	1.0	6/7/2019	20.7	8.5	0.0
6/14/2019	25.0	7.2	0.0	6/8/2019	21.6	10.8	0.0
6/15/2019	21.7	17.2	12.4	6/9/2019	17.3	6.0	0.0
6/16/2019	27.2	17.2	3.0	6/10/2019	7.5	1.3	0.0
6/17/2019	23.3	15.6	0.0	6/11/2019	17.2	3.1	0.0
6/18/2019	27.8	17.8	0.0	6/12/2019	12.1	5.6	0.4
6/19/2019	27.8	17.2	37.6	6/13/2019	11.0	0.1	0.0
6/20/2019	20.6	12.8	1.5	6/14/2019	4.4	0.1	0.5
6/21/2019	23.9	12.2	0.8	6/15/2019	11.4	-0.2	0.0
6/22/2019	24.4	16.7	1.8	6/16/2019	22.5	7.6	0.0
6/23/2019	27.8	17.8	29.7	6/17/2019	22.3	8.9	0.0
6/24/2019	26.1	17.8	5.1	6/18/2019	18.4	4.4	0.2
6/25/2019	27.8	16.1	0.0	6/19/2019	5.2	2.9	0.0
6/26/2019	31.1	17.2	0.0	6/20/2019	11.5	1.1	0.0
West Lafayette, 2020				**Wanatah, 2020**			
5/13/2020	17.5	4.9	0.0	6/2/2020	22.6	7.1	0.0
5/14/2020	19.8	2.7	0.0	6/3/2020	33.0	16.6	0.0
5/15/2020	23.3	12.1	9.6	6/4/2020	28.8	16.6	0.0
5/16/2020	23.2	14.6	8.6	6/5/2020	28.3	17.8	0.0
5/17/2020	27.0	12.4	0.0	6/6/2020	30.9	17.6	0.0
5/18/2020	22.5	15.8	32.8	6/7/2020	24.5	13.3	0.0
5/19/2020	20.1	13.6	1.8	6/8/2020	27.7	11.7	0.0

Table 2-8 continued

5/20/2020	20.7	13.1	4.5	6/9/2020	30.8	16.0	0.0
5/21/2020	15.7	11.8	0.0	6/10/2020	31.5	19.9	1.8
5/22/2020	25.7	13.9	0.0	6/11/2020	29.5	15.9	0.3
5/23/2020	27.8	15.3	0.0	6/12/2020	26.7	13.7	0.0
5/24/2020	31.8	18.1	0.0	6/13/2020	27.6	11.8	0.0
5/25/2020	32.5	18.8	17.0	6/14/2020	21.5	12.2	2.8
5/26/2020	31.5	18.1	0.0	6/15/2020	22.0	10.0	0.0

Table 2-9. Means of stand and plant height for UTC, AMS (21.6 kg S/ha), ATS (4 S rates x 2 placements), KTS (4 S rates x 2 placements), K-Fuse (4 S rates x 2 placements), and All (3 Sources x 4 S rates x 2 placements) in 2019 and 2020 near West Lafayette and Wanatah, IN. Single-degree-of-freedom contrasts summarized between UTC vs. AMS, KTS, ATS, K-Fuse, and All; and AMS vs. ATS, KTS, K-Fuse, and All. Symbol in the contrast column (either UTC or AMS) signifies difference compared to that fertility treatment.

	Early Season Population plants ha^{-1}			**Harvest Population** plants ha^{-1}			**Plant Height** cm		
WEST LAFAYETTE 2019									
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	290683		288714	277559		285433	84.2	x	78.8
ATS-all ¶		281143			278051			82.5	
KTS-all ¶		286948			280302			81.2	
K-Fuse-all ¶		287402			276575			81.7	
All-Starters ¶		285164			278309			81.8	
WEST LAFAYETTE 2020									
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	309712		315690	305775		290172	98.0		99.0
ATS-all		307907			303314			99.8	
KTS-all		311352			300689			97.3	
K-Fuse-all		306759			304626			101.3	
All-Starters		308673			302877			99.5	
WANATAH 2019									
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	277559		289042	299869	*	320866	71.3		73.3
ATS-all		277723			296096	**		72.3	
KTS-all		270177	*		300853	*		71.0	x
K-Fuse-all		284929			303314	*		70.6	x
All-Starters		278767			300088	**		71.3	
WANATAH 2020									
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	287777		260280	262467		241689	.	††	.
ATS-all		278021			257850			.	
KTS-all		285606			247790			.	
K-Fuse-all		272675		x	239137			.	
All-Starters		278767			248259			.	

† Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively, between UTC and AMS, UTC vs. ATS, KTS, K-Fuse, and All; and AMS vs. ATS, KTS, K-fuse, and All. The absence of a symbol indicates P>0.10.

†† Plant height measurements were not collected at Wanatah in 2020.

Table 2-10. Early and harvest plant stand response to source-rate interactions in 2019 near West Lafayette. Regression is separated by placement. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regression analyses.

Data	Product	Model	Linear		Quad	Quad + Plateau		Model Significance	R^2
			Y-int.	Rate	Rate SQ	x0	Plateau		
Early Plant Stand	ATS	.	.	.	.	.	.	ns	.
(Plants ha^{-1})	KTS	.	.	.	.	.	.	ns	.
(Single)	K-Fuse	Linear	278478	890.5	.	.	.	X	0.77
Early Plant Stand	ATS	Quad+Plateau	303806	-8726.1	794.8	5.5	279856	X	0.76
(Plants ha^{-1})	KTS	Quad+Plateau	303806	-7191.4	654.5	5.5	284052	X	0.77
(Dual)	K-Fuse	Quad+Plateau	303806	-7987.1	790.4	5.1	283629	X	0.78
Harvest Plant Stand	ATS	.	.	.	.	.	.	ns	.
(Plants ha^{-1})	KTS	.	.	.	.	.	.	ns	.
(Single)	K-Fuse	Quadratic	284402	2875.7	-152.4	.	.	**	0.46
Harvest Plant Stand	ATS	Linear	261155	1148.3	.	.	.	X	0.77
(Plants ha^{-1})	KTS	Linear	266376	997.2	.	.	.	*	0.61
(Dual)	K-Fuse	.	.	.	.	.	.	ns	.

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

Table 2-11. Sulfur starter fertilizers effect on early and harvest plant stands, plant height (~R5), yield, seed weight, protein, and oil in 2019 near Wanatah. Regression models pooled over placement (single and dual) since there was no interaction with fertilizer source and rate. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regression analyses.

			Linear		Quad	Quad + Plateau		Model	R^2
Data	Product	Model	Y-int.	Rate	Rate SQ	x0	Plateau	Significance†	
Early	ATS	.	.	.	.	.	.	ns	.
Plant Stand	KTS	.	.	.	.	.	.	ns	.
(Plants ha^{-1})	K-Fuse	Linear	275200	779.1	.	.	.	X	0.86
Harvest	ATS	.	.	.	.	.	.	ns	.
Plant Stand	KTS	.	.	.	.	.	.	ns	.
(Plants ha^{-1})	K-Fuse	Linear	292836	863.2	.	.	.	X	0.86
Plant Height	ATS	.	.	.	.	.	.	ns	.
(cm)	KTS	.	.	.	.	.	.	ns	.
	K-Fuse	.	.	.	.	.	.	ns	.
Yield	ATS	.	.	.	.	.	.	ns	.
(kg ha^{-1})	KTS	.	.	.	.	.	.	ns	.
	K-Fuse	Quadratic	2790	-56.2	2.6	.	.	*	0.72
Seed Weight	ATS	Quad+Plateau	16.8	0.2	-0.01	9.9	17.8	**	0.54
(g 100 seeds^{-1})	KTS	Quadratic	16.9	0.2	-0.01	.	.	**	0.56
	K-Fuse	Quadratic	16.9	0.02	0.001	.	.	**	0.58
Protein	ATS	.	.	.	.	.	.	ns	.
(%)	KTS	.	.	.	.	.	.	ns	.
	K-Fuse	.	.	.	.	.	.	ns	.
Oil	ATS	Quadratic	20.8	0.01	-0.001	-	.	*	0.74
(%)	KTS	.	.	.	.	.	.	ns	.
	K-Fuse	.	.	.	.	.	.	ns	.

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

Table 2-12. Sulfur starter fertilizers effect on early and harvest plant stands, plant height (~R5), yield, seed weight, protein, and oil in 2020 near Wanatah. Regression models pooled over placement (single and dual) since there was no interaction with fertilizer source and rate. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regression analyses.

			Linear		Quad	Quad + Plateau		Model	R^2
Data	Product	Model	Y-int.	Rate	Rate SQ	x0	Plateau	Significance†	
Early	ATS	.	.	.	.	.	.	ns	.
Plant Stand	KTS	.	.	.	.	.	.	ns	.
(Plants ha^{-1})	K-Fuse	.	.	.	.	.	.	ns	.
Harvest	ATS	.	.	.	.	.	.	ns	.
Plant Stand	KTS	.	.	.	.	.	.	ns	.
(Plants ha^{-1})	K-Fuse	Quad+Plateau	257546	-7051.2	675.2	5.2	239137	X	0.80
Yield	ATS	.	.	.	.	.	.	ns	.
(kg ha^{-1})	KTS	.	.	.	.	.	.	ns	.
	K-Fuse	.	.	.	.	.	.	ns	.
Seed Weight	ATS	.	.	.	.	.	.	ns	.
(g 100 $seeds^{-1}$)	KTS	.	.	.	.	.	.	ns	.
	K-Fuse	Quad+Plateau	14.2	0.4	-0.04	5.0	15.1	*	0.76
Protein	ATS	.	.	.	.	.	.	ns	.
(%)	KTS	Quad+Plateau	40.2	0.2	-0.004	21.7	42.5	*	0.73
	K-Fuse	.	.	.	.	.	.	ns	.
Oil	ATS	.	.	.	.	.	.	ns	.
(%)	KTS	Quadratic	20.1	-0.2	0.004	.	.	*	0.74
	K-Fuse	.	.	.	.	.	.	ns	.

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

Table 2-13. Means of leaf N, P, K, S and N:S ratios for UTC, AMS (21.6 kg S/ha), ATS (4 S rates), KTS (4 S rates), K-Fuse (4 S rates), and All (3 Sources x 4 S rates) in 2019 and 2020 near West Lafayette and Wanatah, IN. Orthogonal contrasts summarized between UTC vs. AMS, KTS, ATS, K-Fuse, and All; and AMS vs. ATS, KTS, K-Fuse, and All. Contrasts use the single placement within each fertilizer source since not all rates were sampled from dual placement. Symbol in the contrast column (either UTC or AMS) signifies difference compared to that fertility treatment.

	N g kg^{-1}			P g kg^{-1}			K g kg^{-1}			S g kg^{-1}			N:S		
WEST LAFAYETTE 2019															
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	48.9		50.3	2.9		2.6	16.3		16.5	3.2		3.3	15.5		15.2
ATS-all ¶		50.7			3.0			15.9			3.2			16.0	
KTS-all ¶		50.0			2.8			16.5			3.1	x	x	16.3	*
K-Fuse-all ¶		48.4			2.8			16.4			3.1	x		15.8	
All-Starters ¶		49.7			2.8			16.3			3.2			16.1	
WEST LAFAYETTE 2020															
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	61.6		59.9	3.8		4.0	13.8		15.2	3.3		3.5	18.6	**	17.3
ATS-all		60.2			3.9			14.8			3.4		x	17.9	
KTS-all		61.4			3.9			14.7			3.4			18.1	*
K-Fuse-all		59.7			3.8			14.8			3.3	x		18.2	*
All-Starters		60.4			3.9			14.8			3.3	x	x	18.0	
WANATAH 2019															
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	51.9	*	48.6	3.3		3.2	17.0		18.1	3.1		3.2	16.8	*	15.3
ATS-all		50.5			3.3			15.9	*		3.2		*	15.7	
KTS-all	x	49.7			3.2			16.8			3.1		x	15.9	
K-Fuse-all		51.5	*		3.4	*		16.7			3.2			16.0	
All-Starters		50.6			3.3			16.4	x		3.2		x	15.9	
WANATAH 2020															
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	54.4		54.8	4.2	**	3.7	19.6		20.3	3.1		3.0	17.7		18.1
ATS-all		53.3		*	4.0			21.1			3.1			17.4	
KTS-all		53.2			4.1	x		21.0			3.1			17.2	
K-Fuse-all		55.0			4.2	**		20.5			3.2			17.2	
All-Starters		53.8			4.1	*		20.9			3.1			17.3	

† Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively, between UTC and AMS, UTC vs. ATS, KTS, K-Fuse, and All; and AMS vs. ATS, KTS, K-fuse, and All. The absence of a symbol indicates P>0.10.

Table 2-14. Means of leaf Ca, Mg, Zn, Mn, Fe, Cu and B for UTC, AMS (21.6 kg S/ha), ATS (4 S rates), KTS (4 S rates), K-Fuse (4 S rates), and All (3 Sources x 4 S rates) in 2019 and 2020 near West Lafayette and Wanatah, IN. Orthogonal contrasts summarized between UTC vs. AMS, KTS, ATS, K-Fuse, and All; and AMS vs. ATS, KTS, K-Fuse, and All. Contrasts use the single placement within each fertilizer source as not all rates were sampled from dual placement. Symbol in the contrast column (either UTC or AMS) signifies difference compared to that fertility treatment.

	Ca			**Mg**			**Zn**			**Mn**			**Fe**			**Cu**			**B**		
	g kg^{-1}			g kg^{-1}			mg kg^{-1}			mg kg^{-1}			mg kg^{-1}			mg kg^{-1}			mg kg^{-1}		
WEST LAFAYETTE 2019	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	11.8		11.4	5.8		5.9	43.8		42.2	56.2		66.0	115.6		124.0	11.4		11.6	64.2	*	57.6
ATS-all ¶		11.6			5.9			42.0			55.4			108.0	*		10.9			61.4	
KTS-all ¶		11.3			5.6			40.9			60.8			109.6	*	*	10.3	*		60.2	
K-Fuse-all ¶		11.5			5.5			40.8			62.5			114.2		x	10.5	*	x	59.8	
All-Starters ¶		11.5			5.7			41.2			59.5			110.6			10.6			60.5	
WEST LAFAYETTE 2020	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	11.5		11.0	6.4		6.1	44.1		43.7	33.9		30.2	106.7		104.6	11.6	x	10.8	66.4	x	61.8
ATS-all		11.6			6.3			44.2			34.0			106.0		*	10.9			64.8	
KTS-all		11.4			6.3			44.6			36.8	*		103.1		x	11.0			64.6	
K-Fuse-all		11.6			6.2			42.9			31.3			101.2		**	10.6			63.7	
All-Starters		11.5			6.3			43.9			34.0			103.4			10.8			64.4	
WANATAH 2019	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	9.9		10.4	5.2		4.9	46.4		44.8	128.6	x	160.6	100.4		101.2	9.0		8.2	42.2		39.2
ATS-all		10.1			5.3			45.6			135.9	x		105.3			8.8			40.1	
KTS-all		9.9	x		5.0			46.0			146.9			104.0		*	8.1			39.4	
K-Fuse-all		9.8	x		5.1			46.8			132.6	*		106.1			8.8			41.1	
All-Starters		9.9			5.1			46.1			138.5	x		105.1			8.5			40.2	
WANATAH 2020	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	10.3		10.9	5.3		5.2	41.3		41.2	27.5	*	35.4	95.5		96.4	9.7	x	9.4	63.8		61.2
ATS-all		11.0			5.3			42.3			29.3	*		95.3			9.5			63.7	
KTS-all		10.7			5.1			42.1		*	33.5			96.4			9.5			62.9	
K-Fuse-all		10.6			5.2			43.6		x	32.5			96.3			10.3	*		64.3	
All-Starters		10.8			5.2			42.7			31.8			96.0			9.7			63.7	

† Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively, between UTC and AMS, UTC vs. ATS, KTS, K-Fuse, and All; and AMS vs. ATS, KTS, K-fuse, and All. The absence of a symbol indicates P>0.10.

Table 2-15. Sulfur starter fertilizers effect on R3 most recent mature leaf nutrient concentrations in 2019 and 2020 near West Lafayette. Regression models use the single placement within each fertilizer source and rate since not all rates were sampled from dual placement. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regression analyses. N, P, K, S, N:S, Ca, Mg, Zn, Mn, Fe, Cu and B concentrations were all tested for, however only significant regressions are presented.

Site Year	Data	Product	Model	Linear		Quad	Quad + Plateau		Model Significance	R^2
				Y-int.	Rate	Rate SQ	x0	Plateau		
West Lafayette 2019	Leaf Mn † ($mg\ kg^{-1}$)	ATS	.	.	.	.	.	.	ns	.
		KTS	Linear	50.9	0.80	.	.	.	*	0.61
		K-Fuse	.	.	.	.	.	.	ns	.
	Leaf Fe † ($mg\ kg^{-1}$)	ATS	.	.	.	.	.	.	ns	.
		KTS	Quadratic	115.1	-2.33	0.12	.	.	X	0.61
		K-Fuse	.	.	.	.	.	.	ns	.
	Leaf Cu † ($mg\ kg^{-1}$)	ATS	.	.	.	.	.	.	ns	.
		KTS	Quad+Plateau	11.4	-0.47	0.05	4.62	10.3	X	0.77
		K-Fuse	.	.	.	.	.	.	ns	.
	Leaf B † ($mg\ kg^{-1}$)	ATS	.	.	.	.	.	.	ns	.
		KTS	Linear	63.4	-0.22	.	.	.	X	0.77
		K-Fuse	.	.	.	.	.	.	ns	.
West Lafayette 2020	Leaf N:S †	ATS	Quadratic	18.9	-0.12	0.003	.	.	X	0.62
		KTS	.	.	.	.	.	.	ns	.
		K-Fuse	.	.	.	.	.	.	ns	.
	Leaf Cu † ($mg\ kg^{-1}$)	ATS	.	.	.	.	.	.	ns	.
		KTS	.	.	.	.	.	.	ns	.
		K-Fuse	Quadratic	11.6	-0.21	0.01	.	.	*	0.53

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

Table 2-16. Sulfur starter fertilizers effect on R3 most recent mature leaf nutrient concentrations in 2019 and 2020 near Wanatah. Regression models use the single placement within each fertilizer source and rate since not all rates were sampled from dual placement. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regression analyses. N, P, K, S, N:S, Ca, Mg, Zn, Mn, Fe, Cu and B concentrations were all tested for, however only significant regressions are presented.

				Linear		Quad	Quad + Plateau			
Site Year	Data	Product	Model	Y-int.	Rate	Rate SQ	x0	Plateau	Model Significance	R^2
Wanatah 2019	Leaf N † ($g\ kg^{-1}$)	ATS	.	.	.	.	.	.	ns	.
		KTS	Linear	51.4	-0.11	.	.	.	*	0.52
		K-Fuse	.	.	.	.	.	.	ns	.
	Leaf P † ($g\ kg^{-1}$)	ATS	.	.	.	.	.	.	ns	.
		KTS	Linear	3.3	-0.01	.	.	.	X	0.52
		K-Fuse	.	.	.	.	.	.	ns	.
	Leaf Mn † ($mg\ kg^{-1}$)	ATS	Quadratic	118.9	5.61	-0.26	.	.	*	0.45
		KTS	.	.	.	.	.	.	ns	.
		K-Fuse	.	.	.	.	.	.	ns	.
	Leaf Fe † ($mg\ kg^{-1}$)	ATS	Quadratic	100.6	1.73	-0.08	.	.	*	0.58
		KTS	.	.	.	.	.	.	ns	.
		K-Fuse	.	.	.	.	.	.	ns	.
	Leaf Cu † ($mg\ kg^{-1}$)	ATS	.	.	.	.	.	.	ns	.
		KTS	Quad+Plateau	9.2	-0.41	0.04	5.7	8.1	**	0.52
		K-Fuse	.	.	.	.	.	.	ns	.
	Leaf B † ($mg\ kg^{-1}$)	ATS	Quadratic	44.9	-0.98	0.04	.	.	X	0.66
		KTS	Linear	43.6	-0.28	.	.	.	**	0.52
		K-Fuse	Linear	44.9	-0.27	.	.	.	**	0.55
Wanatah 2020	Leaf Mn † ($mg\ kg^{-1}$)	ATS	Linear	1.0	0.004	.	.	.	*	0.67
		KTS	.	.	.	.	.	.	ns	.
		K-Fuse	.	.	.	.	.	.	ns	.
	Leaf Fe † ($mg\ kg^{-1}$)	ATS	.	.	.	.	.	.	ns	.
		KTS	Quad+Plateau	27.5	0.64	-0.01	24.4	35.3	X	0.99
		K-Fuse	Linear	28.3	0.25	.	.	.	X	0.53
	Leaf Cu † ($mg\ kg^{-1}$)	ATS	.	.	.	.	.	.	ns	.
		KTS	Quadratic	9.9	-0.15	0.01	.	.	X	0.88
		K-Fuse	.	.	.	.	.	.	ns	.

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

Table 2-17. Means of yield, moisture, seed weight, oil, and protein for UTC, AMS (21.6 kg S/ha), ATS (4 S rates x 2 placements), KTS (4 S rates x 2 placements), K-Fuse (4 S rates x 2 placements), and All (3 Sources x 4 S rates x 2 placements) in 2019 and 2020 near West Lafayette and Wanatah, IN. Orthogonal contrasts summarized between UTC vs. AMS, KTS, ATS, K-Fuse, and All; and AMS vs. ATS, KTS, K-Fuse, and All. Symbol in the contrast column (either UTC or AMS) signifies difference compared to that fertility treatment.

	Yield kg ha^{-1}			**Moisture** %			**Seed Weight** g 100 seeds^{-1}			**Oil** %			**Protein** %		
WEST LAFAYETTE 2019															
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	3752	x	3225	12.6		12.5	19.2		19.0	22.3		22.5	36.7		37.0
ATS-all ¶		3646	x		12.5			19.2			22.3			36.9	
KTS-all ¶		3520			12.5			19.1			22.2	x		37.0	
K-Fuse-all ¶		3576			12.5			19.1			22.4			36.8	
All-Starters ¶		3581	x		12.5			19.1			22.3			36.9	
WEST LAFAYETTE 2020															
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	3925		4001	10.6		10.5	17.0		17.0	20.1		20.5	40.4		41.2
ATS-all	x	4137			10.6			17.0			19.9	*	**	41.5	
KTS-all	***	4301	**		10.6			17.2			20.2		*	41.3	
K-Fuse-all	*	4181			10.5		x	17.4			19.8	*	**	41.6	
All-Starters	**	4206	x		10.6			17.2			20.0	x	**	41.5	
WANATAH 2019															
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	2666		2820	12.6		12.6	17.1	**	18.0	20.8	**	20.2	35.5	*	36.0
ATS-all		2745			12.8		**	17.8			20.7	***	*	35.9	
KTS-all		2681			12.6		**	17.8			20.7	***		35.7	
K-Fuse-all		2607	x		12.6			17.4	*		20.6	*	x	35.8	
All-Starters		2678			12.7		*	17.7			20.7	***	x	35.8	
WANATAH 2020															
	UTC		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**	**UTC**		**AMS**
CONTRASTS:	3164		3241	11.5		11.7	14.4		15.1	20.0		19.6	40.5		41.3
ATS-all		3056			11.6			14.7			19.3			41.9	
KTS-all		3051			11.6			14.7		x	19.2			41.9	
K-Fuse-all		3137			11.4			15.1			19.4			41.3	
All-Starters		3081			11.5			14.8		x	19.3			41.7	

† Significance at P ≤ 0.10, 0.05, 0.01, and ≤ 0.001 is denoted by x, *, **, and ***, respectively, between UTC and AMS, UTC vs. ATS, KTS, K-Fuse, and All; and AMS vs. ATS, KTS, K-fuse, and All. The absence of a symbol indicates P>0.10.

Table 2-18. Sulfur starter fertilizers effect on plant height (~R5), yield, protein, and oil in 2019 near West Lafayette. Regression models pooled over placement (single and dual) since there was no interaction with fertilizer source and rate. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regression analyses.

			Linear		Quad	Quad + Plateau		Model	
Data	Product	Model	Y-int.	Rate	Rate SQ	x0	Plateau	Significance†	R^2
Plant Height	ATS	.	.	.	.	.	.	ns	.
(cm)	KTS	Linear	84.8	-0.26	.	.	.	*	0.79
	K-Fuse	.	.	.	.	.	.	ns	.
Yield	ATS	.	.	.	.	.	.	ns	.
(kg ha^{-1})	KTS	Quadratic	3666	35.7	-2.6	.	.	X	0.76
	K-Fuse	.	.	.	.	.	.	ns	.
Protein	ATS	.	.	.	.	.	.	ns	.
(%)	KTS	.	.	.	.	.	.	ns	.
	K-Fuse	.	.	.	.	.	.	ns	.
Oil	ATS	.	.	.	.	.	.	ns	.
(%)	KTS	.	.	.	.	.	.	ns	.
	K-Fuse	.	.	.	.	.	.	ns	.

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

Table 2-19. Sulfur starter fertilizers effect on plant height (~R5), yield, protein, and oil in 2020 near West Lafayette. Regression models pooled over placement (single and dual) since there was no interaction with fertilizer source and rate. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regression analyses.

			Linear		Quad	Quad + Plateau		Model	R^2
Data	Product	Model	Y-int.	Rate	Rate SQ	x0	Plateau	Significance	
Plant Height	ATS	.	.	.	.	.	.	ns	.
(cm)	KTS	.	.	.	.	.	.	ns	.
	K-Fuse	Quad+Plateau	97.7	0.45	-0.01	20.2	102.3	X	0.98
Yield	ATS	Quad+Plateau	3925	90.7	-9.7	4.7	4138	X	0.87
(kg ha^{-1})	KTS	Quad+Plateau	3925	101.9	-6.8	7.5	4308	**	0.67
	K-Fuse	Quad+Plateau	3925	49.5	-2.3	11.0	4198	X	0.79
Protein	ATS	Quad+Plateau	40.4	0.71	-0.11	3.3	41.5	**	0.78
(%)	KTS	Quadratic	40.2	0.18	-0.01	.	.	**	0.67
	K-Fuse	Quad+Plateau	40.4	0.13	0.00	21.1	41.8	*	0.70
Oil	ATS	.	.	.	.	.	.	ns	.
(%)	KTS	.	.	.	.	.	.	ns	.
	K-Fuse	.	.	.	.	.	.	ns	.

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

Table 2-20. Sulfur starter fertilizer effect on seed weight in 2019 and 2020 near West Lafayette. Regression models in 2019 are pooled over placement (single and dual) since there was no interaction with fertilizer source and rate. Regression models in 2020 are separated by placement. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regression analyses.

			Linear		Quad	Quad + Plateau			
Data	Product	Model	Y-int.	Rate	Rate SQ	x0	Plateau	Model Significance	R^2
2019									
Seed Weight	ATS	Quadratic	19.2	-0.04	0.002	.	.	X	0.76
(g 100 seeds^{-1})	KTS	.	.	.	.	.	.	ns	.
	K-Fuse	.	.	.	.	.	.	ns	.
2020									
Seed Weight	ATS	Quadratic	16.9	-0.03	0.003	.	.	X	0.64
Single	KTS	.	.	.	.	.	.	ns	.
(g 100 seeds^{-1})	K-Fuse	.	.	.	.	.	.	ns	.
Seed Weight	ATS	.	.	.	.	.	.	ns	.
Dual	KTS	Linear	17.0	0.03	.	.	.	X	0.77
(g 100 seeds^{-1})	K-Fuse	.	.	.	.	.	.	ns	.

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

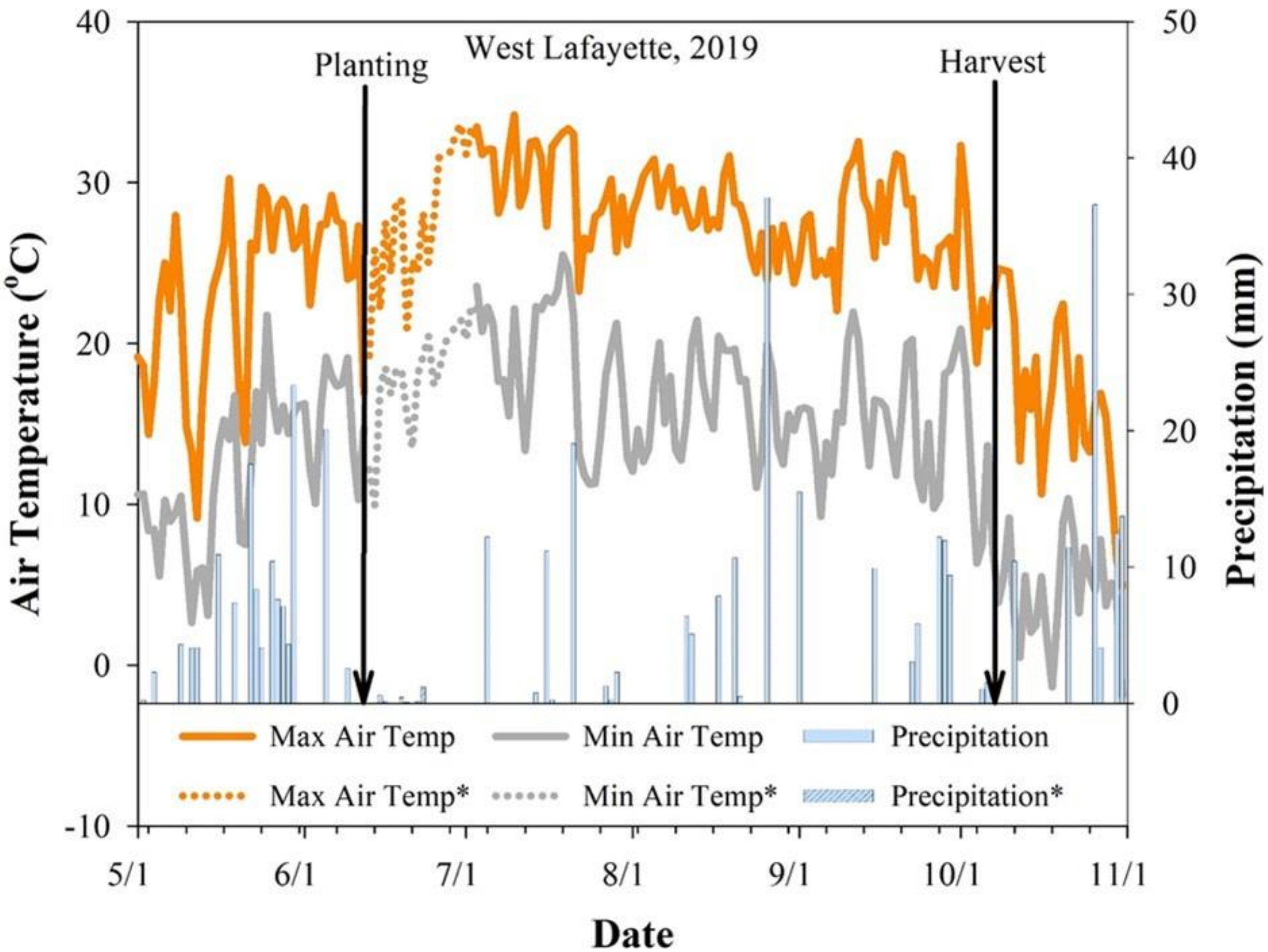

Figure 2-1. West Lafayette 2019 maximum and minimum daily air temperatures and total daily precipitation with planting and harvest dates indicated. Dotted temperature line and dashed precipitation bars are from Lafayette (Throckmorton Purdue Agriculture Center) in 2019 due to lost data from West Lafayette.

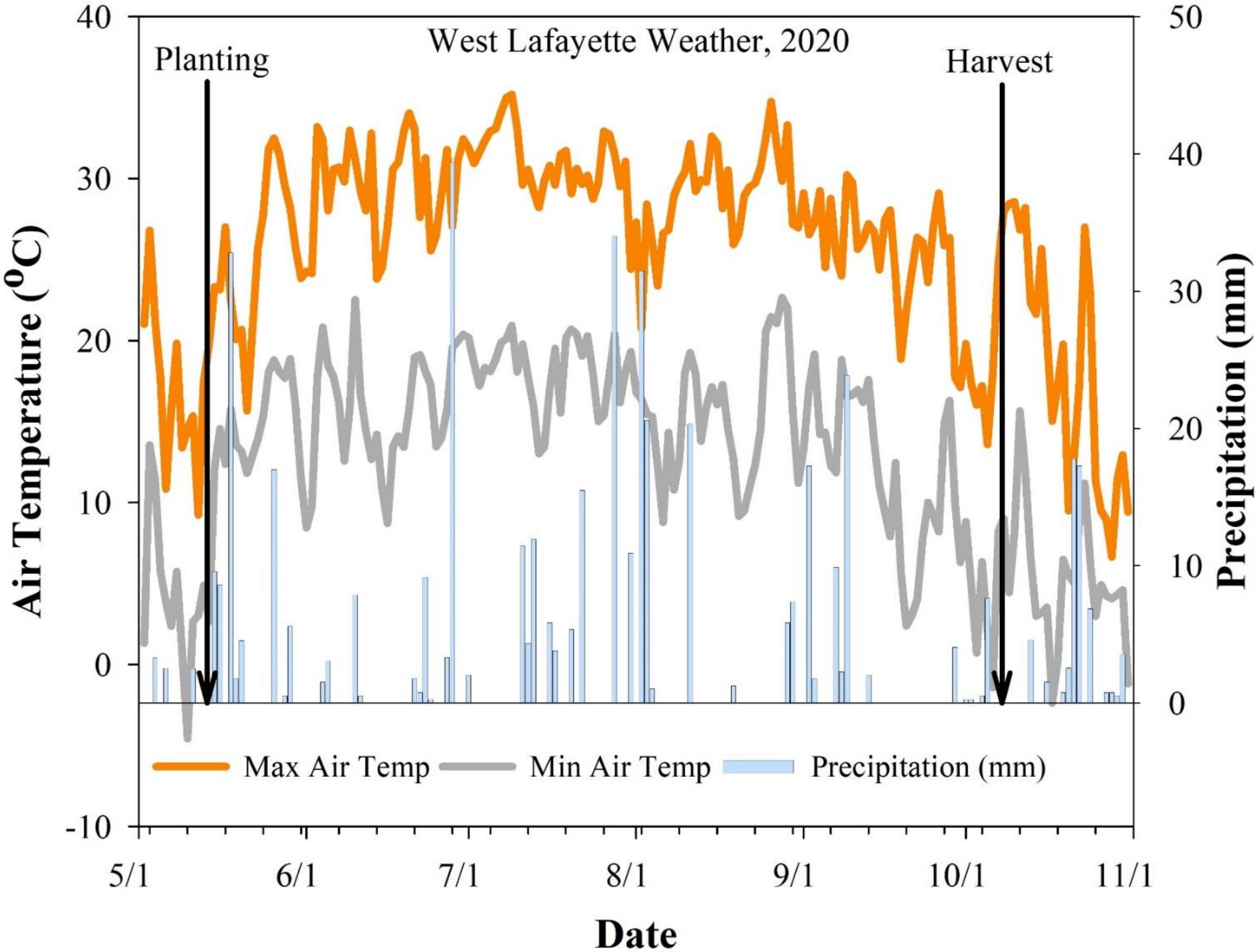

Figure 2-2. West Lafayette 2020 maximum and minimum daily air temperatures and total daily precipitation with planting and harvest dates indicated

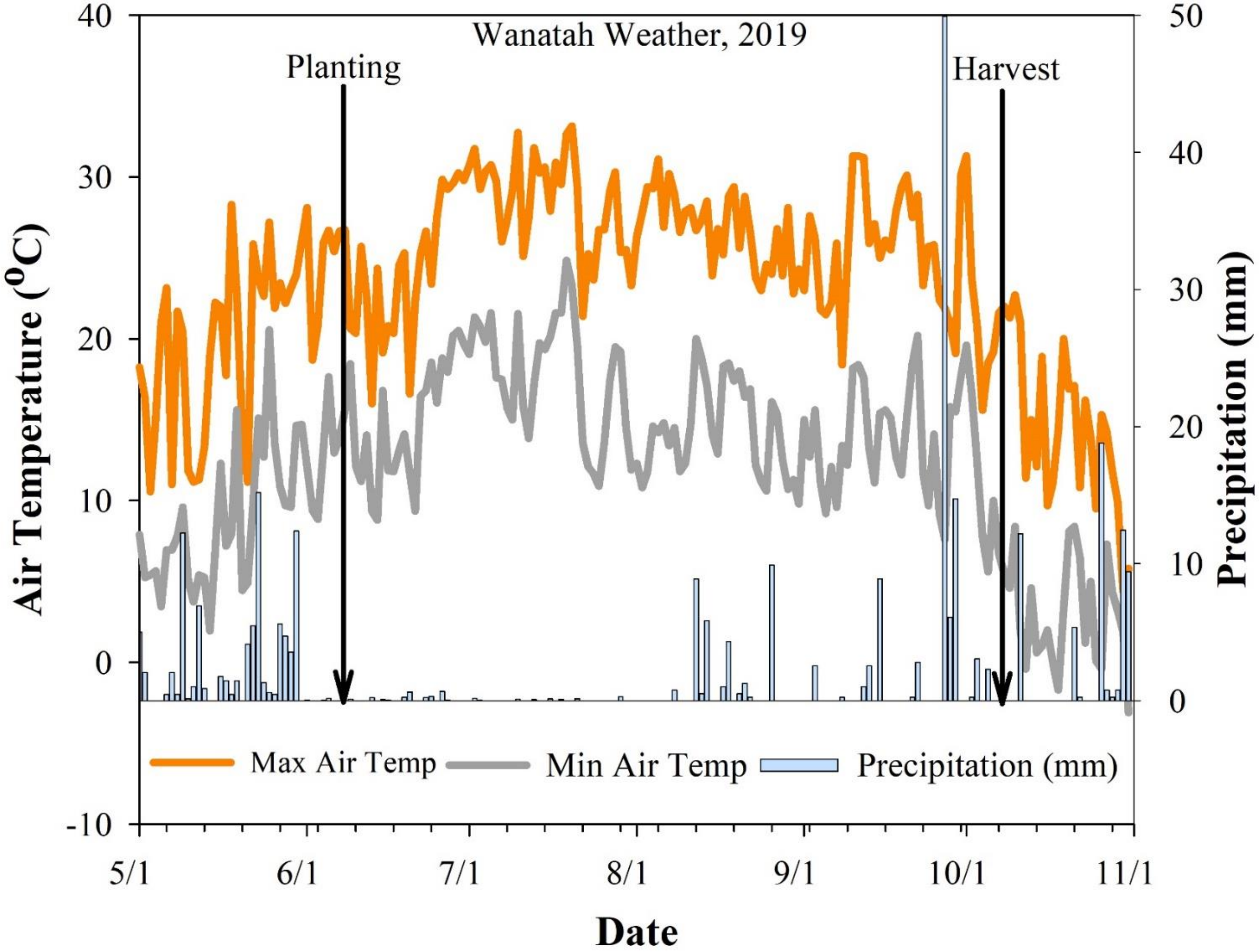

Figure 2-3. Wanatah 2019 maximum and minimum daily air temperatures and total daily precipitation with planting and harvest dates indicated.

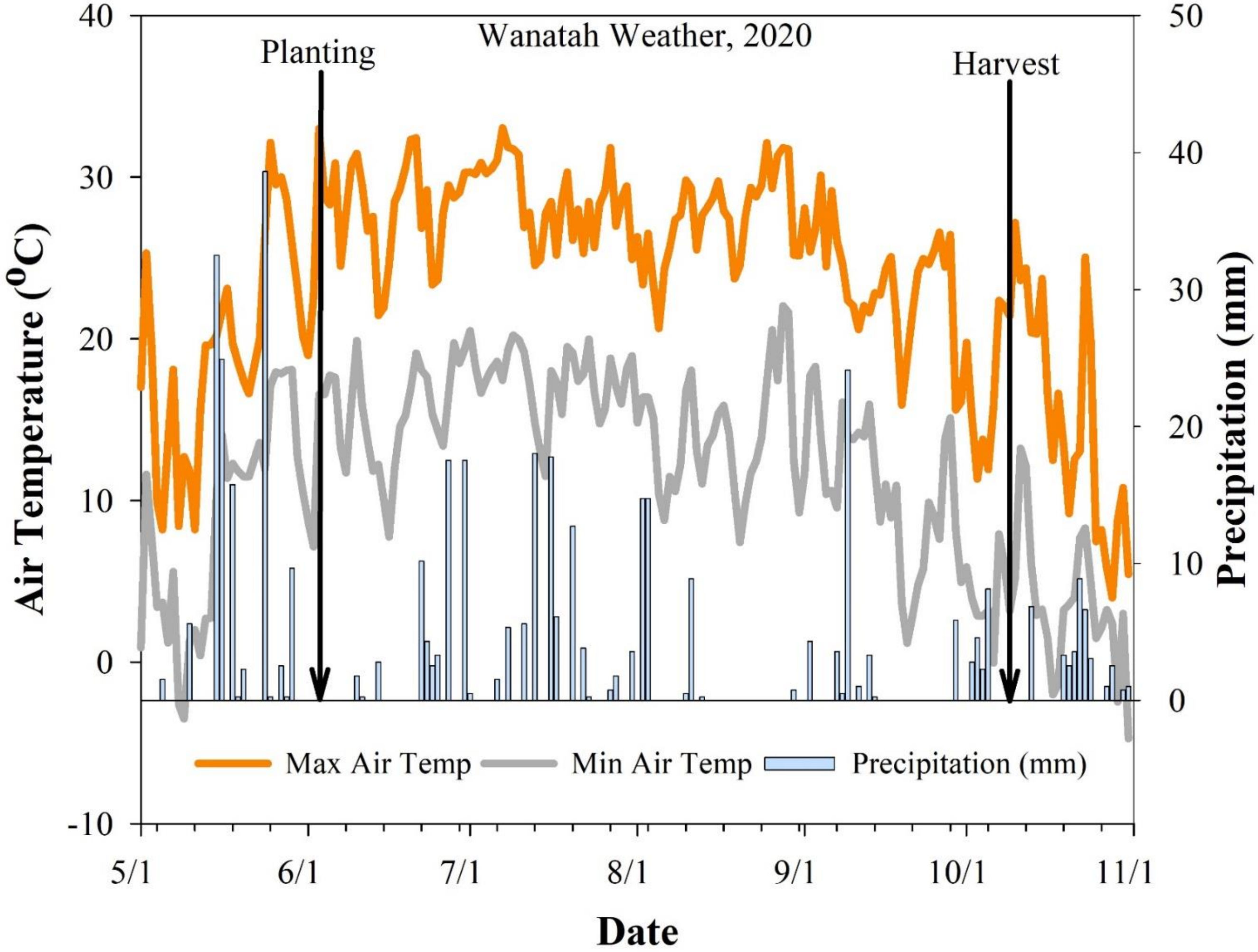

Figure 2-4. Wanatah 2020 maximum and minimum daily air temperatures and total daily precipitation with planting and harvest dates indicated.

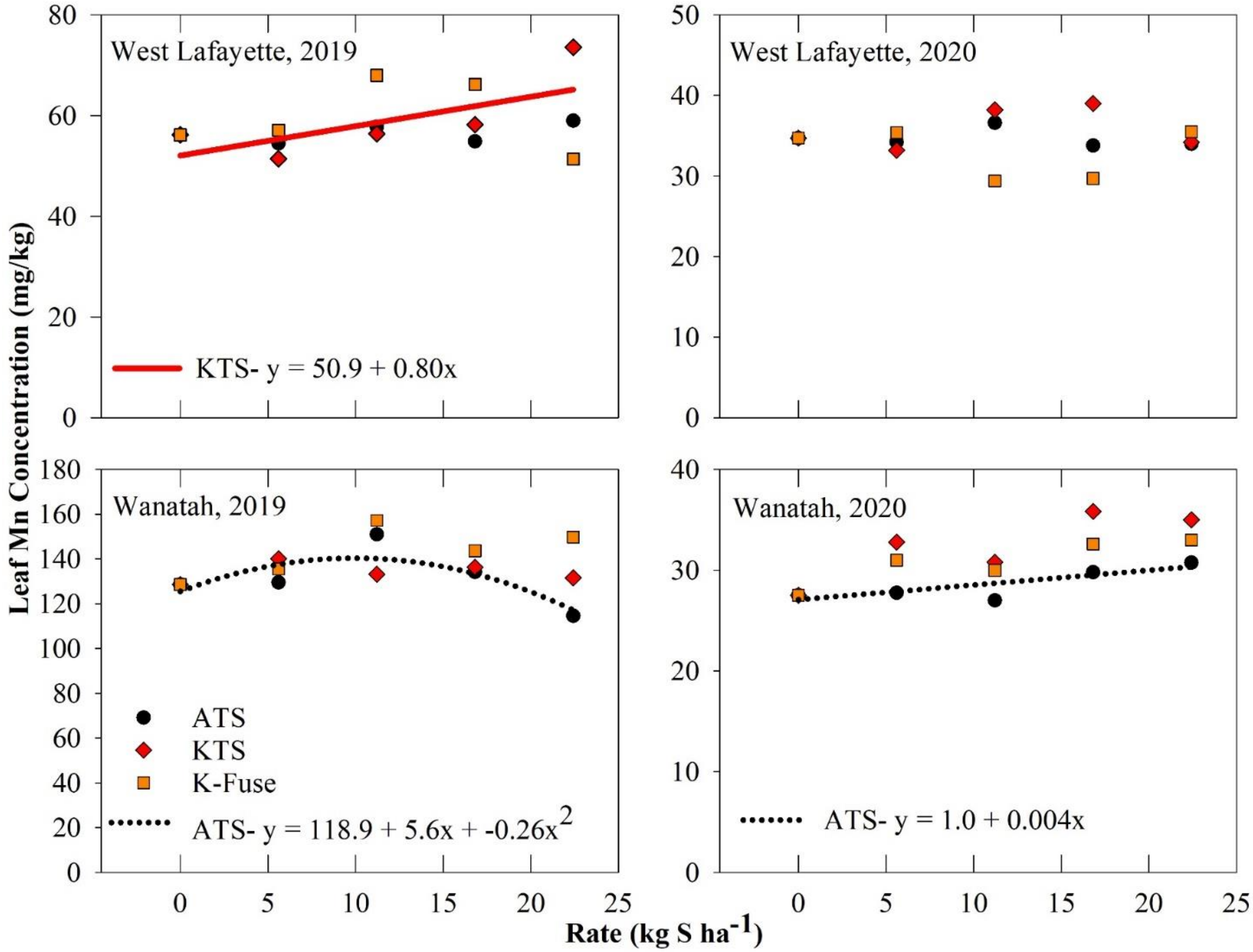

Figure 2-5. Leaf concentrations of Mn in response to starter sulfur fertilizer treatments at West Lafayette and Wanatah in 2019 and 2020. Regression models use the single placement since there was no interaction with fertilizer source and rate, and a reduced data set for dual placement. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regression analyses.

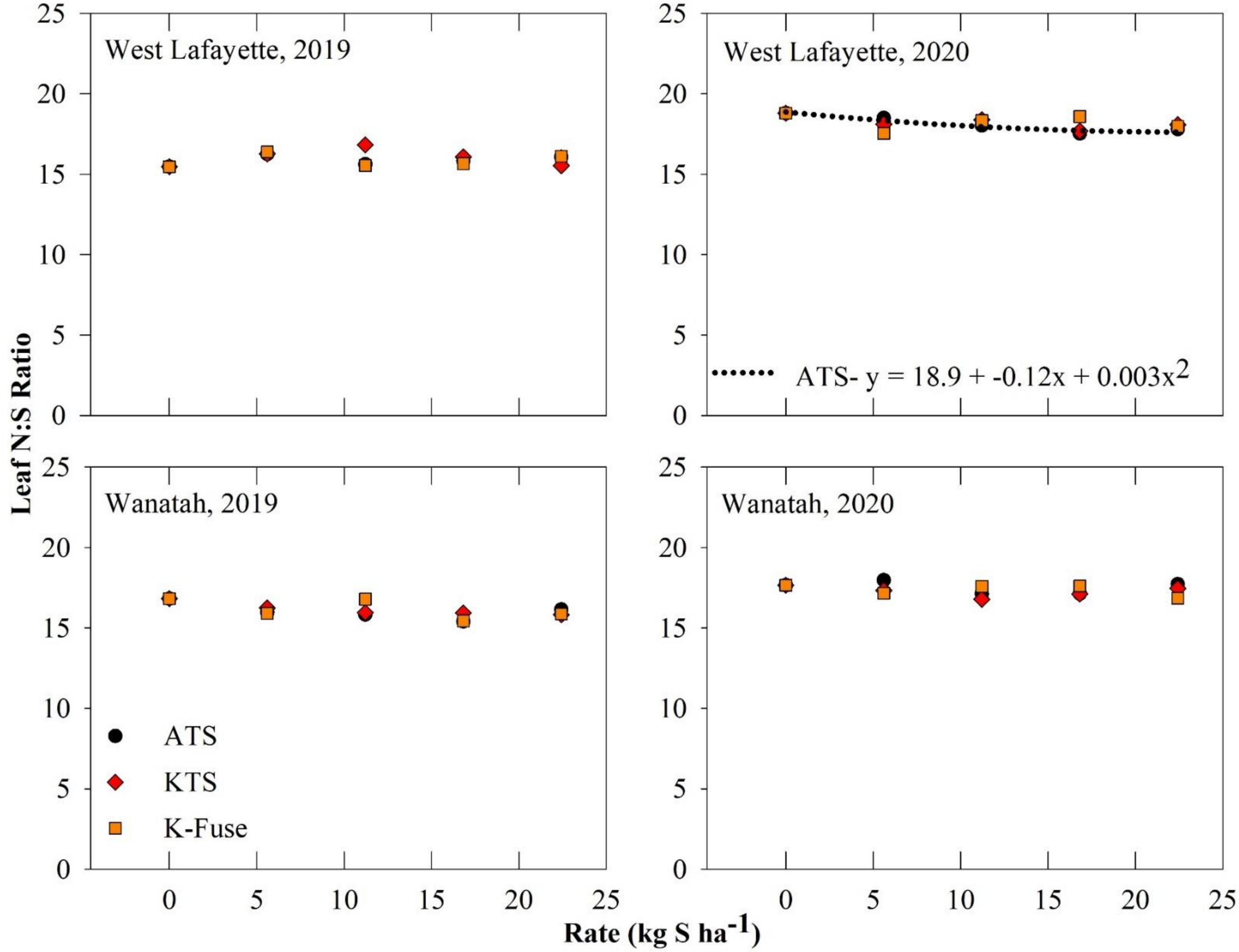

Figure 2-6. Leaf N:S ratio in response to starter sulfur fertilizer treatments at West Lafayette and Wanatah in 2019 and 2020. Regression models use the single placement since there was no interaction with fertilizer source and rate, and a reduced data set for dual placement. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regressions.

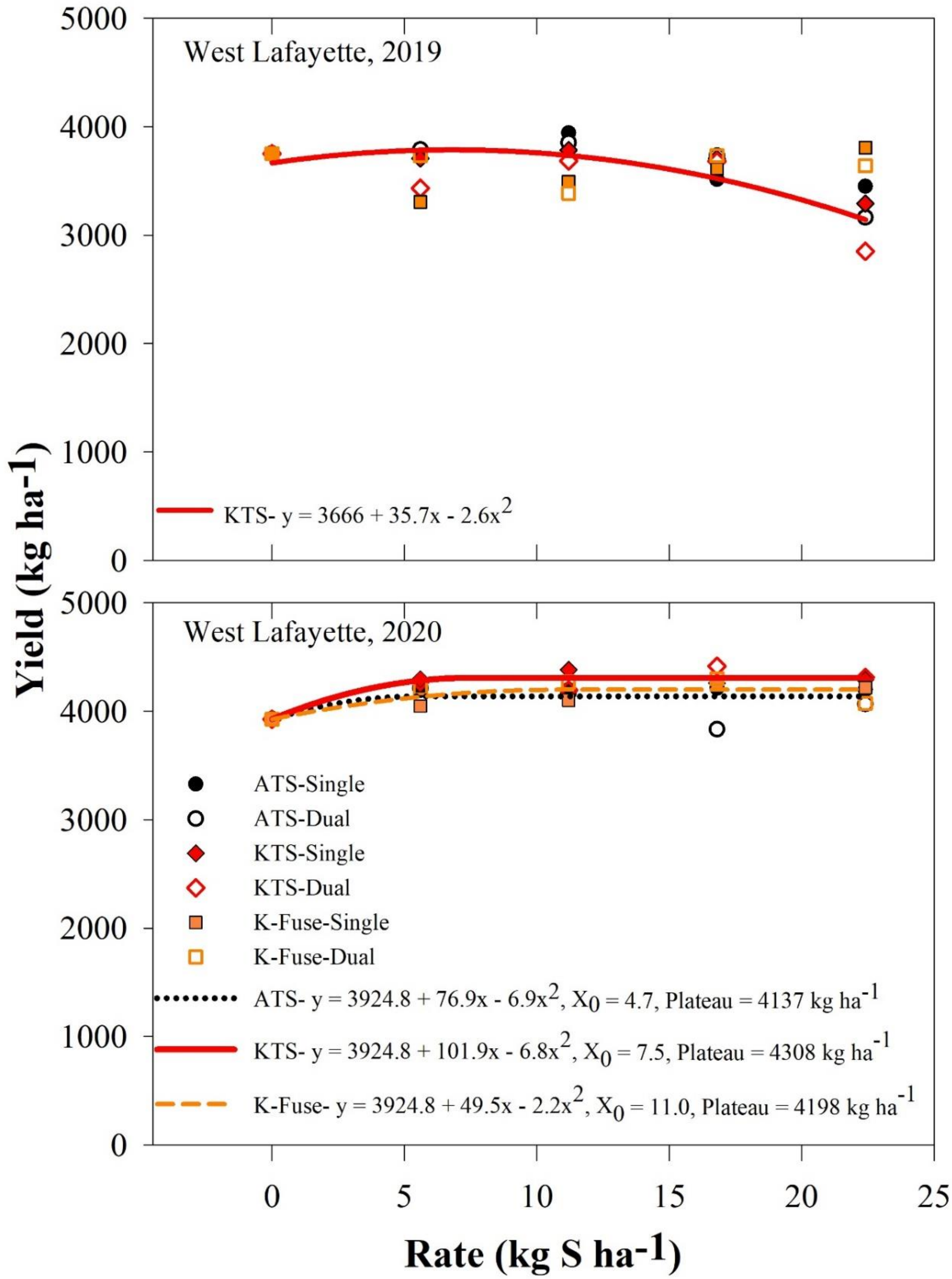

Figure 2-7. Yield response to starter sulfur fertilizer at West Lafayette in 2019 and 2020. Regression models pooled over placement (single and dual) since there was no interaction with fertilizer source and rate. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regression analyses.

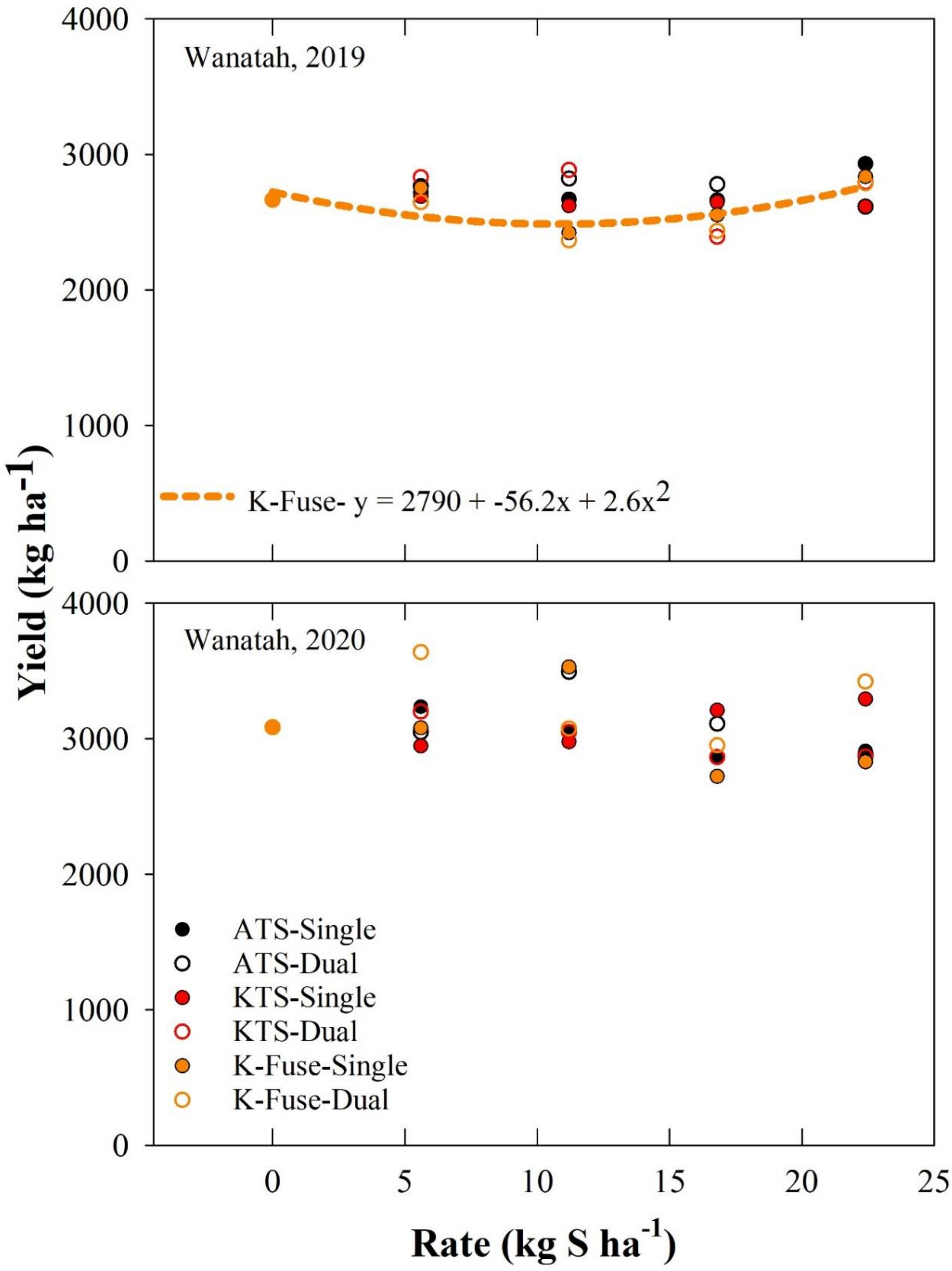

Figure 2-8. Yield response regression to starter sulfur fertilizer at West Lafayette in 2019 and 2020. Regression models pooled over placement (single and dual) since there was no interaction with fertilizer source and rate. Models were chosen based on best fit and significance among linear, quadratic, and quadratic+plateau regression analyses.

CHAPTER 3: YIELD AND QUALITY RESPONSE OF SOYBEAN (*GLYCINE MAX* (L.) MERR. TO STARTER SULFUR FERTILIZER BY PLANTING DATE AND MATURITY GROUP

3.1 Abstract

With the increased interest in planting soybean earlier to maximize the growing season, new tools to improve yield, and the necessity in increasing sulfur supply, starter sulfur (S) fertilizers impact on earlier compared to later planting dates must be thoroughly evaluated. The objectives of this study were to evaluate the effects of starter fertilizer between early and late planting dates, as well as early and late maturing soybean varieties. The six fertility treatments used were: ammonium thiosulfate (ATS, 12-0-0-26S), potassium thiosulfate (KTS, 0-0-25-17S), K-Fuse (derived from potassium acetate, ammonium thiosulfate and urea, 6-0-12-12S, NACHURS), 28% urea ammonium nitrate, (UAN, 28-0-0), ammonium sulfate (AMS, 21-0-0-treatments in West Lafayette, IN in 2020, while two varieties (P24A80X, P35A33X) were crossed with six fertility treatments in Wanatah, IN in 2020. Sulfur fertilizers were applied at 16.8 kg S ha^{-1} and 28% UAN was applied at a 7.9 kg Nitrogen (N) ha^{-1} rate, all in a single (0x5x1 cm) placement.

Early planting yielded more and had more plants than the late planting. Starter fertilizers did not impact yield but did improve oil compared to untreated control. KTS increased protein the most. Leaf S was improved by a Planting x Fertility interaction, where more S was in the leaves of the early planting soybean treated with KTS compared to UTC and any late planted counterparts. Variety impacted protein, but yield was not affected by variety, fertilizer, or variety x fertilizer interaction. Protein was higher in P24A80X compared to P35A33X. There was no

fertilizer effect on any essential nutrient concentration. Additional site-year evaluation will aid in determining planting and varietal influence on starter S fertilizer response.

3.2 Introduction

Along with high yielding varieties and fertilizer applications, a key agronomic management tool used in the Midwest to increase yields in soybean is early planting. According to Conley and Santini (2007), based on survey results, growers in the Midwest have moved to planting soybeans earlier in the season (Conley & Santini, 2007; Robinson et al., 2009). Planting soybeans in April or early May is considered to be an effective strategy to increase yields in Indiana. Yields were projected to decrease by as much as 39 kg per ha per day (0.58 bu per acre per day) when comparing yields from May to June (Hankinson et al., 2015). As more growers plant early, fluid starter fertilizer applications may be required. Starter fertilizer applies nutrients to the root zone for soybean seedlings. At an early planting, insufficient nutrient levels, cold and/or wet soils, root restricting barriers, and slow mineralization are all reasons why a grower would consider a starter sulfur (S) fertilizer application (Touchton & Rickerl, 1986). Despite the interest in starter S fertilizer applications, the effect of starter S fertilizers in early versus late planting dates has yet to be properly quantified. Therefore, our objectives are to determine the effects of starter S fertilizer on soybean yield and yield components when applied at an early (Late April- Mid May) planting date compared to a late (Early-Mid June) planting date, and when using early and late maturing soybean varieties.

3.3 Materials and Methods

3.3.1 Site Characterization

Field activity was conducted over the 2020 growing season at the Pinney Purdue Agricultural Center (PPAC, 41°26'42.1"N, 86°55'48.5"W) in Wanatah, IN and the Purdue Agronomy Center for Research and Education (ACRE, 40°28'18.8"N, 86°59'28.3"W) in West Lafayette, IN. The West Lafayette site was designed as a planting date study with two separate planting dates, while the Wanatah site was designed as a maturity group study on the same planting date. This discrepancy was due to a lack of viable early planting dates at the Wanatah site.

The Wanatah site soil was characterized as a Sebewa loam, shaley sand substratum (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Argiaquolls). The previous crop was corn (*Zea Mays* (L.)), and the field was chisel plowed in the fall of 2019 and field cultivated in the spring of 2020. The West Lafayette site soil was characterized as a Drummer soil (Fine-silty, mixed, superactive, mesic Typic Endoaquolls). The previous crop was corn, and the field was disked in the fall of 2018 and chisel plowed in 2019. Soil fertility levels for both locations before planting and fertilization applications can be seen in

Table 3-1.

3.3.2 Experimental Design

A split-plot design was used to evaluate 2 planting dates crossed by 6 fertility treatments (2 x 6 factorial) (Table 3-2) at West Lafayette in 2020. The main plot factor was planting date (early, May 13 vs. late, June 8) (Table 3-2) and the subplot factor was fertility treatments with 5 replications. Sub-plots were four 76-cm rows wide by 12-m long. The main plot was 76-cm wide by 36-m in length. Pioneer soybean variety P31A22X was used for both planting dates. The first planting date was on May 13, 2020. The second planting date was on June 8, 2020. Both planting dates were planted at populations of 345,000 seeds ha^{-1} in 76-cm rows.

Planting was delayed due to a wet spring at Wanatah in 2020, and thus, the planting date factor was converted to variety. The treatment structure was still a 2 x 6 factorial with two varieties and the six fertility treatments (Table 3-2). The variety trial was arranged in a randomized complex block design (RCBD) with five replications. Plots were four 76-cm rows wide by 12-m long. Pioneer varieties P24A80X (relative maturity 2.4) and P35A33X (relative maturity 3.5) were used to provide different reproductive durations that would simulate a planting date difference with differences in growing conditions during seed fill. The varieties were planted June 2, 2020, at a population of 345,000 seeds ha^{-1} in 76-cm rows.

3.3.3 Starter Fertilizer Treatments

Starter fertilizers were applied using a modified Kincaid Voltra planter with the capability of applying two fluid starter treatments in one planting pass. Exchange plots between main plots were employed to flush, prime, and prepare the planter for the next treatment. The exchange plots were the same length and width as the test plots. Starter fertilizers were applied in a single surface band (0x5x1 cm).

The six fertility treatments were three S starter fertilizers: ammonium thiosulfate (ATS, 12-0-0-26S, Hydrite Chemical), potassium thiosulfate (KTS, 0-0-25-17S, Hydrite Chemical), and K-Fuse (derived from potassium acetate, urea, and ammonium thiosulfate, 6-0-12-12S, NACHURS). This study also included one nitrogen (N) starter fertilizer (28% urea ammonium nitrate, UAN, 28-0-0), one dry S fertilizer (granular ammonium sulfate, AMS, 21-0-0-24S), and one untreated control (UTC). Sulfur-based fertility targeted 16.8 kg S ha^{-1}, which resulted in 7.8 kg ha^{-1} additional N for ATS, 24.7 kg ha^{-1} additional K_2O for KTS and 8.4 kg ha^{-1} additional N and 16.8 kg ha^{-1} additional K_2O for K-Fuse. Broadcast AMS provided 19.6 kg ha^{-1} additional N. A 28% urea ammonium nitrate (UAN) treatment was applied at a 7.9 kg N ha^{-1} to evaluate the potential N effects of starter fertilizer. This rate of N is similar to the amount of N provided by ATS and K-Fuse treatments. Treatments can also be noted in Table 3-2.

3.3.4 Data Collection

Stands were assessed at both studies during the V2-V3 growth stages and at harvest. Three sets of 1-m lengths of plants from the inside two rows were counted in the front, middle and back of each plot. These three counts were averaged to assess plot population. Most recent mature leaf samples were collected from these studies during the R3 growth stage, which occurred first for the May 13 planting at West Lafayette and the P24A80X variety at Wanatah. Samples were taken for the first planting date on July 13, and on August 3 for the second planting date. Samples at Wanatah were collected on July 23 for the 2.4 early maturing group and on July 29 for the 3.5 later maturing group. Samples were collected from the uppermost, fully expanded trifoliate leaf (usually 3rd to 4th leaf from the terminal bud) at R3 (First Pod). Samples were taken by walking the length of the plot between the 2nd and 3rd rows, collecting samples from both side until ~15 trifoliate samples were collected. Samples were dried at 60°C for 3-5 days, ground to a 0.5 mm powder, then sent to A&L Great Lakes Laboratories (3505 Conestoga Dr, Fort Wayne, IN 46808) to assess leaf macro- and micronutrient content.

Upon reaching R8 (full maturity) all plots were first end-trimmed at about 1-m from both sides of the plot to reduce border effects. The two center rows were harvested using a Kincaid 8-

XP plot combine. Plot grain yield, grain moisture, and a grain subset was collected from each plot. The first planting date at West Lafayette was harvested on October 7, 2020, and the second planting date on October 14, 2020. The maturity group study at Wanatah was harvested in its entirety on October 8, 2020. Grain yield was calculated per plot and adjusted to 13% moisture. NIR (Perten Model DA7350 NIR) was used on a subset of the grain samples to assess protein and oil content, and seed weight was determined by counting two 100-seed subsamples of each plot and adjusting to 13% moisture.

3.3.5 Field Management

Wanatah received two post-emergence herbicide applications. The first was on June 18of 0.28 L ha^{-1} glyphosate, 0.007 L ha^{-1} cloransulam-methyl, and 0.76 L ha^{-1} of glyphosate activator adjuvant. The second application occurred on July 6 and consisted of 0.38 L ha^{-1} glyphosate, 2.3 L ha^{-1} Eezy Man, at a rate of 142.9 g Mn ha^{-1}, and 0.76 L ha^{-1} of glyphosate activator adjuvant. The West Lafayette location received one post-emergence herbicide application of 0.38 L ha^{-1} glyphosate and 0.05 L ha^{-1} fluazifop-P-butyl on June 18.

3.3.6 Statistical Analysis

Analysis of variance (ANOVA) using PROC GLM in SAS (SAS Institute, version 9.4) was used to test the main effect at each site. Planting x Starter Fertility in West Lafayette was a split-plot, so planting date was the whole plot factor and starter fertility was the subplot factor. Fisher’s protected LSD was used to compare significant mean separations at an alpha level of 0.05. Variety x Starter Fertility in Wanatah was a RCBD and was analyzed in a factorial structure with variety, starter fertility, and variety x starter fertility. Fisher’s protected LSD was used to compare significant mean separations at an alpha level of 0.05.

3.4 Weather

Past growing season averages from 2002-2018 were compiled and calculated and presented in Table 2-6 and Table 2-7. Weather data for 2020 from both locations can be seen in Figure 3-1 and Figure 3-2.

3.5 Results and Discussion: Starter by Planting Date - West Lafayette

3.5.1 Early and Harvest Population

Early season stands were impacted by planting date. The early planting (May 13) had 15,500 more plants ha^{-1} than late planting (June 8) (Table 3-4). Since the same variety was used for both planting dates, field conditions associated with the two planting dates was the underlying difference in stand establishment. Limited rainfall in June, compared to May (Table 2-6, Figure 3-1), likely influenced stand establishment. However, early plant populations of both planting dates were within acceptable ranges, and by harvest plant stands did not differ between planting dates (Table 3-4). Fertility did not impact plant stands in early season (approximately 324,000 plants ha^{-1}) or at harvest (approximately 255,000 plants ha^{-1}).

3.5.2 Plant Nutrient Concentration- N, P, K, S, N:S, Ca, Mg

Planting date influenced leaf N, potassium (K), and the N:S ratio. The interaction of planting date x fertility impacted leaf S and calcium (Ca). Leaf magnesium (Mg) was influenced by planting date and fertility, but not the interaction. Leaf N was greater in the late versus early planting dates (Table 3-5). The highest S concentration was with KTS in early planting, while the lowest S concentration was with broadcast AMS in late planting (Table 3-5). KTS increased S from UTC in the early planting date (3.1 vs. 2.7 g S kg^{-1}), but not in the late planting date. In the late planting date, S concentrations of K-Fuse and 28% UAN were higher than UTC (Table

3-5). Leaf N:S ratio did not differ from planting date or fertility treatment and ranged from 17.8 (KTS) to 19.3 (UTC). Leaf Ca in UAN and AMS in the early planting date were higher than the late planting (Table 3-5). Leaf Ca in late planting was approximately 9.0 g kg^{-1} regardless of fertility (Table 3-5). Leaf Mg was higher in the earlier planting date than the later, and only UAN resulted in leaf Mg higher than the other treatments (Table 3-5).

With regards to N, it is likely that in an earlier planting date, with a longer growth period, this treatment was in a more advanced reproductively compared to the later plant date, and therefore had partitioned more of the nutrients out of the leaves and into the developing reproductive organs, like pods and seeds. Additional N provided by the starter fertilizers did not impact leaf concentrations. In contrast, leaf S changed with fertility, but was not consistent between the two planting dates. The soil within the early planting was likely cool compared to the late planting, and thus, there limited mineralization of soil organic matter to supply N and S. The responsiveness of soybean to additional S (and N) in early planting was only noted from KTS. The maximum air temperature was approximately 10^{o}C cooler during the first 30 days of early planting vs. the first 30 days of late planting (Table 2-6, Figure 3-1). KTS provided the most additional K, and when combined with the S rates, may have increased S uptake (Table 3-2). In a study evaluating starter fertilizer on soybean in Alabama, Touchton and Rickerl (1986) found that starter fertilizer, particularly that of N, stimulated early season root growth. This could explain the increased S uptake, wherein the later planting, the additional N in K-Fuse and UAN may have increased root biomass growth, effectively leading to increased S uptake (Touchton & Rickerl, 1986). However, this effect was not seen in other N containing fertilizers, therefore exact nature of why this effect was only seen in K-Fuse and UAN is unknown at this time. The lack of a difference in N or S leaf concentrations explains the lack of a corresponding response in the N:S ratio.

The differences in Ca uptake could be explained through greater biomass growth experienced when planting earlier, allowing for more Ca uptake from the soil. Additional N may have further increased biomass development, explaining how UAN may have influenced the increase in Ca uptake greater than other sources (Touchton & Rickerl, 1986). Given this information, it may seem that liquid S treatments decreased Ca uptake, while the liquid N treatment alone may have enhanced it. The exact nature of this is unclear, although it is possible that S based fertilizers, especially those with thiosulfate, are burning roots or stunting development by salt injury, causing the discrepancy (Camberato, 2019; Mortvedt, 2001).

A similar effect was noted with regards to Mg, where greater biomass development from planting earlier allowed for more Mg uptake, while further N additions from UAN without S increased biomass, thereby increasing Mg uptake (Touchton & Rickerl, 1986). Additionally, Mg uptake can be reduced in the presence of NH_4^+ and K fertilizers. This is described by Havlin (2005) wherein K^+ alters the CEC, displacing Mg^{2+} on the soil particle. Havlin (2005) also noted that and H^+ released when NH_4^+ is absorbed by roots can interfere with Mg^{2+} uptake. UAN, not strictly comprising of only NH_4^+, could be allowing for N additions to contribute to greater biomass growth without competing with Mg.

3.5.3 Plant Nutrient Concentration- Fe, B, Zn, Mn, Cu

Leaf Iron (Fe) and boron (B) concentrations were affected by planting date and fertility alone. Leaf Zinc (Zn) concentrations were impacted by planting date only (

Table 3-6). Leaf Zn, Fe, and B concentrations were greater in the early planting date vs. late planting date. In terms of fertilizer effects, UAN was highest in leaf Fe and B (Table 3-6).

Planting date x fertilizer interaction affected leaf manganese (Mn) and copper (Cu). K-Fuse had the lowest concentration of Mn (21.0 mg kg^{-1}), but was still above the critical level of 10-20 mg kg^{-1} (Broadley et al., 2012). AMS (7.8 mg kg^{-1}) and ATS (8.4 mg kg^{-1}) within the late planting was lower in leaf Cu concentrations than UTC (9.4 mg kg^{-1}) (Table 3-6).

With regards to the greater concentration of Fe, copper (Cu) and Zn in the earlier planting date, it is well known that earlier planting generally leads to more biomass growth and therefore greater nutrient uptake (Hu & Wiatrak, 2012). UAN, showing the greatest increase in concentration could have contributed to increases in early season biomass growth, total growth, and increased uptake like those noted by Touchton and Rickerl (1986). Lastly, a broadcast application of AMS within late planting disrupted Cu uptake, but Cu concentrations did not differ from the UTC in the liquid starter fertilizer treatments.

Planting date x fertilizer interaction on leaf manganese (Mn) was significant where K-Fuse was lowest in the early planting. The combination of all three nutrients, N, K and S, as well as injury from thiosulfate, salt injury, or competition for uptake of other nutrients previously discussed, could have influenced this effect (Camberato, 2019; Mortvedt, 2001).

3.5.4 Yield

Yield was higher in the early planting date than in the late planting date (4862 vs. 3960 kg ha^{-1}) (Table 3-4). Fertility did not influence yield and ranged from 4210 to 4627 kg ha^{-1} for each treatment pooled over plantings. Early planting allows for greater yield potential through more trifoliate nodes, reproductive branches, and reproductive duration (Hankinson et al., 2015; Robinson et al., 2009). Greater precipitation earlier in the season likely allowed greater access to nutrients (Table 2-6). There was less than average rain in June, possibly limiting the yield of the later planting date through a decrease in germination and early plant growth. However, stands in the late plantings were still within agronomically acceptable ranges.

3.5.5 Seed Weight

Planting date did not affect seed weight (Table 3-4). Seed weight with ATS (18.1 g 100 seeds^{-1}) was higher than seed weight with KTS (17.5 g 100 seeds^{-1}) with no other fertility differences (Table 3-4).

With no difference in seed weight, the source of higher yields within early planting were likely due to more pods per plant and/or seeds per pod. The slight fertility difference is puzzling, considering the only difference between ATS and KTS is the addition of N instead of K. A possible explanation is that ATS increased growth, through the addition of N as described by Touchton and Rickerl (1986), compared to KTS, allowing for greater seed weight development. Alternatively, it is possible that KTS reduced growth (e.g., stunting) through salt injury early in the season. Salt injury could result in smaller roots that impeded water and nutrient uptake and thereby, limit seed fill by a reduction in photosynthates from the plant and limited water uptake

3.5.6 Protein and Oil Content

Oil and protein concentrations were not impacted by planting date or fertility (Table 3-4). Interestingly, protein concentration was unchanged with the addition of any fertilizer treatment. Conditions were slightly drier in August through October of 2020, and reduced precipitation may have reduced protein content (Table 2-6).

3.5.7 Summary of Starter x Planting Date

These results were considered preliminary as they only encompass one site-year at one location. Yield benefited from an early planting, but the full impact of starter fertilizer was not clear. Several factors may help to clarify the starter fertilizer potential, such as an earlier planting date, multiple locations with early vs. late planting, and more replications. Starter S fertilizer applied in an early planting improved leaf S concentration (i.e., KTS applied with early planting), but none of the other products improved leaf S from UTC. Sulfur supply did not appear to be limited, therefore reducing the potential benefits of the starter S fertilizer treatments. The current study was also conducted in a nutrient-rich soil and thus, starter S fertilizer experiments should be evaluated in S-deficient fields.

3.6 Results and Discussion: Starter Sulfur by Variety- Wanatah

3.6.1 Early and Harvest Populations

Early season stands in Wanatah were not impacted by variety or fertility treatment. Average stands were higher in the 3.5 maturity group than the 2.4 group, at 318,898 plants ha^{-1} in 35A33X and 311,899 plants ha^{-1} in 24A80X (Table 3-7). Stands at harvest were not affected by variety or fertility treatment (Table 3-7).

These varieties were planted on the same day under the same settings and conditions; therefore, the lack of a variety response was to be expected. The lack of a fertilizer treatment effect at both site years was fairly consistent with the findings of the starter by placement study, in that a single placement of starter S fertilizer often did not affect early season and harvest plant stands.

3.6.2 Plant Nutrient Concentration

Many nutrient concentrations differed by variety. Concentrations of N, S, Ca, Zn, Mn, Fe, Cu were all higher in the 3.5 maturity group variety than the 2.4 variety (Table 3-8, Table 3-9). In contrast, leaf Mg was lower in variety 35A33X compared to variety 24A80X.

Overall, the general nutrient trend observed was likely a result of the early maturity group remobilizing and partitioning nutrients to reproductive development earlier than the later maturing variety. The earlier maturing variety had likely entered R3 earlier, resulting in lower leaf nutrient concentrations of most essential nutrients as nutrients were remobilized out of the leaves and into developing pods. Fertility and variety x fertility did not influence any of the nutrients (Table 3-8, Table 3-9). Additionally, planting at this location was delayed until June 2 reducing the overall effectiveness of starter S fertilizer. Finally, the site experienced droughty conditions in August through October, which could have had an adverse effect on nutrient uptake through the lack of nutrient movement via water (Table 2-7). Sulfur concentrations of all planting date and fertility treatments were above critical levels at 2.5 g S kg^{-1} (Table 3-8) (Hitsuda et al., 2008b).

3.6.3 Yield

Yield at Wanatah was not impacted by variety, fertility, or variety x fertility interaction (Table 3-7). Yields at this site were fairly low compared to the starter x planting date trial, averaging around 3000 kg ha^{-1} for both varieties under all fertility treatments (Table 3-4, Table 3-7). This is likely an effect of the limited rainfall later in the growing season, coupled with a later planting date. This is similar to Robinson's (2009) study, where soybean plantings into late June in Indiana greatly reduced yield (Robinson et al., 2009). The total precipitation in 2020 at Wanatah was less than the past averages in August, September, and October (Table 2-7). In

August alone, the field only received 40 mm of rain, compared to a 94 mm average in past seasons.

3.6.4 Seed Weight

Seed weight was not influenced by variety, fertility, or variety x fertility interaction at Wanatah in 2020. Seed weight averaged across both varieties and all fertility treatments was 12.8 g 100 seeds^{-1}. As with yield, the droughty conditions during seed fill likely reduced the chance of any perceivable response (Table 3-7).

3.6.5 Protein and Oil Content

Oil concentration was not impacted by variety, fertility, or variety x fertility interaction, but protein concentration was impacted by variety. Protein concentration of variety 24A80X was higher than variety 35A33X (41.6% vs, 40.2% protein) (Table 3-7) with no fertility or variety x fertility interaction.

Hammond (2005) described how protein synthesis is increased late season droughts and later plantings (Hammond et al., 2005). Later, Mourtzinis (2017) found that late plantings of maturity groups ranging from 0.6-2.0 resulted in the greatest protein content compared to earlier plantings of the same varieties, where the earliest maturity groups had the highest oil content of the varieties planted in the later planting date. The earlier maturity varieties having high protein content is likely a result of faster maturation, leading to increased protein synthesis (Table 2-7). Incidentally, as protein synthesis is favored, oil production is decreased, thus reducing the possibility of fertility x variety effects on oil content (Hammond et al., 2005; Mourtzinis et al., 2017).

3.6.6 Summary of Starter x Variety

Starter fertility had no impact on plant stand, leaf nutrition, yield, protein, and oil in 2020 at Wanatah. This single site-year was planted late and experienced droughty conditions during seed fill. Varietal differences were present in leaf nutrition and protein. The early maturing group matured faster, resulted in more protein, and less nutrient concentrations in the leaf at R3, while the later maturing group had less protein and greater leaf nutrient concentrations. These results were compatible with previously studied maturing group effects on functional traits like protein described by Mourtzinis (2017) and the movement of nutrients described by Bender (2015). No yield differences were observed, likely due to later planting and dry conditions during seed fill, reducing yield overall and impacting the effectiveness of starter fertilizers.

The effectiveness of starter S fertilizer on leaf nutrition, yield, and quality likely diminished as planting was delayed into June. In other words, mineralization was active in the warmer soils. A continuation of this study should utilize an earlier planting date as well as fields that have a history of S deficiency and/or S responsiveness. Another possible avenue of research is to investigate multiple varieties representing short and full season varieties.

Table 3-1. Soil fertility analyses for Starter Sulfur x planting date in 2020 from locations near West Lafayette and Wanatah, Indiana. Samples were taken prior to fertilizer applications and planting in each respective site. The mean values are averaged over replications. (+ standard deviation)

Soil Analyses	**West Lafayette†**			**Wanatah**		
OM ($g\ kg^{-1}$)	39	±	2	32	±	2
pH	6.8	±	0.2	6.0	±	0.1
CEC ($cmol\ kg^{-1}$)	24.5	±	0.9	17.0	±	0.8
P ($mg\ kg^{-1}$)	25	±	4	47	±	3
K ($mg\ kg^{-1}$)	147	±	6	145	±	4
Mg ($mg\ kg^{-1}$)	827	±	14	496	±	12
Ca ($mg\ kg^{-1}$)	3199	±	101	1822	±	81
S ($mg\ kg^{-1}$)	5	±	1	5	±	1
Zn ($mg\ kg^{-1}$)	2.5	±	0.2	2.9	±	0.1
Mn ($mg\ kg^{-1}$)	11	±	2	11	±	3
Fe ($mg\ kg^{-1}$)	141	±	6	179	±	18
Cu ($mg\ kg^{-1}$)	3.7	±	0.1	2.3	±	0.1
B ($mg\ kg^{-1}$)	0.6	±	0.2	0.2	±	0.1

† Soil Fertility data from this site year was lost, so fertility data presented is from an adjacent trial in the same field as Starter Sulfur x planting date at West Lafayette.

Table 3-2. Treatments and resulting nutrients of Starter Sulfur by Planting Date at West Lafayette and Starter by Variety at Wanatah.

Treatment	**Planting Date / Variety**	**Treatment**	**Rate L/ha**	**kg S ha^{-1}**	**kg N ha^{-1}**	**kg K_2O ha^{-1}**
1	**Early 5/13/2020**	Broadcast_AMS	93.9 kg ha^{-1}	16.8	19.6	.
3		ATS_15	47.3	16.8	7.8	.
5		K-Fuse_15	104.9	16.8	8.4	16.8
7		KTS_15	64.9	16.8	.	24.7
9		28% UAN	22.5	.	7.9	.
11		UTC	0	0	0	0
2	**Late 6/8/2020**	Broadcast_AMS	93.9 kg ha^{-1}	16.8	19.6	.
4		ATS_15	47.3	16.8	7.8	.
6		K-Fuse_15	104.9	16.8	8.4	16.8
8		KTS_15	64.9	16.8	.	24.7
10		28% UAN	22.5	.	7.9	.
12		UTC	0	0	0	0

Table 3-3. Planting, sampling dates and harvest at West Lafayette and Wanatah in 2020.

West Lafayette, IN	
Date	**Field Activity**
05/13/2020	First planting date planted
06/08/2020	Second planting date planted
07/13/2020	First planting date MRML samples collected (~R3)
08/02/2020	Second planting date MRML samples collected (~R3)
10/02/2020	First planting date harvested
10/14/2020	Second planting date harvested
Wanatah, IN	
Date	Field Activity
06/02/2020	Planted both varieties
07/23/2020	2.4 maturity group MRML samples collected (~R3)
07/29/2020	3.5 maturity group MRML samples collected (~R3)
10/08/2020	Harvested

Table 3-4. Soybean stand and seed quality responses to planting date across fertility treatments. Study was located near West Lafayette, IN in 2020. Analysis uses Fishers protected LSD, α=0.05. Pdate refers to Planting Date, fertility refers to Fertility treatment.

	Early Season Stand and Seed Quality Response						
Main Effect	**Early Season Population** Plants ha^{-1}	**Harvest Population** Plants ha^{-1}	**Yield** kg ha^{-1}	**Moisture** %	**Seed Weight** g 100 seeds^{-1}	**Oil** %	**Protein** %
Early- 5/13/2020	324366 A	255680	4862 A	13.3 B	18.4	20.1	39.7
Late- 6/8/2020	308837 B	252625	3960 B	14.2 A	17.3	19.4	39.8
UTC	324147	265092	4210	13.5	18.1 ab	19.6	40.2
AMS	319554	262467	4391	13.6	18.0 ab	20.1	39.3
ATS	318242	248032	4627	14.1	18.1 a	19.4	40.1
KTS	317586	236221	4439	13.8	17.5 b	20.1	39.5
K-Fuse	310368	250073	4212	13.6	17.7 ab	19.6	39.4
28% UAN	309712	262467	4524	13.9	17.7 ab	19.7	39.7
Pdate †	*	ns	**	*	ns	ns	ns
Fertility †	ns	ns	ns	ns	*	ns	ns
Pdate x Fertility †	ns	ns	ns	ns	ns	ns	ns
CV (%)	5.8	10.5	10.7	5.0	4.4	4.8	2.4

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

Table 3-5. Most recently matured leaf nutrient concentrations in responses to different planting dates across fertility treatments. Study was located near West Lafayette, IN in 2020. Analysis uses Fishers protected LSD, α=0.05. Pdate refers to planting date, Fertility refers to fertility treatment, PD1 and PD2 specify interactions within specific planting dates.

	R3 MRML Nutrient Concentrations Response														
	N		**P**	**K**	**S**				**N:S**	**Ca**				**Mg**	
Main Effect	g kg^{-1}		g kg^{-1}	g kg^{-1}	g kg^{-1}					g kg^{-1}				g kg^{-1}	
Early- 5/13/2020	51.8	B	3.2	18.7	2.9				18.0	11.7				5.2	A
Late- 6/8/2020	53.0	A	3.5	20.6	2.8				18.7	8.9				3.7	B
Fertility					**PD1**		**PD2**			**PD1**		**PD2**			
UTC	52.9		3.3	19.6	2.8	cde	2.7	de	19.3	11.8	bc	8.6	d	4.4	b
AMS	51.3		3.4	20.1	2.9	bcd	2.7	e	18.5	12.3	ab	9.1	d	4.3	b
ATS	51.8		3.4	19.5	2.9	abc	2.8	cde	18.0	11.1	c	9.1	d	4.4	b
KTS	52.4		3.5	20.2	3.1	a	2.8	cde	17.8	11.0	c	9.4	d	4.3	b
K-Fuse	53.0		3.2	19.4	2.9	bcd	3.0	ab	18.0	11.3	bc	8.8	d	4.4	b
28% UAN	52.8		3.4	19.1	2.8	cde	2.9	abc	18.4	12.8	a	8.7	d	5.0	a
Pdate †	*		ns	ns	ns				ns	**				**	
Fertility †	ns		ns	ns	**				ns	ns				*	
Pdate x Fertility †	ns		ns	ns	**				ns	**				ns	
CV (%)	4.9		2.4	8.0	4.0				6.9	7.3				5.0	

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

Table 3-6. Most recently matured leaf nutrient concentrations in responses to different planting dates across fertility treatments. Study was located near West Lafayette, IN in 2020. Analysis uses Fishers protected LSD, α=0.05. Pdate refers to planting date, Fertility refers to fertility treatment, PD1 and PD2 specify interactions within specific planting dates.

	R3 MRML Nutrient Concentrations Response													
	Zn		Mn				Fe		Cu				B	
Main Effect	mg kg^{-1}		mg kg^{-1}				mg kg^{-1}		mg kg^{-1}				mg kg^{-1}	
Planting Date														
Early- 5/13/2020	49.6	A	26.7				85.0	A	9.7				60.0	A
Late- 6/8/2020	41.3	B	26.5				71.3	B	9.1				56.1	B
Fertility			**PD1**		**PD2**				**PD1**		**PD2**			
UTC	45.3		28.6	ab	22.6	bc	75.0	bc	10.0	a	9.4	ab	57.6	b
AMS	46.8		27.0	abc	28.4	ab	73.0	c	9.4	ab	7.8	c	56.4	b
ATS	44.0		24.2	bc	29.0	ab	78.6	abc	10.0	a	8.4	bc	58.6	ab
KTS	44.0		26.6	abc	27.4	abc	79.6	ab	9.8	a	9.0	ab	58.4	ab
K-Fuse	45.9		21.0	c	27.4	ab	80.6	ab	9.8	a	10.0	a	56.9	b
28% UAN	46.7		31.6	a	24.0	bc	82.1	a	9.4	ab	10.0	a	60.4	a
Pdate †	**		ns				*		ns				*	
Fertility †	ns		ns				*		*				*	
Pdate x Fertility †	ns		*				ns		*				ns	
CV (%)	12.7		21.7				8.0		9.0				5.1	

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

Table 3-7. Stand and seed quality responses to variety across fertility treatments. Study was located near Wanatah, IN in 2020. Analysis uses Fishers protected LSD, α=0.05.

	Early Season Stand and Seed Quality Response						
	Early Season Population	**Harvest Population**	**Yield**	**Moisture**	**Seed Weight**	**Oil**	**Protein**
Main Effect	Plants ha^{-1}	Plants ha^{-1}	kg ha^{-1}	%	g 100 $seeds^{-1}$	%	%
Variety							
24A80X	311899	271435	3028	12.6 A	12.6	18.0	41.6 A
35A33X	318898	263373	2974	13.1 B	13.1	18.7	40.2 B
Fertility							
UTC	318898	276903	3075	12.2	12.4	18.1	40.3
AMS	322179	261155	2957	12.1	12.5	18.5	41.2
ATS	309055	273622	2968	12.1	12.8	18.3	40.9
KTS	305775	256562	2966	12.2	13.0	18.0	41.3
K-Fuse	314961	261738	2987	12.2	13.0	18.4	41.7
28% UAN	321523	274279	3052	12.1	13.5	18.7	39.8
Variety †	ns	ns	ns	*	ns	ns	**
Fertility †	ns	ns	ns	ns	ns	ns	ns
Variety x Fertility †	ns	ns	ns	ns	ns	ns	ns
CV (%)	7.8	13.7	14.4	2.2	14.7	4.3	3.6

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

Table 3-8. Most recently matured leaf macronutrient and sulfur concentrations in responses to different varieties across fertility treatments. Study was located near Wanatah, IN in 2020. Analysis uses Fishers protected LSD, α=0.05.

	R3 MRML Nutrient Concentrations Response						
	N	**P**	**K**	**S**	**N:S**	**Ca**	**Mg**
Main Effect	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}		g kg^{-1}	g kg^{-1}
Variety							
24A80X	51.9 B	3.9	20.8	3.0 B	17.4	10.9 B	5.4 A
35A33X	58.1 A	4.2	19.2	3.4 A	17.2	12.9 A	5.1 B
Fertility							
UTC	54.9	4.0	19.6	3.1	17.8	11.9	5.3
AMS	53.2	4.0	21.0	3.2	16.8	12.1	5.3
ATS	54.9	4.0	20.3	3.2	17.4	12.3	5.2
KTS	54.8	3.9	19.7	3.3	16.9	12.0	5.2
K-Fuse	56.1	4.0	19.7	3.3	17.2	12.1	5.3
28% UAN	56.1	4.1	19.7	3.1	17.9	11.3	5.3
Variety	**	ns	ns	**	ns	*	*
Fertility	ns	ns	ns	ns	ns	ns	ns
Variety x Fertility	ns	ns	ns	ns	ns	ns	ns
CV (%)	6.2	6.8	ns	8.2	5.5	8.4	10.2

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

Table 3-9. Most recently matured leaf micronutrient concentrations in responses to different varieties across fertility treatments. Study was located near Wanatah, IN in 2020. Analysis uses Fishers protected LSD, α=0.05.

	R3 MRML Nutrient Concentrations Response				
Main Effect	**Zn** mg kg^{-1}	**Mn** mg kg^{-1}	**Fe** mg kg^{-1}	**Cu** mg kg^{-1}	**B** mg kg^{-1}
Variety					
24A80X	48.4 B	32.5 B	89.4 B	9.7 B	58.2
35A33X	55.6 A	42.7 A	103.5 A	11.7 A	57.1
Fertility					
UTC	51.7	35.5	95.6	10.8	58.1
AMS	52.9	36.9	89.7	10.7	56.6
ATS	52.3	38.8	95.3	10.8	57.7
KTS	52.0	40.8	98.0	10.5	57.2
K-Fuse	51.4	38.7	106.2	10.8	58.0
28% UAN	51.8	35.1	93.9	10.6	58.1
Variety	**	**	*	**	ns
Fertility	ns	ns	ns	ns	ns
Variety x Fertility	ns	ns	ns	ns	ns
CV (%)	8.2	12.9	14.7	10.3	8.3

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

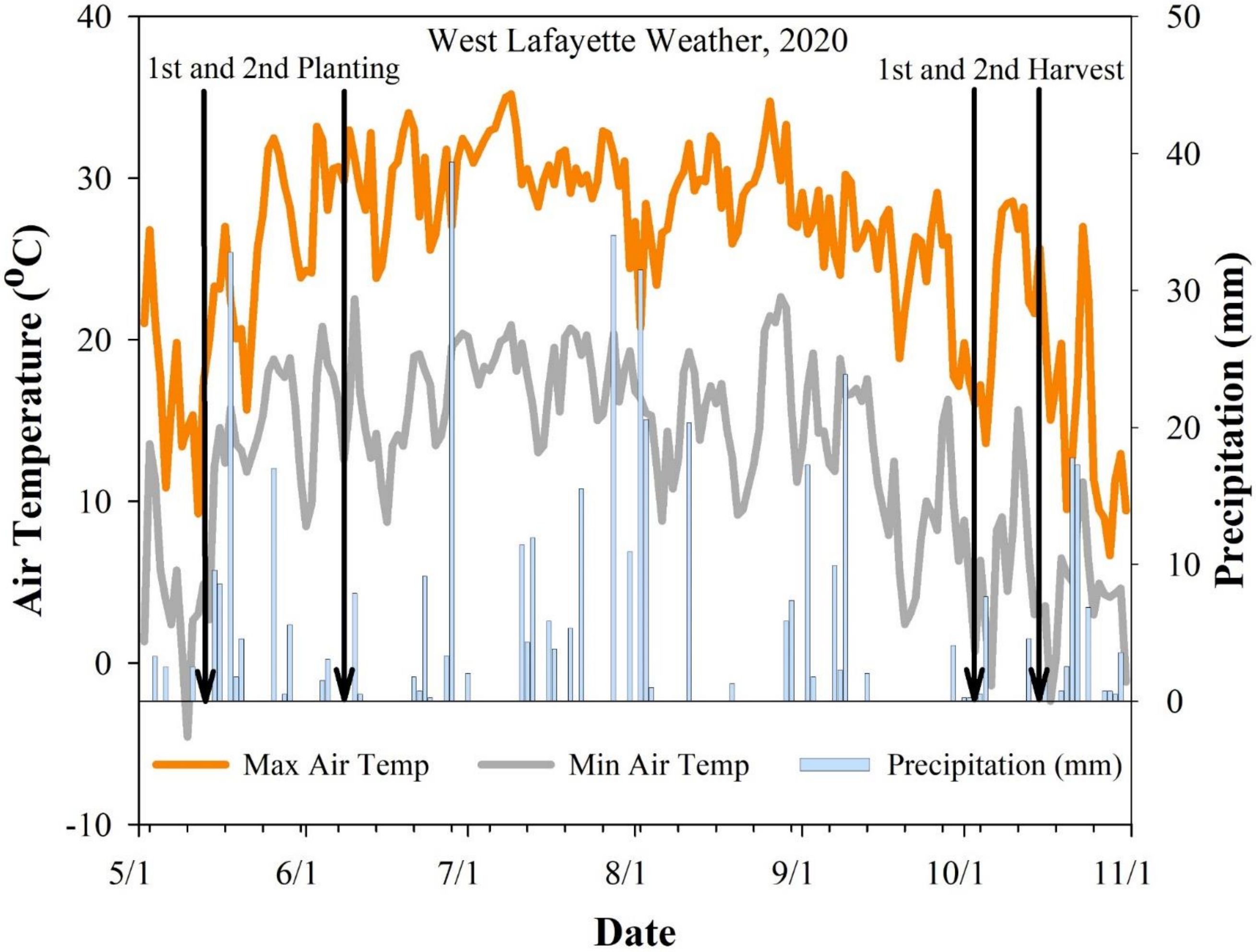

Figure 3-1. West Lafayette 2020 maximum and minimum daily air temperatures and total daily precipitation with planting and harvest dates indicated.

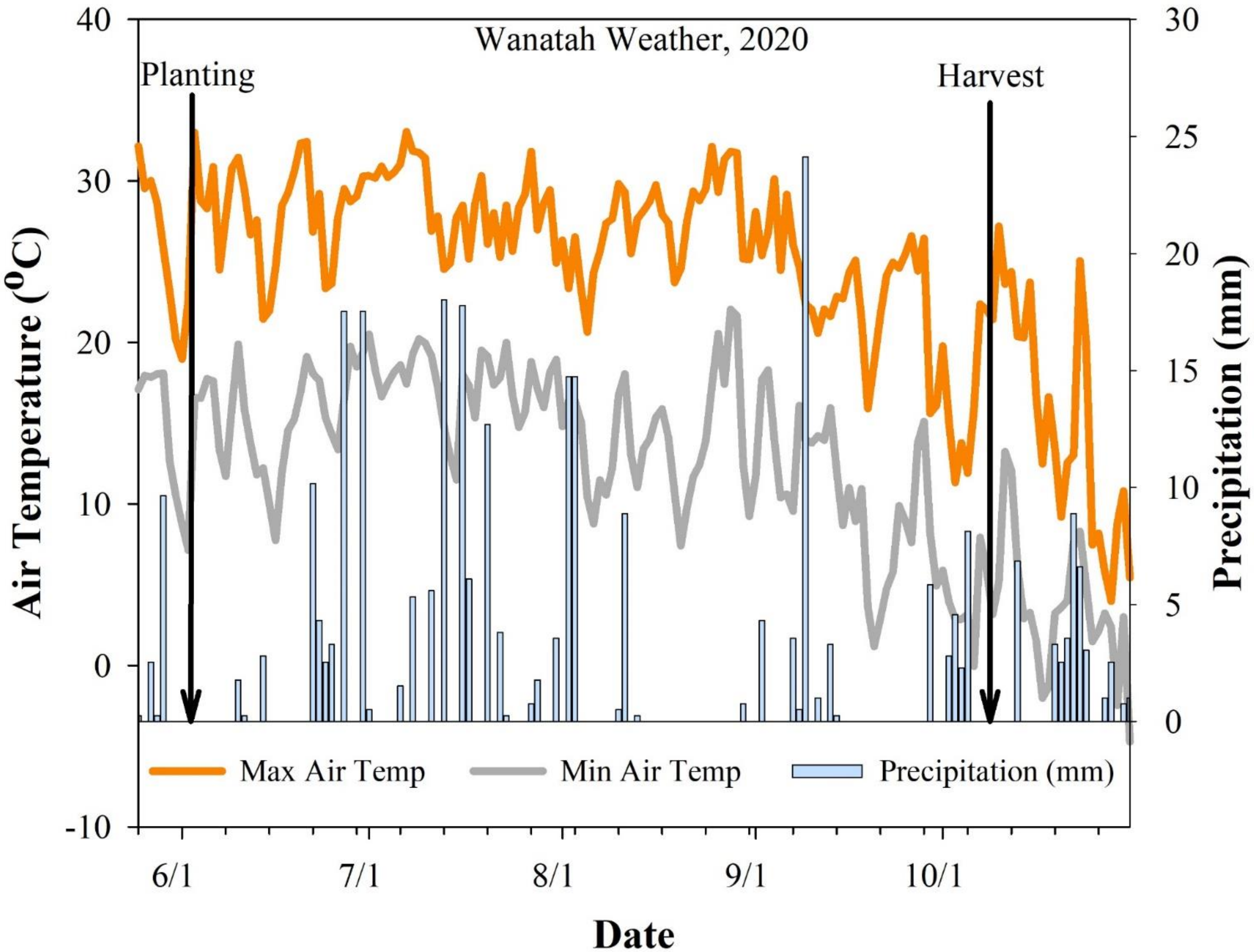

Figure 3-2. Wanatah 2020 maximum and minimum daily air temperatures and total daily precipitation with planting and harvest dates indicated.

APPENDIX A

Table A-1. Resulting Plots following emergence issues, Wanatah 2020.

Fertilizer Treatment	Plots	Placement	
		one	TWO
ATS 5	4	2	2
ATS 10	5	3	2
ATS 15	5	3	2
ATS 20	9	4	5
ATS Total	**23**	**12**	**11**
KTS 5	9	4	5
KTS 10	10	5	5
KTS 15	6	3	3
KTS 20	10	5	5
KTS Total	**35**	**17**	**18**
K-Fuse 5	6	3	3
K-Fuse 10	3	1	2
K-Fuse 15	5	2	3
K-Fuse 20	8	4	4
K-Fuse Total	**22**	**10**	**12**
AMS	5	.	.
UTC	7	.	.
Total Plots	**92**		

Table A-2. Sulfur starter fertilizers treatment average whole plant biomass including leaves, stems, branches, and pods collected at R5 in 2019 near West Lafayette. Means are for the single placement as the dual placement was not collected. Samples were collected from a 1-meter length of the 2nd row by cutting the stem at the soil surface. Samples were kept at 4.4°C until they could be partitioned into their subsequent parts, including stems, leaves, branches, petioles, and pods. Partitioned plant parts were dried at 60°C for 3-5 days, then all biomass weights were recorded.

Data	**Product**	Rate kg S/ha			**Source Mean †**
		0	**5.6**	**16.8**	
Leaves	ATS	14.8	14.3	15.6	15.0
(g/m²)	KTS		15.8	13.8	14.8
	K-Fuse		13.3	13.6	13.4
Stems	ATS	11.2	10.4	11.6	11.0
(g/m²)	KTS		11.6	10.3	10.9
	K-Fuse		10.1	10.4	10.3
Branches	ATS	11.2	9.2	10.1	9.7
(g/m²)	KTS		10.1	9.4	9.8
	K-Fuse		9.1	9.4	9.2
Pods	ATS	15.9	14.1	15.5	14.8
(g/m²)	KTS		15.0	14.5	14.7
	K-Fuse		13.8	14.4	14.1

† Source mean is averaged across 5.6, and 16.8 kg S/ha rate.

Table A-3. Sulfur starter fertilizers treatment average whole plant biomass including leaves, stems, branches, and pods collected at R5 in 2020 near West Lafayette. Means are for the single placement as the dual placement was not collected. Samples were collected from a 1-meter length of the 2nd row by cutting the stem at the soil surface. Samples were kept at 4.4°C until they could be partitioned into their subsequent parts, including stems, leaves, branches, petioles, and pods. Partitioned plant parts were dried at 60°C for 3-5 days, then all biomass weights were recorded.

Data	**Product**	Rate kg S/ha			**Source Mean †**
		0	**5.6**	**16.8**	
Leaves	ATS	24.9	24.7	23.7	24.2
(g/m²)	KTS		29.5	24.1	26.8
	K-Fuse		28.3	25.5	26.9
Stems	ATS	27.6	27.5	27.3	27.4
(g/m²)	KTS		33.9	27.7	30.8
	K-Fuse		31.8	31.4	31.6
Branches	ATS	26.6	25.7	24.3	25.0
(g/m²)	KTS		30.4	24.9	27.6
	K-Fuse		29.5	27.9	28.7
Pods	ATS	11.6	13.3	13.2	13.2
(g/m²)	KTS		16.3	13.0	14.7
	K-Fuse		16.2	15.1	15.7

† Source mean is averaged across 5.6, and 16.9 kg S/ha rate.

Table A-4. UAV imagery dates. Visible Atmospherically Resistant Index (VARI) readings taken from a Phantom 4 Pro UAV flown at approximately 56 meters above the ground between 10 am and 3 pm, with 75-75% overlap in image capture. Images were stitched and plant health maps created using VARI algorithm in Dronedeploy.

Location	Date
West Lafayette, IN 40°28'18.8"N, 86°59'28.3"W	7/26/2019 7/31/2019 8/9/2019 8/13/2019 6/19/2020 6/22/2020 6/25/2020 7/22/2020 7/28/2020 8/6/2020 8/10/2020 8/19/2020 8/21/2020 9/4/2020 9/11/2020 9/22/2020
Wanatah, IN 41°26'42.1"N, 86°55'48.5"W	7/12/2019 8/2/2019 8/7/2019 6/24/2020 6/25/2020

APPENDIX B

Table B-1. Sulfur starter fertilizers and variety effect on average whole plant biomass including leaves, stems, branches, and pods collected at R5 in 2020 near Wanatah. Samples were collected from a 1-meter length of the 2nd row by cutting the stem at the soil surface. Samples were kept at 4.4°C until they could be partitioned into their subsequent parts, including stems, leaves, branches, petioles, and pods. Partitioned plant parts were dried at 60°C for 3-5 days, then all biomass weights were recorded.

Variety	**Fertilizer Treatment**	**Leaves** *(g/m^2)*	**Stems** *(g/m^2)*	**Branches** *(g/m^2)*	**Pods** *(g/m^2)*
24A80X	UTC	22.8	25.3	23.7	17.4
	AMS	27.3	28.6	27.2	20.4
	UAN	22.4	24.9	22.3	16.8
	ATS	25.7	26.3	25.8	19.0
	KTS	23.0	24.7	23.9	18.1
	Fuse	25.5	27.4	26.1	16.6
35A33X	UTC	23.4	26.8	22.4	12.4
	AMS	23.3	26.1	22.9	12.5
	UAN	23.0	25.4	22.4	12.6
	ATS	24.1	27.0	23.5	13.0
	KTS	22.3	25.2	21.8	13.5
	Fuse	24.2	27.2	23.5	13.4

† Source mean is averaged across 5.6, and 16.9 kg S/ha rate.

Table B-2. UAV imagery dates. Visible Atmospherically Resistant Index (VARI) readings taken from a Phantom 4 Pro UAV flown at approximately 56 meters above the ground between 10 am and 3 pm, with 75-75% overlap in image capture. Images were stitched and plant health maps created using VARI algorithm in Dronedeploy.

Location	Date
West Lafayette, IN 40°28'18.8"N, 86°59'28.3"W	6/19/2020 6/22/2020 6/25/2020 7/22/2020 7/28/2020 8/6/2020 8/10/2020 8/21/2020 9/11/2020 9/22/2020
Wanatah, IN 41°26'42.1"N, 86°55'48.5"W	7/12/2019 8/2/2019 8/7/2019 6/24/2020 6/25/2020

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