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## Effect of Different Sources and Application Rates of Sulfur on Corn and Soybean Production Systems in Louisiana

Diego Mayorga Valladares

*Louisiana State University and Agricultural and Mechanical College*

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# **EFFECT OF DIFFERENT SOURCES AND APPLICATION RATES OF SULFUR ON CORN AND SOYBEAN PRODUCTION SYSTEMS IN LOUISIANA**

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

in

The School of Plant, Environmental, and Soil Sciences

by  
Diego Manuel Mayorga Valladares  
B.S., Zamorano University, 2015  
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## Abstract

Sulfur (S) is a structural component of amino acids such as cysteine and methionine and is involved in important functions within the plant like photosynthesis, carbon and nitrogen metabolism, protein synthesis of oils, and detoxification mechanisms. Sulfur deficiency in crops has intensified around the world. Some of the reasons are due to improvement in controlling SO<sub>2</sub> emissions from industries, growing usage of high analysis S-free fertilizers and augmented cropping intensity. Sulfur fertilization has become an important factor in crop production systems and fertilization guidelines for S need to be up-to-date to improve use efficiency and compensate for rising prices of fertilizers. This study was conducted in 2019 and 2020 at two locations at the LSU AgCenter Central Research Station in Baton Rouge, LA to: (1) evaluate the potential of elemental- and sulfate-based S sources, and (2) establish the optimal S application rate to maximize production in a soybean-corn rotation system in Louisiana. A 3 [sources: calcium sulfate (CaSO<sub>4</sub>), ammonium thiosulfate (ATS), and 50% CaSO<sub>4</sub> + 50% elemental S] x 4 (rates: 0, 23, 45, and 90 kg ha<sup>-1</sup>) factorial treatment structure was arranged in a randomized complete block design with four replications. Grain yield, soil S at midseason and harvest, leaf and grain S, and S removal rate were measured. Source had a significant effect on soybean yield in 2019 with ATS obtaining the highest yield. Sulfur source had a significant effect on soybean seed S removal rate with ATS obtaining the highest average S removal. Rates had no effect on yield of corn and soybean for both years but increased corn grain S content and removal rate. An estimate of optimal S rate using Cate-Nelson method showed that yield tended to decline with application rate below 23 kg S ha<sup>-1</sup> and maximize at an application rate not exceeding 45 kg S ha<sup>-1</sup>. The estimated critical soil S was 40 and 52 mg kg<sup>-1</sup> based on fitted linear plateau model using leaf S and grain/seed S removal rate as response variables to soil S. The lack of yield response



despite the improvement in soil S content, subsequently, corn and soybean S uptake possibly was an indication of either luxury consumption of S and/or weather interference during the critical crop growth stages that masked the effects of S source and rate.

## Chapter 1. Introduction

Corn or maize (*Zea mays*) is a cereal widely grown for food and livestock fodder, together with rice (*Oryza sativa*) and wheat (*Triticum aestivum*) are the world's principal grain crops, and it is the largest crop industry in the U.S. (World Book Inc., 2021). In 2020, US had a total area of 36.1 million ha planted to corn producing 374 million Mg grain and 125 million Mg silage (USDA, 2021). Total corn grain production for Louisiana in 2020 was 2.29 million Mg (USDA, 2020). One third of this production is used to feed hogs, cattle (dairy and beef cows), and poultry and is the main source of carbohydrate for these animals' diets (USDA 2021). Corn grain is also used to produce ethanol, that is added to gasoline that serve as fuel for vehicles while the rest is destined for human consumption (food and drinks), industrial uses in the U.S. or export to other countries. Some of the corn-based products include breakfast cereal, tortilla chips, soda, beer, grits, medicine, and packing materials (USDA, 2021).

Moisture is vital to establish a good corn stand. Seeds sown in moist soil immediately absorb water. Water is needed to dissolve the nutrients stored in the endosperm. The nutrients are absorbed by the embryo through the scutellum. A few days after sowing, the radicle will then emerge from the seed and will become the root system. Leaves also start to grow at this time. Corn growth stages can be classified into vegetative (V) and reproductive (R). For every vegetative stage, the letter V is followed by the number of uppermost collared leaf. The last vegetative stage is tasseling and called VT (Espinoza and Ross, 2003). Depth of planting, soil moisture and soil temperature are factors that affect seedling emergence time. Depending on these conditions, it takes between 5 and 21 days for a seedling to emerge (Espinoza and Ross, 2003). In Louisiana, corn is typically planted in March. While temperature may be too cold for corn establishment during this month, this has become a practice to avoid insect problems. It is

recommended to plant from the last week of February to 3<sup>rd</sup> week of March 20th. Yield potential starts to decline if planting date goes later than April 15 (Arledge and Kenneth, 2015).

Varietal selection is fundamental for obtaining a good yield. Factors to consider when choosing a variety include soil type, planting date, environmental condition, location, irrigation practices, and crop rotation system. Annual varietal testing is done at the LSU AgCenter research stations which also include validations of fertilizer recommendation mainly for nitrogen (N). These trials are established to identify best performing varieties in Louisiana growing conditions and cropping systems. For example, in 2019 commercial corn seed companies provided 54 hybrids to be tested at the stations and 10 hybrids evaluated on 13 on-farm core block demonstrations at different locations in Louisiana. Based on these trials, Armor, Dekalb and Dyna-Gro came out as amongst the best yielding varieties (Fromme et al., 2019).

Soybean (*Glycine max*) belongs to the Fabaceae (legume) family with annual growth habit (Kumudini, 2010). It is cultivated across the world with Brazil, U.S., and Argentina being the major producers with production of 128.5, 96.67 and 48.8 million Mg, respectively in the year 2019/2020 (USDA, 2021). On the basis of harvested area, soybean is the largest among the field crops produced in Louisiana. In 2019, soybean harvested area was recorded at 348,000 ha returning a total production of 1.12 million Mg (USDA, 2020).

Soybean is very important because of its seed protein (40%) and oil (20%) content. With the high-quality protein and digestible energy of soymeal, most of it is turned into animal feed and the rest is used to make some soyfoods like tofu. Soybean oil is used for production of cooking oil, biodiesel, and industrial supplies such as paints and cleaners. Fehr and Caviness (1977) established a system to stage soybean development. Briefly, the earliest vegetative stage is VE (emergence) where the soybean cotyledons are above the soil surface followed by VC

wherein the primary leaves have expanded. Following vegetative stages are called V<sub>n</sub> where, n means the number of nodes on the main stem. The reproductive stage begins with the appearance of the first open flower and is called R1- wherein R1 and R2 refer to flowering stages, R3 and R4 refer to pod development, R5 and R6 refer to seed development and R7 and R8 refer to plant maturation (Kumudini, 2010).

Like corn, the LSU AgCenter has active On-farm Variety Trials or OVT for soybean. Typically, several seed companies participate in the annual OVT involving more than 100 varieties. The OVTs are established at the LSU AgCenter research stations on different soil types and on-farm core-block demonstration plots on producers' fields. Varieties that are high-yielding under Louisiana growing conditions are identified. Recent OVTs identified Pioneer P46A86X, LS 4795XS, and Dyna-Gro S43XS70 as good performing varieties recording an average yield of 3.4 Mg ha<sup>-1</sup> (Moseley et al., 2020).

One important cultural practice is crop rotation. Long-term implementation of crop rotations is a fundamental component of sustainable farming systems. Rotation with a legume such as soybean provides N credit for the following corn crop (Gentry et al., 2001). While N credit is the most sought-after benefit from rotating a crop with a legume, in general the improvement in yields of crops in rotation can be attributed to a long list of benefits such as reduction in insect and disease pressure, enhanced soil physical properties and root growth, and release of growth-promoting or inhibiting substances from the residue of the previous crop (Crookston et al., 1991; Nickel et al., 1995; Riedell et al., 2009). Some factors that affect corn yield have been linked to continuous cropping involving allelopathy and other inexplicable adverse effects that persist in this cropping system (Meese et al., 1991).

Higher crop yields are attained by choosing the right cultivar and implementation of proper pest and nutrient management, soil and water management, and cultural practices. Production and usage of fertilizer and pesticides are directly related to increased crop production in the U.S. Depending on soil type, crops, and climate, 40 to 90% of crop productivity is attributed to fertilizer application and to attain the same level of production without fertilizer, 30 to 40% more land would be required (Havlin et al., 1999).

Arnon and Stout (1939) established the criteria for essentiality of mineral element. A nutrient is deemed plant-essential if (a) its absence will cease the plant cycle or will result in plant's death, (b) its function(s) is(are) unique and cannot be replaced by another element, and (c) if it is involved directly in plant metabolism (Kirkby, 2012). There are seventeen elements that are essential for plant growth. Carbon, hydrogen, and oxygen are the most abundant elements in plants and classified as non-mineral nutrients; the sources are mainly the air and water. The other fourteen essential elements are classified mineral nutrients with the soil as the principal source via root absorption. The mineral nutrients are grouped into macronutrients and micronutrients considering their relative abundance in plants. The primary macronutrients are N, phosphorus (P), and potassium (K) and the secondary macronutrients include sulfur (S), calcium (Ca), and magnesium (Mg). The micronutrients are iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), chlorine (Cl), molybdenum (Mo), and nickel (Ni). Sodium (Na), cobalt (Co), vanadium (V), and silicon (Si) are considered essential nutrients for some plants.

Sulfur is vital for many reactions in living cells. While S has essential roles in plant and animal nutrition, it has been associated with different types of air, water, and soil pollution such as acid precipitation, forest decline, acid mine drainage, acid sulfate soils, and toxic properties in drinking water destined for humans and livestock intake (Brady and Weil, 1999). Sulfur is a

component of different amino acids such as methionine, cysteine and cystine, lack of which in human diet will result in malnourishment. The vitamins biotin, thiamine, and B1 contain S, as well as a lot of proteins and enzymes that control important activities for plant functioning such as photosynthesis and N fixation (Brady and Weil, 1999).

Total S content in plant dry matter varies from 0.3 to 7.6 %. Plant takes up S by root absorption as sulfate ( $\text{SO}_4^{2-}$ ). The quantity of  $\text{SO}_4^{2-}$  in the pedosphere fluctuates widely and is produced from weathering of rocks, mineralization of organic S, ground or runoff water, atmospheric deposition of S gases and fertilization (Zhao et al., 2008). Nitrogen use efficiency is influenced by S supply such that on average every Kg of S lost to satisfy the plant S requirements results in a possible N loss of 15 Kg to the environment (Haneklaus et al., 2003). Nitrogen and S are indispensable for crop growth and quality because both are required for amino acid and protein synthesis. The organic N/S ratio on a molar basis is usually about 20. The consumption and assimilation of S and N by plants are strongly complementary, since the major amount of the reduced N and S in plants is incorporated into amino acids and then into proteins (Stulen and De Kok, 2012).

Atmospheric S comes from both natural and anthropogenic sources. Volcanic and geothermic activity naturally emits sulfur dioxide ( $\text{SO}_2$ ) and hydrogen sulfide ( $\text{H}_2\text{S}$ ). Also, dimethyl sulfide, carbonyl sulfide and carbon disulfide and  $\text{H}_2\text{S}$  are produced over oceans, wetlands, salt marshes and estuaries by algae and bacteria. Anthropogenic S is primarily released as  $\text{SO}_2$  from coal, oil, industrial processes, and biomass burning. In 2000 anthropogenic S global emission was estimated at  $68 \text{ Tg S year}^{-1}$  which was twice-higher than the natural S emission at  $34 \text{ Tg S year}^{-1}$  (De Kok et al., 2007). Formation of sulfuric acid follows when atmospheric  $\text{SO}_2$

reacts with water and oxygen. The atmospheric SO<sub>2</sub> together with nitrogen oxides (NO<sub>2</sub>) precipitates back to the earth in the form of acid rain (De Kok et al., 2007).

Both SO<sub>2</sub> and NO<sub>2</sub> are among the air pollutants in the U.S. The passage of the Clean Air Act (CAA) in 1963 gave federal support for efforts to reverse and regulate air pollution. In 1970, congress approved the Clean Air Act Extension, adjusting the CAA to provide firm national ambient air quality standards and their enforcement. The 1970 and later amendments have focused on reducing atmospheric ozone, carbon monoxide, SO<sub>2</sub>, NO<sub>2</sub>, hydrocarbons, lead, and particulate matter (Theilmann, 2019). At the beginning of the 1980's, CAA came into force and atmospheric S depositions were reduced rapidly in Western Europe. On production fields, S deficiency symptoms were observed since the early 1980's, and the deficiency condition escalated with decreasing atmospheric S inputs. Widespread symptoms of macroscopic S deficiency can be observed consistently over time in cereals since 1992. Since then, severe S deficiency has become the main nutrient disorder in agronomic crops (Haneklaus et al., 2008).

Another factor that contributes to S deficiency is increased yields which eventually result in greater crop S removal from the field. Corn grain comprises about 4.5 kg of S for every 2.54 Mg of grain, consequently about 11.2 kg of S per hectare is removed by corn that yields 12.6 Mg ha<sup>-1</sup>. According to Sawyer (2020), from about 150 trials conducted in Iowa since 2005, 50% of trials have had a statistically significance yield increases as a response to S applications. Yield responded to application rates of 17 kg S ha<sup>-1</sup> in fine textured soils and 28 kg S ha<sup>-1</sup> in coarse textured soils for corn and soybean.

Chlorosis or yellowing of leaves is a typical visual symptom of S deficiency in corn. Unlike N, S is not mobile in the plant thus deficiency symptoms will show in the upper canopy mainly the younger group of leaves. Deficiency can cause leaf striping as well, which is

confused with Mg, Mn, and Zn deficiency (Camberato et al., 2012). Sulfur deficiencies on soybean show up like in corn, in the younger leaves and these will be smaller than usual with a pale yellow-green color. Also, stems are thinner, hard, and elongated (Gatiboni and Crozier, 2020). Sulfur deficiencies normally occur in deep, coarse-textured soils with low organic matter (OM) in which S leaches easily. Sulfate leaches through sandy surface soils but accumulates in clay subsoil where some plants can take it. Heavy, lengthy rains during the growing season can leach S to lower profiles. Other conditions besides leaching that can decrease crop rooting depth, such as cold weather, high soil moisture, low soil pH, and soil compaction (Gatiboni and Crozier, 2020) could lead to S deficiency.

One of the sources of S in most soils is organic S. One (1) % OM in the plow layer is equivalent to 112 kg S ha<sup>-1</sup>. Organic S has to be mineralized to SO<sub>4</sub><sup>2-</sup> in order to be taken up by plants. Therefore, whenever the OM of a soil is low, S deficiencies are more likely to occur (Camberato et al., 2012). Sulfur fertilization is recommended in soil testing low in SO<sub>4</sub><sup>2-</sup>, the form of S taken up by plants. Sulfur in inorganic fertilizers is usually present as SO<sub>4</sub><sup>2-</sup> (e.g., ammonium sulfate, calcium sulfate or gypsum). Sulfate is readily available to plants but is vulnerable to leaching. Another source of S is the elemental S (S<sup>0</sup>); it is considered a slow-release S fertilizer since it must undergo oxidation process, thus does not leach with percolating water and has the advantage of a low transport cost due to its high analysis (Degryse et al., 2015). Factors like particle size, rate, method, and time of application affect the efficacy of S<sup>0</sup> in providing S to plants (Havlin, 1999). The most commonly use S fertilizer is gypsum which contains 23% Ca and 19% S. It is also used as a calcium source for crops with large Ca requirements. Besides mined gypsum and gypsum retrieved from flue gases when coal is burned, gypsum is also generated from a range of processes including neutralization of sulfuric acid



(Dick et al., 2008). Ammonium thiosulfate (ATS) contains 12% N and 26% S and it can be mixed with liquid fertilizers. When ATS is applied to the soil, colloidal S and  $(\text{NH}_4)_2\text{SO}_4$  are formed making  $\text{SO}_4^{2-}$  instantly accessible by plants.

The S critical level for Louisiana soils is 12 mg  $\text{kg}^{-1}$  based on Mehlich-3 extraction procedure (Parvej, 2021). Sulfur application at 22 kg  $\text{ha}^{-1}$  rate is recommended when Mehlich-3 soil test S concentration falls below this critical level. The application is commonly done at or after crop establishment as gypsum, ammonium sulfate, potassium thiosulfate, and magnesium thiosulfate. Ammonium sulfate is not recommended for soybeans since its N content will negatively impact nodulation (Parvej, 2021).

The main sources of N for high yielding soybeans are biological  $\text{N}_2$  fixation (BNF) and mineral soil or fertilizer N. However, there is a known antagonistic effect of nitrate on  $\text{N}_2$  fixation process in the root nodules making this a major constraint for increasing N uptake when no other abiotic stress arises (Streeter, 1988). Maximum  $\text{N}_2$  fixation happens between R3 and R5 stages of soybean development, and any differences between crop N necessity and N supply by  $\text{N}_2$  fixation should be met by N uptake from other sources. If N supply does not meet soybean requirement, the crop will remobilize the N accumulated in leaves to the grain, which reduces the photosynthetic capacity of the canopy and therefore limits yield potential (Zapata et al., 1987). Applying N fertilizer has been proposed as an aid for increasing available N in the soils. Studies on nodulating soybeans showed significant yield response to frequent N additions when the  $\text{N}_2$  fixation system could not meet the N demand (Thies et al., 1995). However, yield response of soybean to N fertilizer has been inconsistent at economically acceptable levels (Barker and Sawyer, 2005; Gan et al., 2003; Schmitt et al., 2001).

In Pennsylvanian soils under corn and soybean production, S fertilization is recommended once the Mehlich-3 soil test S falls below  $15 \text{ mg kg}^{-1}$  (White et al., 2021). However, they recorded more than 60% of the sites (27 trials) with Mehlich-3 soil test S below  $15 \text{ mg kg}^{-1}$  were not responsive to S fertilization possibly due to higher S levels in the lower soil layers or high organic matter (OM) mineralization rates. Sulfur fertilizer rate can be estimated based on crop S removal rate (Oldham, 2021).

The cases of S deficiency in crops have been increasing due to low level of available S in the soil and higher crop S removal rate. The low level of soil S is triggered by several factors which include the significant reduction in S emission from industrial sources, decrease in use of organic fertilizers, and use of high yielding cultivar coupled with intensive farm practices (Scherer, 2001). For these reasons, S fertilizer has become a vital component of fertilization program for most crop production systems which also means that fertilization guidelines for S need to be up-to-date and effective to increase use efficiency and offset the soaring prices of fertilizers. This study was established to 1) evaluate the efficacy of different S fertilizer sources, 2) evaluate the relationship between S fertilizer rates, soil S level, yield, and plant S, and 3) determine and validate the optimal S application rate to maximize production in a corn-soybean rotation system in Louisiana.

## Chapter 2. Effect of Sulfur Rates and Sources on Corn and Soybean Yield

### 2.1. Introduction

For worldwide corn (*Zea mays*) production in 2019/2020, the U.S. was responsible for over a third of the world's corn production (31.06%), followed by China (23.42%), and Brazil (9.07%) (Shabandeh, 2022). Corn is grown in most of the U.S. agricultural land, but production is concentrated in the Heartland region which includes Illinois, Iowa, Indiana, eastern South Dakota and Nebraska, Western Kentucky and Ohio, and northern Missouri. Corn production has risen throughout the years and higher yields have been obtained because of improvements in technology (seed varieties, pesticides, fertilizers, machinery) and production practices (reduced tillage, irrigation, crop rotation, and pest management systems) (USDA, 2022). According to USDA-NASS (2014), corn is the third most produced grain crop in Louisiana, and most of it is produced in the state's northeastern region. In 2021, about 235,000 hectares were planted to corn obtaining a production of 2.8 million Mg and 43,545 Mg for grain and silage, respectively. The average grain yield was 12.3 Mg ha<sup>-1</sup> and the price per unit was \$204 Mg<sup>-1</sup> reaching a total production value of \$537,654,000 (USDA, 2022).

Optimizing corn yields begins with a good stand. Both temperature and soil moisture are contributing factors for establishing a good stand in Louisiana. Seed germination requires a minimum soil temperature of 10°C soil moisture, ideally, should be at field capacity. Excessive moisture can lead to diseases and not enough moisture can cause spotty emergence (Williams, 2021). According to Foster (2022), a good germination is achieved when a given soil within its 5-cm depth has reached about 13°C by 9 a.m. for three consecutive days. In Louisiana this usually happens in late February and March. On average years, the planting window for South Louisiana is from last week of February to 3<sup>rd</sup> week of March while it comes later for north

Louisiana between early March till first week of April. However, soil temperature is a better basis of planting date rather than the calendar date (Arledge et al., 2015). According to Arledge et al. (2015) corn is usually fertilized before planting in non-irrigated soils with 67 kg nitrogen (N) ha<sup>-1</sup>, and phosphorus (P) and potassium (K) based on the soil test recommendation with seeding rate ranging from 76,000 to 91,000 seeds ha<sup>-1</sup> depending on the target population.

Soybeans are amongst the main agricultural crops planted in the U.S. Soybeans belong to the legume family but is considered an oilseed crop. Soybean produces high-quality protein and digestible energy of soymeal that is used for production of animal feed and soyfoods whereas soybean oil is used for production of cooking oil, biodiesel, and industrial supplies. Generally, soybean in the U.S. is sown in May and early June and is harvested in late September and October depending on maturity group (MG). Most soybeans production is in the upper Midwest. Illinois, Iowa, and Indiana were the top soybean producing states in 2016 in the U.S. (Shabandeh, 2022). The U.S. was the top global producer of soybeans with a production amount of 120.5 million metric tons in 2018/2019. In 2020, Brazil surpassed the U.S. production reaching around 138 million metric tons (Shabandeh, 2022).

Soybean is a major row-crop and consistently has the largest production area among the field crops grown in Louisiana. More than 400,000 hectares of soybeans were harvested in 2021, achieving a total production of 1.5 million Mg. The average yield in the U.S. was 2,600 kg ha<sup>-1</sup> and the cost per unit was \$0.52 kg<sup>-1</sup> (USDA, 2022). Soybean planting methods differ throughout Louisiana, due in big part to differing weather and cropping systems. Early planting requires soils that reach temperature between 13 to 16°C by 10 a.m. The optimal planting window typically falls between mid-April to mid-May. Seed must be planted from 2 to 4 cm on sandy or silt loam soils and 2.5 to 5 cm on clay soils depending on soil moisture (Brown and Stephenson,

2021). Soybeans can be cultivated on a broad range of well-drained soils but achieve higher yields on clay loam soils. Soybean favors a somewhat acidic soil (pH 6.0 – 6.5) and is typically cultivated in environments with temperatures between 10°C and 40°C (Subba and Sammi, 2010).

Soybean crop bearing 2.5 Mg seed ha<sup>-1</sup> takes about 124 kg N, 23 kg P, 101 kg K, 22 kg sulfur (S), 35 kg calcium (Ca), 19 kg magnesium (Mg), 192 g zinc (Zn), 866 g iron (Fe), 208 g manganese (Mn), and 74 g copper (Cu) from the soil (Subba and Sammi, 2010). Nitrogen is needed in the highest amount of all nutrients taken up from the soil. Soybean plants can utilize N released by mineralization, residual soil N, fertilizer, or atmospheric N, which is transformed into a usable form in root nodules by way of its symbiotic relationship with *Bradyrhizobium japonicum* bacteria. Even though the soil is the main resource of N for several crops, soybeans get 65 – 85% of its demand through symbiotic fixation. A high amount of N fertilizer application prevents N fixation, and most experts suggest either no fertilizer or a small treatment of 30-50 kg N ha<sup>-1</sup> at sowing or before flowering (Subba and Sammi, 2010).

Sulfur is a component of cysteine and methionine, and therefore of proteins. Sulfur is a structural component of these amino acids or acts as a functional group, and engaged in several metabolic reactions (Hawkesford et al., 2012). Cysteine and methionine are also precursors to produce complexes as well as glutathione and a broad variety of enzymes, vitamins, cofactors, and S compounds implicated in the growth and development of plant cells. Sulfur is involved in important functions such as photosynthesis, carbon (C) and N metabolism, protein synthesis of oils in oilseed crops, and detoxification mechanisms (Franzen and Grant, 2008). Sulfate (SO<sub>4</sub><sup>2-</sup>) is the form of S that plants uptake which is less than 5% of the total S in the soil (Scherer, 2009). Sulfur can also be absorbed by plants as thiosulfate (Schoenau and Malhi, 2008). The main site of plant SO<sub>4</sub><sup>2-</sup> uptake is via root absorption. Sulfur cycle is very complex and involves the

following processes: immobilization, mineralization, atmospheric deposition, reduction, oxidation, leaching, runoff, volatilization with the release of  $\text{SO}_4^{2-}$  from organic matter (mineralization) being fundamental for the S supply to crops (Scherer, 2009).

The S deficiency in crops has intensified around the world due to the improvement in controlling S dioxide emissions from industries, growing usage of high analysis S-free fertilizers, declining use of pesticides and fungicides containing S, use of newer cultivars that demands a high fertility requirement resulting in higher nutrient removal from soils, and augmented cropping intensity (Assefa and von Tucher, 2013). In the USA, in recent years, S deficiency has been detected in corn, soybean, alfalfa (*Medicago sativa*), and wheat (*Triticum aestivum*) in the Midwest. Camberato and Casteel (2017) noted that these were due to a decrease in atmospheric S deposition, increased crop removal of S, and extensive use of no-tillage practice. Sulfur deficiency is commonly observed in soils with low organic matter content and in coarse-textured soils or those with high leaching potential. Sandy soils are particularly vulnerable to S deficiency because they have a tendency to be low in organic matter content and because  $\text{SO}_4^{2-}$ -S will easily leach out of the plant root zone (Franzen and Grant, 2008).

Fertilization is an agronomic practice to avoid yield losses due to nutrient deficiency. Calcium sulfate ( $\text{CaSO}_4$ , 17% S) is a common S fertilizer source. This source is either natural (gypsum) or a product of  $\text{SO}_2$  neutralization and is also useful for Ca fertilization (Zielewics et al., 2022). Calcium sulfate is a chemically neutral salt, therefore does not harm leaves and it can be used for top dressing or as a dust fertilizer. Calcium sulfate application improves aggregations in heavy clay soil thus, improves water infiltration and decreases soil surface crusting (Zielewicz et al., 2022).

Another source of S is ammonium thiosulfate (ATS, 26% S). This is a transparent liquid that also contains 12% N. Being a liquid, ATS can be mixed with N and N-P-K liquid solutions that are neutral to somewhat acidic in pH. Ammonium thiosulfate can be applied to the soil, in mixes, or to sprinkler and open-ditch irrigation systems. Colloidal S and  $(\text{NH}_4)_2\text{SO}_4$  are formed when ATS is applied to the soil. The  $\text{SO}_4^{2-}$  is instantly accessible. Potassium thiosulfate (KTS, 25% K and 17% S) performs in the same way as ATS (Havlin et al., 1999).

Elemental sulfur ( $\text{S}^0$ ) is another source of S with the highest analysis (99% S) but less soluble. When  $\text{S}^0$  is ground and mixed with soil it is oxidized to  $\text{SO}_4^{2-}$  by soil microorganisms. This is a slow process thus,  $\text{S}^0$  is considered a slow-release fertilizer. Various factors such as particle size, rate, method, and time of application affects the efficacy of  $\text{S}^0$  in providing S to plants. The higher the application rates and the finer the particle size of  $\text{S}^0$  the higher the amount of S that quickly becomes available for plant uptake (Havlin et al., 1999). Broadcast incorporation of  $\text{S}^0$  is better than banding since it creates larger exposure of  $\text{S}^0$  particles to oxidizing microorganisms and reduce problems caused by extreme acidity. Examples of granular  $\text{S}^0$  fertilizers include S-bentonite (90% S) and micronized granular S (95% S) (Havlin et al., 1999).

As S fertilization becomes routine in most crop production systems, the potential of sources of S other than  $\text{CaSO}_4$  should be evaluated. This study was conducted to evaluate the effect of different sources of S applied at increasing rates on corn and soybean yield under rotation system in Louisiana.

## **2.2. Materials and Methods**

### **2.2.1. Site Description, Planting Method, Treatment Structure and Trial Establishment**

This study was conducted in 2019 and 2020 at the LSU AgCenter Central Research Station in Baton Rouge, LA (Latitude 30°, 15', 06.8'' N; Longitude 91°, 10', 11.7'' W). The field was divided into two blocks, half was planted to corn and the other half was planted to soybean. The following year, these crops were rotated in the 2-block field. The soil in this field is a Cancienne silt loam, consist of a mixture of Cancienne (90%), and minor components such as Carville, Thibaut, and Gramercy (10%) (Fine-silty, mixed, superactive, nonacid, hyperthermic Fluvaquentic Epiaquepts). The varieties used in 2019 and 2020 for corn was DEKALB 6826 and DEKALB 6869, respectively. The variety used in 2019 and 2020 for soybean was ASGROW 5535.

Before planting, soil was tilled and leveled using a Wil-Rich 1400 field cultivator. After that, seeds of corn and soybean were planted at 92,000 and 185,000 seeds ha<sup>-1</sup> with row spacing of 0.96 m using a John Deere 1700 Max Emerge plus vacuum planter. For corn, N was knifed-in at 200 kg ha<sup>-1</sup> using urea-ammonium nitrate (UAN, 32%N) solution around V3 leaf stage. Phosphorus and K fertilizers were not applied since soil test P and K came back as medium to high. Based on Mehlich-3 extraction procedure, the nutrient levels of the initial soil samples were: 86 mg P, 142 mg K, 1649 mg Ca, 356 mg Mg, 15.2 mg S, 4.8 mg Cu, and 1.35 mg Zn per kg soil. The S treatment (or fertilization) was made between V4 to V5 leaf stage. The treatment structure consisted of twelve combinations of different S sources (CaSO<sub>4</sub>, ATS, and 50% CaSO<sub>4</sub> + 50% S<sup>o</sup>) applied at 23, 45, and 90 kg ha<sup>-1</sup>, including an untreated check plot or 0 (Table 2.1). Each treatment was replicated four times and arranged in a randomized complete block design. The experimental units (plots) consisted of four 12.2 m long rows with a 3.05 m alleyway.



Granular/ solid sources ( $\text{CaSO}_4$  and  $\text{S}^0$ ) were broadcast-applied by hand while ATS was surface band applied using a Solo 425 15-L professional piston backpack sprayer. The dates of major field operations are summarized in Table 2.2.

Table 2.1. Description of the treatment structure for corn and soybean implemented in this study at the LSU AgCenter Central Research Station in Baton Rouge, LA in 2019 and 2020.

Trt. No	S Source	S Rate ( $\text{kg ha}^{-1}$ )	Type	Application method
1	Control	0	-	-
2	$\text{CaSO}_4$	23	Granular	Broadcast
3		45		
4		90		
5	Control	0	-	-
6	Ammonium Thiosulfate	23	liquid	Dribble in (surface)
7		45		
8		90		
9	Control	0	-	-
10	$\text{CaSO}_4 + \text{S}^0$	23	Granular/pellets	Broadcast
11		45		
12		90		

Table 2.2. Timeline of agronomic activities accomplished during the two-year study at the LSU AgCenter Central Research Station in Baton Rouge, LA in 2019 and 2020.

Site	Year	Crop	Planting Date	S Application Date	Harvest Date
1	2019	Corn	20-Mar-19	1-May-19	27-Aug-19
2	2019	Soybean	13-May-19	24-May-19	30-Sep-19
1	2020	Soybean	25-Mar-20	21-Apr-20	3-Aug-20
2	2020	Corn	25-Mar-20	21-Apr-20	4-Aug-20

### 2.2.2. Corn and Soybean Yield

Corn and soybean plots were harvested using a Kincaid 8-XP research plot combine harvester equipped with loadcells and infrared moisture sensor to determine plot grain yield and

grain moisture content, respectively. For each plot, the two middle rows were cut; both the grain/seed weight and moisture content were recorded. Grab samples of corn grains and soybean seeds were collected from each plot for nutrient content analysis. Plot yield was converted to per hectare basis and adjusted at 15.5% and 13% moisture content for corn and soybean, respectively.

### **2.2.3. Grain and Seed Nutrient Analysis**

The nitric acid-hydrogen peroxide ( $\text{HNO}_3\text{-H}_2\text{O}_2$ ) digestion method followed by ICP analysis was used for analysis of grain/seed S content. For this, ground samples (0.5 g) were weighed in kimwipes and placed into digestion tubes followed by the addition of 5 mL of trace metal grade nitric acid ( $\text{HNO}_3$  67-70%), making sure that the acid washed any remaining of ground samples on the side of the tube. After that, the samples were allowed to set undisturbed for 50 minutes then mixed using a vortex mixer before placing them in a digestion block (previously turned on and set to a temperature between 152-155°C) for 2-3 minutes to vigorously boil the samples. When a brown fume appeared, the tubes were removed from the digestion block and let cool for 10 minutes. Three (3) mL of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was added to the samples before placing them back to the digestion block for 2 hours and 45 minutes. Samples were then taken out from the digestion block and allowed to cool before transferring the digested samples to 15 mL centrifuge tubes, adjusting to a volume of 12.5 mL with deionized water. The final solution was filtered using Whatman No. 1 filter paper and transferred into 10-mL tubes for inductively coupled plasma optical emission spectrophotometry (ICP-OES) analysis. Blank and reference samples were integrated in each batch of digestion for quality assurance. The grain/seed S removal rate was computed as the product S concentration and yield.

#### **2.2.4. Data Analysis**

R project (R Core Team, 2022) was used for the statistical analysis through the integrated development environment (IDE) of R Studio. Analysis of variance (ANOVA) was run with the corresponding function in native R. Tukey's HSD test was performed using agricolae package in R (Mendiburu F, 2021). Sulfur source and rate were treated as fixed effects in each crop and each year for yield, grain/seed S concentration, and removal rate. Alpha of 0.1 was used for significance in all the analysis.

### **2.3. Results**

#### **2.3.1. Climatic Conditions**

Average monthly precipitation and temperature for the two crop-years (2019 and 2020) are presented in Figure 2.1. and 2.2., respectively. The highest average monthly precipitation was recorded in October 2020 with 252 mm; similar amounts were recorded in April 2019 (252) and May 2019 (246 mm) (Figure 2.1.). Given that S application for 2019 was done in the month with the second highest average monthly precipitation (May), a minimal effect could have been occurred to both corn and soybean yield due to potential S leaching, and other applied nutrient (N) for that matter. Overall, the 2020 crop season had more precipitation compared to 2019. The average monthly temperature from May to September was very similar across the cropping years, between 30°C and 31°C (Figure 2.2.).

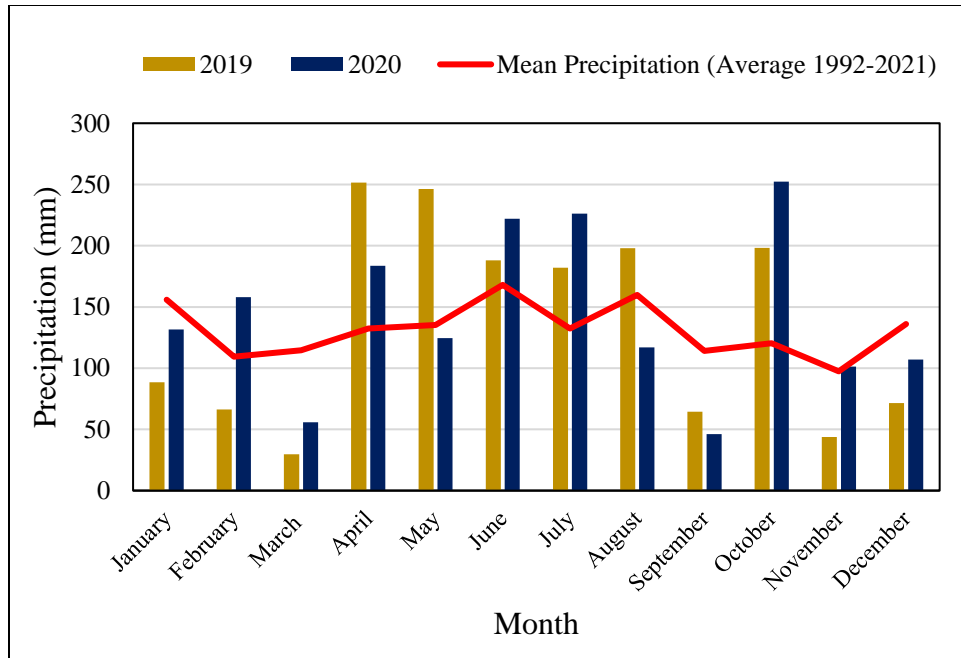


Figure 2.1. Average monthly precipitation from January to December in 2019 and 2020 at the LSU AgCenter Central Research Station in Baton Rouge, LA.

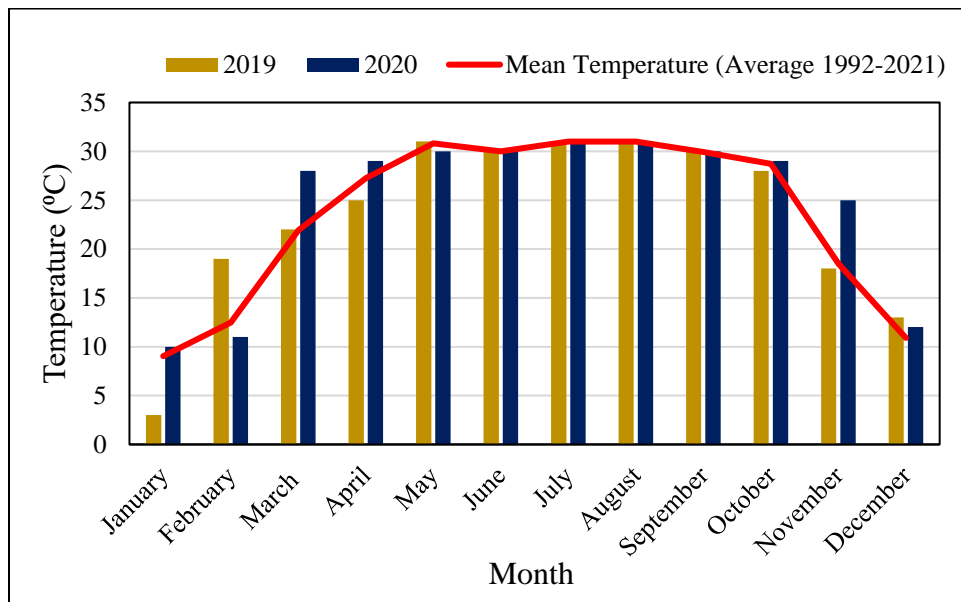


Figure 2.2. Average monthly temperature from January to December in 2019 and 2020 at the LSU AgCenter Central Research Station in Baton Rouge, LA.

### 2.3.2. Analysis of Variance on All Measured Variables

The effect of S source, S rate, and their interaction on yield and grain/seed S content and removal rate for corn and soybean in 2019 and 2020 is shown in Table 2.3 and Table 2.4, respectively. In 2019, there was a significant source effect on soybean yield and soybean seed S removal rate ( $p < 0.1$ ). Significant effect of S rate on grain S content and S source on grain S removal rate was recorded. Both corn and soybean yield did not respond to S rate. In 2020, only S rate treatment recorded a significant effect on corn yield, grain S content and removal rate ( $p < 0.1$ ). There was no significant interaction effect between S source and rate for both crops and years across all the variables measured.

Table 2.3. Analysis of variance for the effect of S source and rate and their interaction on yield and grains/seed S content and removal rate for corn and soybean in 2019 at the LSU AgCenter Central Research Station in Baton Rouge, LA.

Crop	Treatment	Yield	Grain/Seed Sulfur Content	Grain/Seed Sulfur Removal Rate
		p-value		
Corn	S Source	NS	NS	0.088
	S Rate	NS	0.065	NS
	Source x Rate	NS	NS	NS
Soybean	S Source	0.028	NS	0.026
	S Rate	NS	NS	NS
	Source x Rate	NS	NS	NS

⌘ NS indicates no significant effect at  $p < 0.1$ .

Table 2.4. Analysis of variance for the effect of S source and rate and their interaction on yield and grains/seed S Content and removal rate for Corn and Soybean in 2020 at the LSU AgCenter Central Research Station in Baton Rouge, LA.

Crop	Treatment	Yield	Grain/Seed Sulfur Content	Grain/Seeds Sulfur Removal Rate
		p-value		
Corn	S Source	NS	NS	NS
	S Rate	0.06	0.010	0.019
	Source x Rate	NS	NS	NS
Soybean	S Source	NS	NS	NS
	S Rate	NS	NS	NS
	Source x Rate	NS	NS	NS

⌘ NS indicates no significant effect at  $p < 0.1$ .

### 2.3.3. Effect of S Source and Rate on Corn and Soybean Yield

The average corn grain yield in 2020 ( $13,000 \text{ kg ha}^{-1}$ ) was higher than in 2019 ( $11,500 \text{ kg ha}^{-1}$ ) (Figure 2.3 and 2.4.). The highest grain yield was recorded in corn which received  $90 \text{ kg S ha}^{-1}$  in both years but it was statistically significant only in 2020 when compared to  $45 \text{ kg ha}^{-1}$  but not with control (Figure 2.3). The S source had no apparent effect on corn yield for both years. The average soybean yield in 2019 was about  $2,900 \text{ kg ha}^{-1}$  compared to 2020 average yield exceeding  $4,000 \text{ kg ha}^{-1}$ . Sulfur application rate had no effect on soybean yield whereas for S source, the thiosulfate-treated soybean attained the highest yield at  $3000 \text{ kg ha}^{-1}$  in 2019. The following year, such effect (of thiosulfate) on soybean yield was not observed (Figure 2.6).

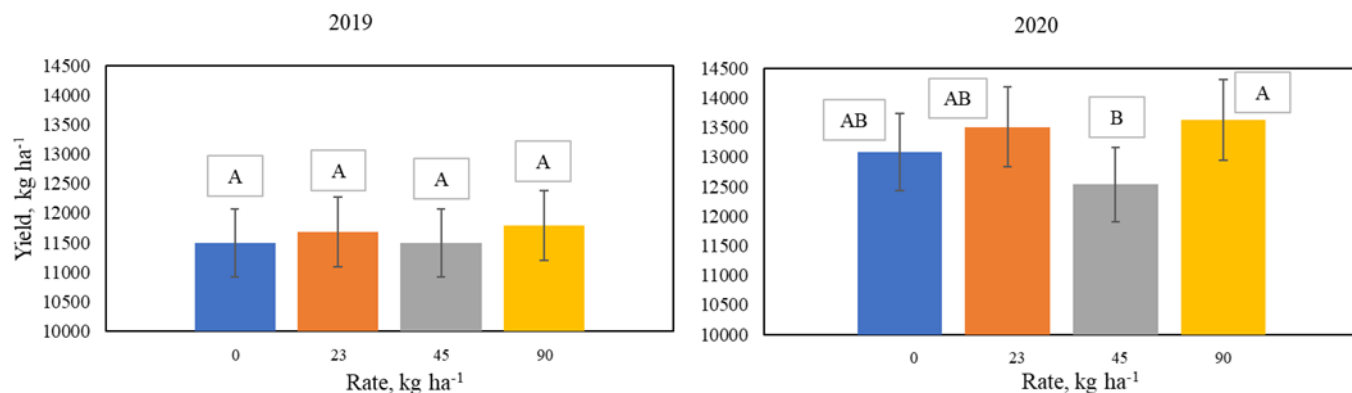


Figure 2.3. Means and analysis of variance on S rate effect on corn yield in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA.

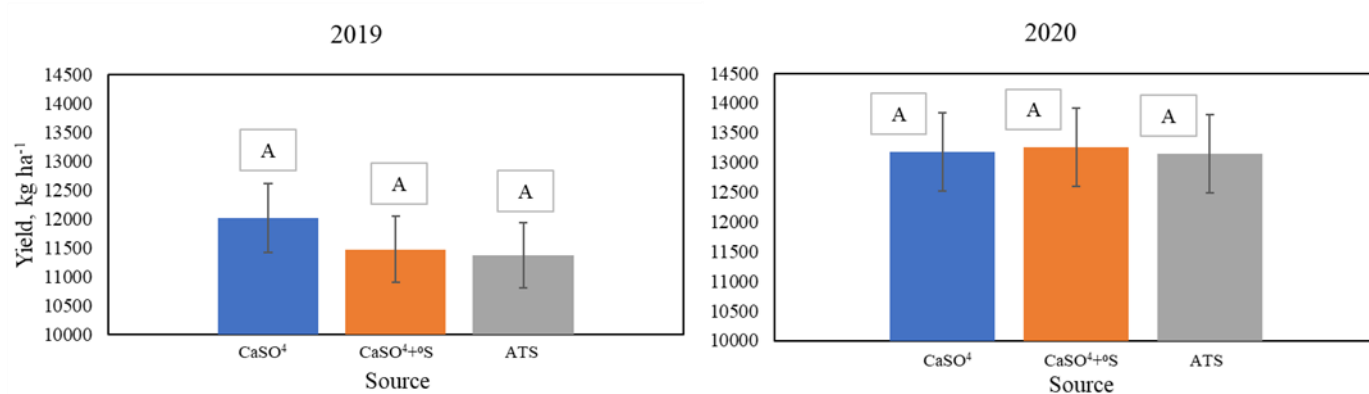


Figure 2.4. Means and analysis of variance on S source effect on corn yield in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA.

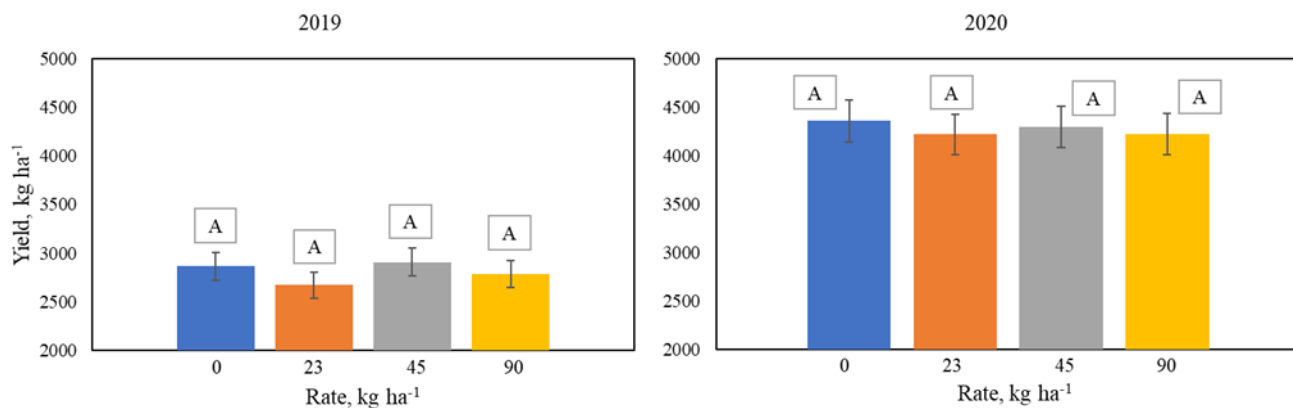


Figure 2.5. Means and analysis of variance on S rate effect on soybean yield in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA.

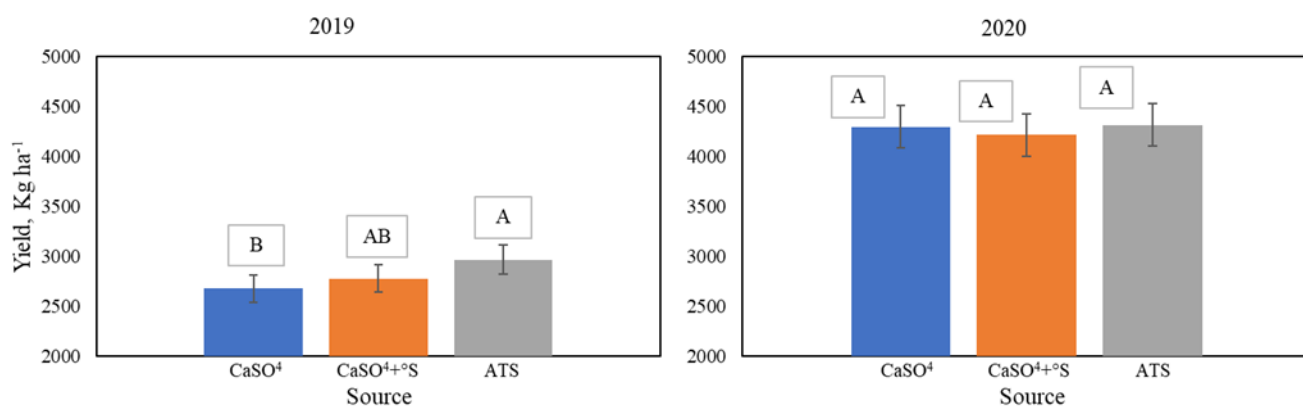


Figure 2.6. Means and analysis of variance on S source effect on soybean yield in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA.



#### 2.3.4. Effect of S Source and Rate on S Content of Corn Grain and Soybean Seed

The S content of corn grain was affected by rate but not the source (Table 2.3 and 2.4). On average, the S grain content in 2019 was higher than in 2020. The application of the highest rate, 90 kg ha<sup>-1</sup>, significantly increased the grain S content to 0.093% and 0.077% compared to the check at 0.087% and 0.072% in 2019 and 2020, respectively. The soybean seed S content did not respond to S source (Figure 2.10) and unlike in corn grain, the increasing S rate did not result in significant increases in seed S content (Figure 2.9). There was a tendency for seed S to increase with increasing S rate but the variation among the replications within each rate was higher than the variation between treatment (rate) means. Between years, the average seed S in 2019 (0.35%) was higher compared to the average seed S in 2020 (0.2%).

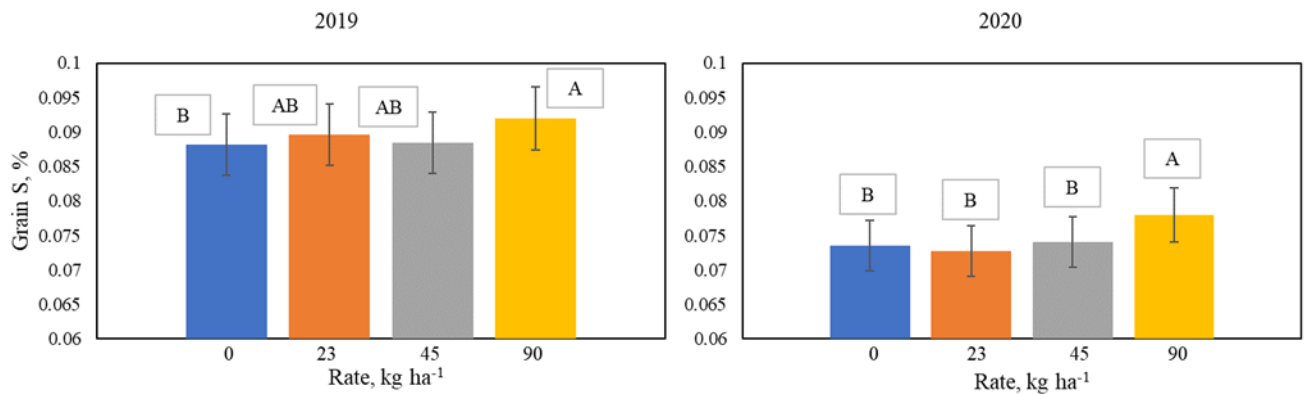


Figure 2.7. Corn grain S content in response to different S rates in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA.

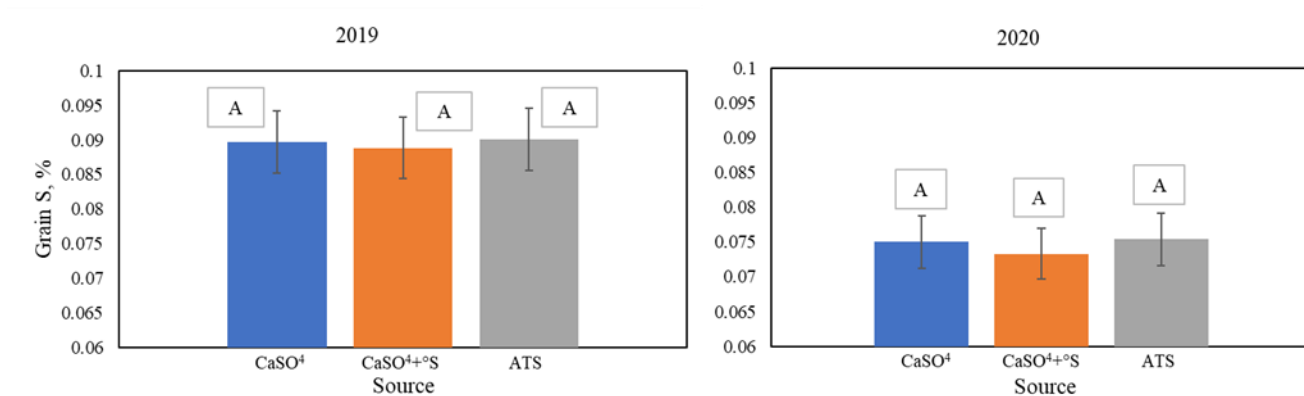


Figure 2.8. Corn grain S content in response to different S sources in 2019 and 2020, LSU AgCenter Central Research Station.

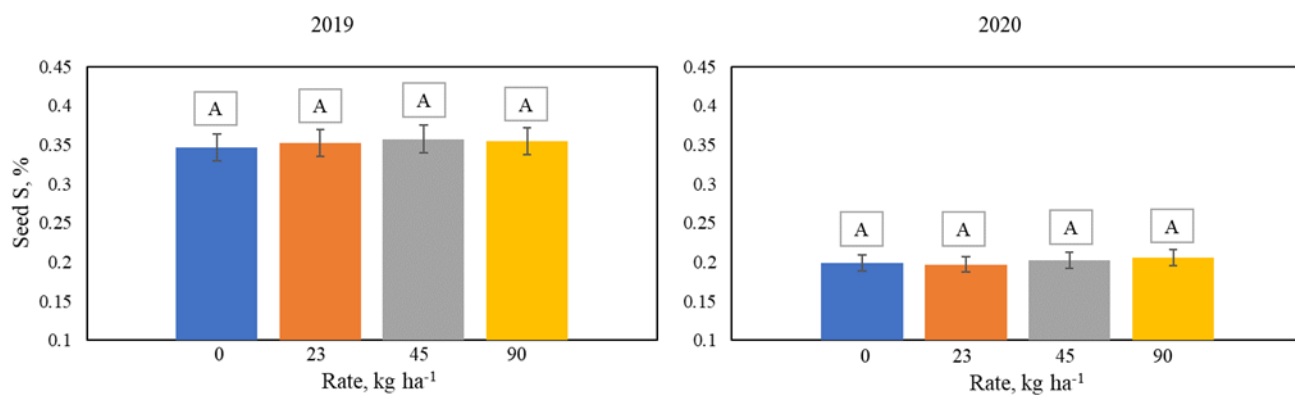


Figure 2.9. Soybean seed S content in response to different S rates in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA.

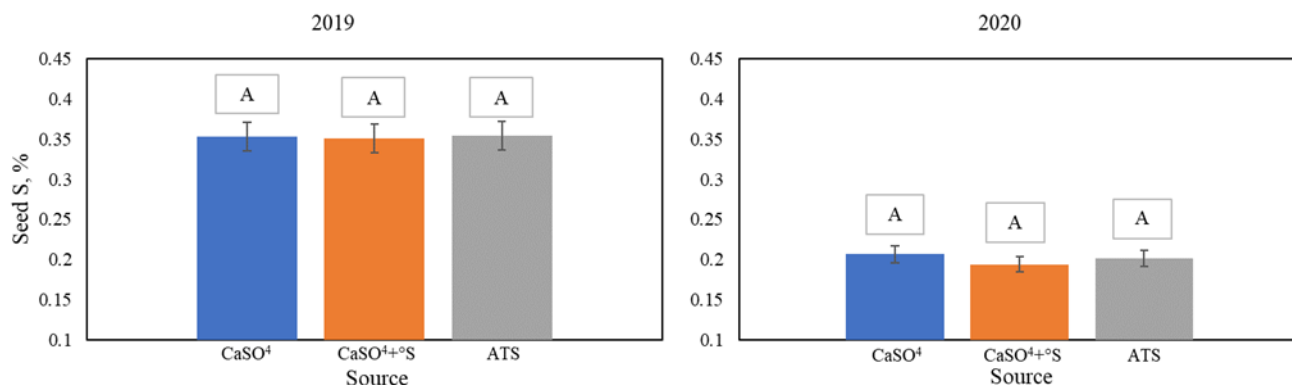


Figure 2.10. Soybean seed S content in response to different S sources in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA.

### 2.3.5. Effect of S Source and Rate on Corn Grain and Soybean Seed S Removal Rate

There was a smaller variation in removal rates between crops, i.e., corn vs. soybean, than between years (Figure 2.11 – 2.14). The average S removed by corn grain and soybean seed was about 10 kg ha<sup>-1</sup>. Between years, S removed by corn grain and soybean seed averaged at 10.2 kg ha<sup>-1</sup> in 2019 and 8.7 kg ha<sup>-1</sup> in 2020. The highest corn grain S was obtained from plots treated with 90 kg S ha<sup>-1</sup> but it was statistically significant only in 2020 (Figure 2.11). For 2019, the numerically highest grain S removal was 10.8 kg ha<sup>-1</sup> while the check plot obtained the lowest at 10 kg ha<sup>-1</sup>. For 2020, the highest grain S removal was 10.6 kg ha<sup>-1</sup> while the plot treated with 45 kg S ha<sup>-1</sup> obtained the lowest grain S removal at 9.3 kg ha<sup>-1</sup>. The S source had no significant effect on grain S removal rate (Figure 2.12). For 2019, CaSO<sub>4</sub>– treated plots obtained the numerically highest grain S removal at 10.8 kg ha<sup>-1</sup> while for 2020 the highest grain S removal rate was 9.9 kg ha<sup>-1</sup> from the thiosulfate-treated plots. The CaSO<sub>4</sub> + S<sup>0</sup> treatment consistently obtained the lowest average S removal for both years.

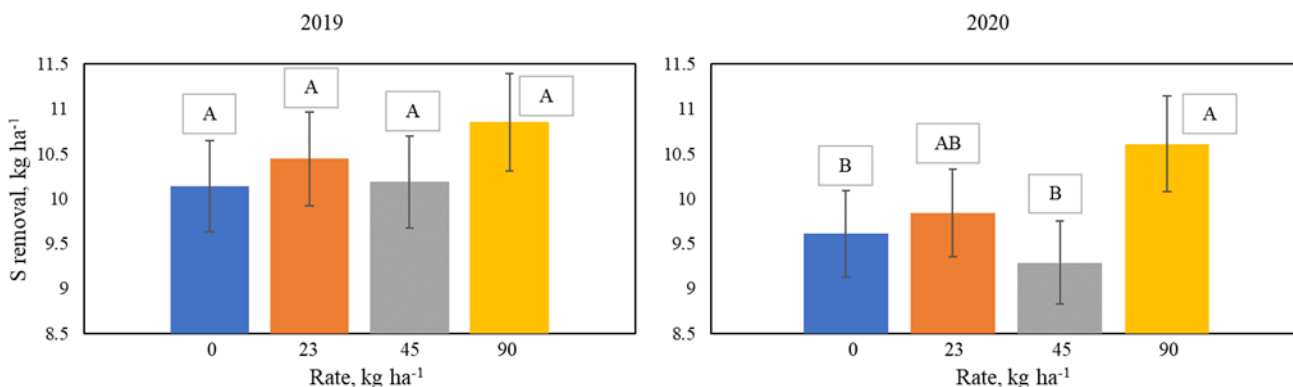


Figure 2.11. Corn grain S removal in response to different S rates in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA.

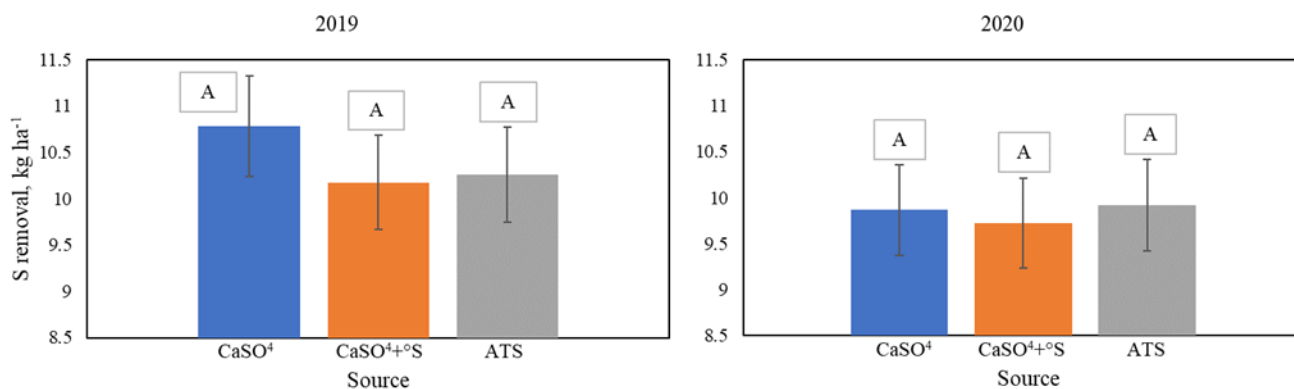


Figure 2.12. Corn grain S removal in response to different S sources in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA.

The amount of S removed by soybean seeds was not influenced by S rate (Figure 2.13). On the other hand, S source had an impact on seed S removal rate in 2019 with thiosulfate-treated plot attaining the highest at 10.5 kg ha<sup>-1</sup>; this was significantly higher than the 9.4 kg ha<sup>-1</sup> removal rate in the CaSO<sub>4</sub>-treated plot.

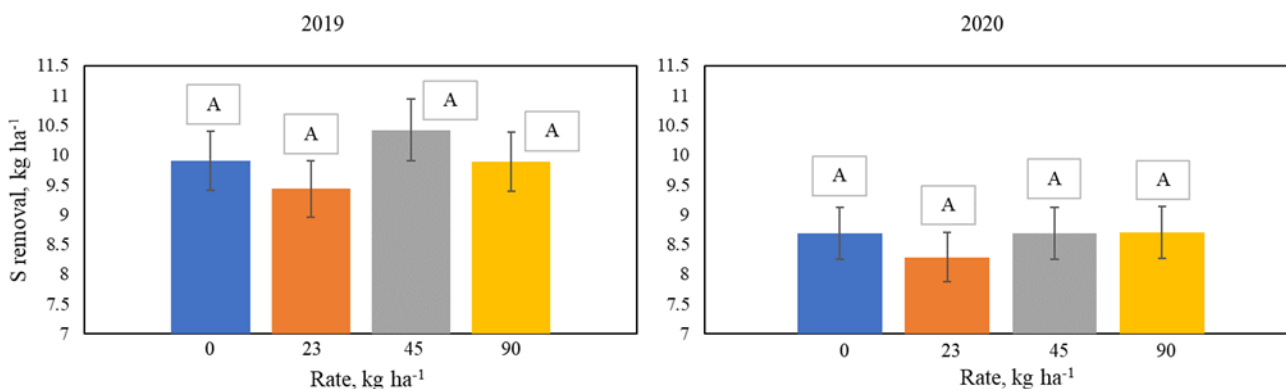


Figure 2.13. Soybean seed S removal in response to different S rates in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA.

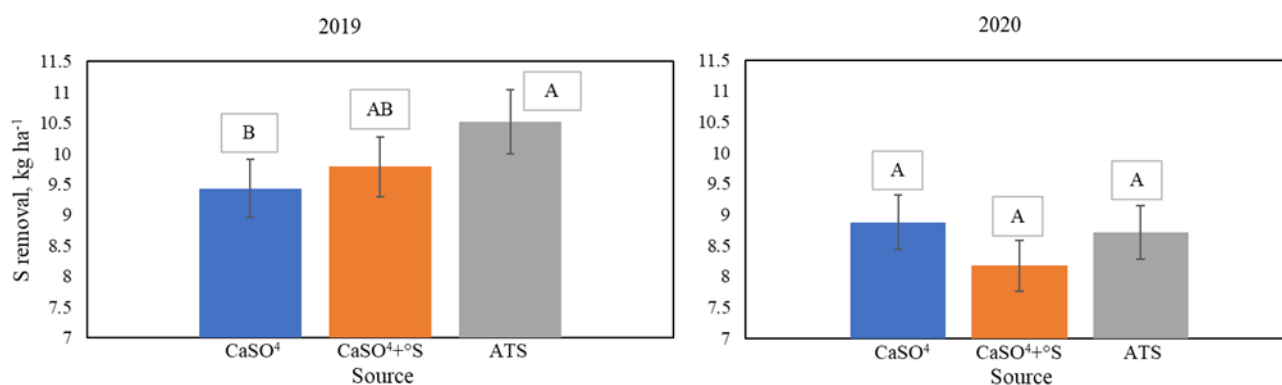


Figure 2.14. Soybean seed S removal in response to different S sources in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA.

## 2.4. Discussion

Yield differed between 2019 and 2020 mainly due to difference in planting date and amount of rainfall received during the vegetative stage. The lower corn and soybean yield recorded in 2019 could be partially attributed to late planting date and high total rainfall received in May at 250 mm. The occurrence of excessive moisture in May 2019 might have compromised the emergence and survival of seedlings resulting in poor stands and reduced yield potential. There was no consistent effect of S source and rate observed on the all the parameters i.e., yield

and grain/seed S content and removal rate, that were evaluated for both corn and soybean. There was no interaction effect of S source and rate observed suggesting that should S rate had significant effect on any of the variables measured, whether positive or negative, this effect is consistent across S source.

The initial soil S test was  $15 \text{ mg kg}^{-1}$  which was above the current recommended critical S level ( $10 \text{ mg kg}^{-1}$ ) for Louisiana soils based on Mehlich-3 procedure. However, studies showed weak association between soil  $\text{SO}_4^{2-}$ -S test level and crop yield (Kim et al., 2013; Sawyer et al., 2009). Soil test interpretation and crop response are purely based on probability, such that low soil test level means there is high probability of crop response to addition of nutrient in questions. Besides, S is a mobile nutrient in the soil and soil testing for estimating plant-available S may be deemed ineffective in certain soils especially in soils with coarse texture and high leaching potential. Sulfur fertilization had a positive effect on corn yield at 62% of the experimental sites established on fine and coarse-textured soils in north-central and northeast Iowa with economic optimum S application rate at  $18 \text{ kg ha}^{-1}$  for fine-textured soils and  $26 \text{ kg ha}^{-1}$  for coarse-textured soils (Sawyer et al., 2011). The results of a study conducted by Rehm (2005) in Southeastern Minnesota suggest that banded fertilizer S improved corn yield at locations with loamy fine sand textured-soil with an optimum application rate of  $6.7 \text{ kg S ha}^{-1}$ . Both studies showed that yield response to S application was not consistently observed across the sites. Even when visual symptoms of S deficiency were evident in alfalfa, only four out of six sites responded to in-season S applications (Sawyer et al., 2009).

Cannon et al. (2020) studied the effect of applying ammonium sulfate (broadcasted) at planting at rates of 0, 11, 22, and  $34 \text{ kg ha}^{-1}$  in corn and soybean under rotation system. Their results came back with no impact of S rate on yield but more on yield difference between years.

A similar scenario occurred in this study where yield differences were more evident between years for both corn and soybean whereas the effect of S source and rate was more sporadic. A study by Goyal et al. (2021) using different S sources applied at 17 kg S ha<sup>-1</sup> showed corn yield was more responsive to interaction of growing season and soil characteristics than S sources. They attributed the lack of response to S treatment to sufficient supply of S in the deep profiles and S release from soil organic matter mineralization. A similar S source and rate study was conducted by Casteel et al. (2019) on soybean production in Indiana. They recorded a significant soybean grain yield response to S application, however, yields of plots treated with monoammonium phosphate blend with 5% S<sup>o</sup> and 90% S<sup>o</sup>+10% bentonite were significantly lower than the plots treated with ammonium sulfate. They attributed this to the slow oxidation process of S<sup>o</sup>, thus the supply of SO<sub>4</sub>-S during the growing season was inadequate.

Soybean seed S content was higher by almost 3.5 folds than corn grain S content. Between years, seed S content was generally higher in 2019 (0.35%) than in 2020 (0.2%). Among the families of crop plants, Leguminosae (0.25-0.30%) in general has higher S content in seeds than Gramineae (0.18-0.19%). Most of the S absorbed by plants is assimilated for production of amino acids, the building blocks of protein. Soybean contains 40% protein which is higher than other legumes and grain crops in general (Carrera et al., 2011; Tessari et al., 2016). With regards to treatments, S rate had a higher and more consistent impact on corn grain S content than S source. This was not the case for soybean seed S wherein neither S source nor S rate had any effect. Sulfur translocation is restricted in the plant in general but for certain plants like soybean the majority of S entering the mature leaves is rapidly loaded into the phloem and translocated to sinks including seeds (Smith and Lang, 1988). This explains the higher S content of soybean seed than corn grain and the higher S rate making a big difference to corn grain S

than soybean S content. Seed S content increased in soybean but not in corn grain S in plots treated with 11 to 34 kg S ha<sup>-1</sup> (Cannon et al., 2020). A study by Kaiser and Kim (2013) investigated the impact of fluid fertilizer combinations containing N, P, and S, on early-growth stage S uptake and removal of soybean on sandy and fine textured soils. The study revealed that soybean seed S concentration was significantly affected by S applications with increases of 31, 7, and 3% at three different sites, indicating a potential for luxury uptake of S into the seed.

Sulfur removal rate is computed as the product of yield and S content. While corn grain S content was substantially lower than soybean seed S content, the higher yield of corn offset this difference which resulted in comparable S removal rates to soybean seeds. Nevertheless, the increasing corn grain S content with increasing S rate was evident enough to carry similar effect on the computed S removal rate. This was not the case for soybean wherein the S source, instead of rate, made a significant impact on seed S removal rate. On the basis of S removal rate, thiosulfate was a better source than CaSO<sub>4</sub> but virtually similar with CaSO<sub>4</sub> + S°. Kaiser et al. (2013) reported that S removal by soybeans was significantly affected by S application across the three sites evaluated regardless of application method, i.e., broadcast and band. The amount of S removed represents a very small portion of the amount applied which were only 1, 3, and 6% of the total S applied for the three sites.

## **2.5. Conclusions**

The response of corn and soybean to S source and rate was sporadic. The impact of the treatments on yield and grain/seed S content and removal rate did not consistently occur in both years for both crops. Source had a significant effect on soybean yield in 2019 with thiosulfate-treated plots obtaining the highest yield at 2,968 kg ha<sup>-1</sup> and was significantly different when



compared to  $\text{CaSO}_4$ -treated plots. Rates had no effect on yield of corn and soybean for both years but increased corn grain S content and removal rate. On the other hand, S source had a significant effect on soybean seed S removal rate in 2020 with thiosulfate treatment obtaining the highest average S removal of  $10.5 \text{ kg ha}^{-1}$  and was significantly higher than the  $9.4 \text{ kg ha}^{-1}$  S removed by  $\text{CaSO}_4$ -treated soybean.

The current S fertilizer recommendation in Louisiana row-crop production is based on soil test such that soils testing below  $10 \text{ mg kg}^{-1}$  is recommended for S application at  $23 \text{ kg ha}^{-1}$ . The outcome of this study was not able to validate this recommendation. The performance of S sources was essentially similar suggesting that any of these (sources) can be used to fertilize corn and soybean. The late planting combined with the high amount of rain received in May 2019 may have interfered with the S source and rate effects on corn and soybean. This excess moisture might have compromised the emergence and survival of seedlings of these late-planted crops. Nutrient losses via leaching and runoff might have occurred which reduced the supply of nutrients, especially the mobile ones like N and S, during the early growth stage of the crops.

## Chapter 3. Optimal Sulfur Application Rates Based on Yield and Plant Sulfur Content

### 3.1. Introduction

In 2019 the U.S. contributed 31% of the world's corn (*Zea mays*) production, followed by China and Brazil (Shabandeh, 2022). Corn production has increased over the years due to improvements in technologies such as seed varieties, pesticides, and fertilizers (USDA, 2022). Corn is an important crop produced in Louisiana, as it is the third most produced crop. In 2021, 235,000 hectares of corn were planted with production level of 2.63 million Mg (USDA-NASS, 2014). Soybean (*Glycine max*) is one of the most important crops produced in the U.S. after corn. From 2015 to 2019, the U.S. was the major global producer of soybeans with 120.5 million Mg production, by 2020 Brazil surpassed the U.S. attaining a production of 138 million Mg (Shabandeh, 2022). In Louisiana, more than 400,000 hectares of soybean were harvested in 2021, achieving a production of 1.4 million Mg.

Sulfur (S) is a plant-essential nutrient and its sufficient availability in soil is vital to plant normal growth and biochemical processes such as biosynthesis of protein and chlorophyll (Brosnan and Brosnan, 2006). Sulfur is a structural component of amino acids such as cysteine and methionine and also performs as a functional group within the plant, participating in important metabolic reactions (Hawkesford et al., 2012). Sulfur is involved in photosynthesis, carbon (C) and nitrogen (N) metabolism, protein synthesis, and detoxification mechanisms (Franzen and Grant, 2008).

Historically, S fertilizer application has not been recommended for corn and soybean production. The soil supply, in combination with sources such as atmospheric deposition, has once met corn and soybean S needs (Tabatai and Bremner, 1972). However, atmospheric S has

been reduced due to the reduction in S emissions from power plants. Thus, occurrence of S deficiency in crops has increased worldwide and in the U.S. (Sulphur Institute, 1982; Bonner et al., 1984; Gravois and Golden, 1984). This has been attributed to reduction in SO<sub>2</sub> emissions from industries, use of S-free fertilizers, declining use of pesticides and fungicides containing S, implementation of cultivars that demands a high fertility requirement resulting in higher nutrient removal from soils, and increased cropping intensity (Assefa and von Tucher, 2013). Further, the new Clean Air Act is expected to result in a permanent 10 million tons reduction in S dioxide (SO<sub>2</sub>) emissions that could further minimize atmospheric S deposition (EPA, 2021).

Sulfur content in plant dry biomass ranges 0.1 and 0.5% depending on plant species (Marschner, 2012). Plants start suffering from S deficiency if S content is below this range. Symptoms of S deficiency include retarded growth, reduced leaf size, and leaf chlorosis occurring in the plant's upper canopy (Ergle and Eaton, 2005). Sulfur is removed from the field with every crop harvesting. Corn removes 0.23 kg of S for every 250 kg grain it produced, so 9 kg S per hectare is removed by corn that yields 10,000 kg per hectare. Soybean grain removes about 0.77 kg S per 250 kg seed – about 9 kg S per hectare at 3,000 kg per hectare (Camberato and Casteel, 2010). Removal rates of S are higher for soybeans because they contain a much higher protein and oil content compared to corn. Unlike corn, soybeans require early-season S uptake to form root nodules for N fixation.

If fertilizer S is not applied, the main source of S in most soils is the S released from mineralization of soil organic matter. Each percent organic matter in the upper 18 to 20 cm of soil contains about 90 kg S per hectare. Organic S must be mineralized to sulfate-S (SO<sub>4</sub>-S) before it becomes plant-available. Sulfate-S is mobile in the soil (similar to nitrate) because it is negatively charged thus, is repelled by the negative charge of the soil. As a result, SO<sub>4</sub>-S is easily

leached from the soils, especially sandy soils. At the field level, the occurrence of S deficiency may be highly variable since soil S availability varies considerably with soil organic matter and texture. Sulfur deficiency is often seen in sandier, lower organic matter, and higher elevation areas of a field.

Crop rotation optimizes productivity arising from a myriad of benefits with the most notable one is the decreased need for N fertilizer for grain (like corn) in sequence with a legume like soybean which fixes  $N_2$  from the atmosphere through symbiotic pathway. Planting a legume after a different crop has resulted in improved yields in comparison to monoculture. The improved yields are attributed to a decrease in pest and disease pressure, enhancement of physical soil properties, and growth-promoting or inhibiting substances in residues of the prior crop (Meese et al., 1991).

There are several S fertilizers sources which come in varying S content, form i.e., dry or liquid, and solubility. These sources include calcium sulfate ( $CaSO_4$ ), ammonium thiosulfate (ATS), and elemental S ( $S^0$ ). Being soluble, when  $CaSO_4$  is incorporated into the soil, it dissociates to  $Ca^{2+}$  and  $SO_4^{-2}$  ions and becomes readily accessible by plants (Zielewicz et al., 2022). Ammonium thiosulfate is a liquid fertilizer containing N and S and when mixed with other fertilizers, the final solution can be neutral to somewhat acidic. Like  $CaSO_4$ , ATS releases readily-available S to the plant. Elemental S on the other hand needs to be oxidized to  $SO_4^{-2}$  by microorganisms, it is a slow process thus S is not readily available for plant uptake. Elemental sulfur is a yellow and water-insoluble solid. Its efficacy in providing S to plants will depend on several factors such as particle size, rate, method, and time of application (Havlin et al., 1999).

Predicting S fertilizer needs is commonly based on soil testing. Critical soil S level is required to derive interpretation and recommendation. Soybean yield response to varying soil

SO<sub>4</sub>-S content was evaluated on different soil types (Embrapa-soja, 2006). The critical SO<sub>4</sub>-S content that was established ranged from 2 to 9 mg kg<sup>-1</sup> in sandy soil and 5 to 35 mg kg<sup>-1</sup> in silty soils. The critical shoot-S concentration for 75% of relative soybean shoot weight was 0.8 g S kg<sup>-1</sup> whereas the optimum leaf-S concentration ranged from 2.0 to 3.1 g kg<sup>-1</sup> and the concentration in the low ranges from 1.5 to 2.5 g S kg<sup>-1</sup> (Hitsuda et al., 2008). In Pennsylvania soils, the critical soil S is 15 mg kg<sup>-1</sup> based on Mehlich-3 procedure (White et al., 2021).

In Louisiana, S fertilization at 22 kg ha<sup>-1</sup> rate is recommended for all field crops when Mehlich-3 soil test S falls below 12 mg kg<sup>-1</sup> (Parvej, 2021). There have been notable improvements in crop yields due to recent adoption of production technologies. With the lower concentration of SO<sub>4</sub> in rainfall than what had been recorded in the past 40 years, soil S in affected regions has been steadily declining followed by increasing cases of S deficiency thus, S fertilization has become routine in many crop production systems (Haneklaus et al., 2006). The occurrence of these changes requires validation and updating of soil S test interpretation and recommendations. The objectives of this study were to (1) evaluate the relationship between S fertilizer rates, soil S level, yield, and plant S content, and (2) determine and validate optimal S fertilizer rates based on these measured variables in corn-soybean rotation system in Louisiana.

## **3.2. Materials and Methods**

### **3.2.1. Site Description, Planting Method, Treatment Structure, and Trial Establishment**

This study was conducted in 2019 and 2020 at the LSU AgCenter Central Research Station in Baton Rouge, LA. The soil type at this site (Latitude 30°, 15', 06.8'' N; Longitude 91°, 10', 11.7'' W) is a silt loam consisting of a mixture of Cancienne (90%) and 10% Carville, Thibaut, and Gramercy (Fine-silty, mixed, superactive, nonacid, hyperthermic Fluvaquentic Epiaquepts).

Prior to crop establishment, composite soil samples were collected for nutrient content analysis. Soil was tilled and leveled using a Wil-Rich 1400 field cultivator. Seeds of corn and soybean were planted at 92,000 and 185,000 seeds ha<sup>-1</sup> with row spacing of 0.96 m using a John Deere 1700 Max Emerge plus vacuum planter. The corn varieties used in 2019 and 2020 were DEKALB 6826 and DEKALB 6869, respectively while soybean variety ASGRO 5535 was used for both years. For corn, N was knifed-in at 200 kg ha<sup>-1</sup> using urea-ammonium nitrate (UAN, 32%N) solution around V3 leaf stage. Phosphorus and K fertilizers were not applied since soil test P and K came back as medium to high. Based on Mehlich-3 extraction procedure (Mehlich, 1984), the nutrient levels of the initial soil samples were: 86 mg P, 142 mg K, 1649 mg Ca, 356 mg Mg, 15.2 mg S, 4.8 mg Cu, and 1.35 mg Zn per kg soil. The S treatment (or fertilization) was made between V4 to V5 leaf stage. The treatment structure was twelve combinations of different S sources (CaSO<sub>4</sub>, ATS, and 50% CaSO<sub>4</sub> + 50% S<sup>0</sup>) applied at 23, 45, and 90 kg ha<sup>-1</sup>, including an untreated check plot (Table 3.1). Each treatment was replicated four times and arranged in a randomized complete block design. Each plot consisted of four 12.2 m long rows with a 3.05 m alleyway. Granular sources (CaSO<sub>4</sub> and S<sup>0</sup>) were broadcast-applied by hand while ATS was dribbled using a Solo 425 15-L professional piston backpack sprayer. The dates of major field operations are summarized in Table 3.2.

Table 3.1. Description of the treatment structure for corn and soybean implemented in this study at the LSU AgCenter Central Research Station in Baton Rouge, LA In 2019 and 2020.

Trt. No	S Source	S Rate (kg ha <sup>-1</sup> )	Type	Application method
1	Control	0	-	-
2	CaSO <sub>4</sub>	23	Granular	Broadcast
3		45		
4		90		
5	Control	0	-	-
6	Ammonium Thiosulfate'	23	liquid	Dribble in (surface)
7		45		
8		90		
9	Control	0	-	-
10	CaSO <sub>4</sub> + S <sup>0</sup>	23	Granular/pellets	Broadcast
11		45		
12		90		

Table 3.2. Agronomic activities accomplished during the two-year study at the LSU AgCenter Central Research Station in Baton Rouge, LA in 2019 and 2020.

Site	Year	Crop	Planting Date	S application Date	Harvest Date
1	2019	Corn	20-Mar-19	1-May-19	27-Aug-19
2	2019	Soybean	13-May-19	24-May-19	30-Sep-19
1	2020	Soybean	25-Mar-20	21-Apr-20	3-Aug-20
2	2020	Corn	25-Mar-20	21-Apr-20	4-Aug-20

### 3.2.2. Soil and Plant Sampling

For each year, soil samples from each plot were collected 30 days after S fertilization (DAF) and at harvest. Soil samples were taken at 0-15 cm depth using a standard soil probe (JMC; Model No. 641-792-8285). Sixteen core samples were taken from each plot and mixed thoroughly before placing them in labeled paper bags. Soil samples were oven-dried (Despatch LBB series; model number LBB2-18-1) at 65°C for a few days, processed using a Humbolt electric flail grinder, and sieved through a 2 mm sieve for analysis.

Leaf samples were collected on the same date the soil samples were collected. The 2<sup>nd</sup> or 3<sup>rd</sup> collared leaves from the top of the canopy (16-20 leaves) were taken from the two middle rows of each plot, oven-dried at 65°C for at least 72 hours, ground using Wiley Mill grinder (Model No. 3, Arthur H. Thomas CO., and Philadelphia, USA) to pass through a 1-mm sieve.

### **3.2.3. Corn and Soybean Yield**

Corn and soybean plots were harvested using a Kincaid 8-XP research plot combine harvester equipped with a load cell and infrared moisture sensor to determine plot grain yield and moisture content, respectively. For each plot, the two middle rows were cut; both the grain/seed weight and moisture content were recorded. Grab samples of corn grains and soybean seeds were collected from each plot, oven-dried and ground for nutrient content analysis. Plot yield was converted to per hectare basis and adjusted at 15.5% and 13% moisture content for corn and soybean, respectively. Relative yield was also calculated using the following equation:

$$\text{Relative yield} = [\text{yield (kg ha}^{-1}\text{)} / (\text{maximum yield} \times 100)]$$

### **3.2.4. Soil and Plant Analysis**

Soil samples were analyzed for nutrient content based on Mehlich-3 extraction (Mehlich, 1984) procedure followed by analysis using inductively coupled plasma optical emission spectrophotometry (ICP-OES). Reference samples and blanks were included in each extraction for quality assurance. The two grams of processed soil samples were placed into a 125 ml plastic bottle and 20 ml of Mehlich-3 solution was added. The samples were shaken for five minutes on a reciprocal shaker at high speed and then filtered using a Whatman No. 42 filter paper. Clear extract samples were poured into 10 ml tubes and analyzed for macro and micronutrient concentration using ICP-OES.



The nitric acid-hydrogen peroxide ( $\text{HNO}_3\text{-H}_2\text{O}_2$ ) digestion method followed by ICP-OES analysis was used for analysis of leaf and grain/seed S content. For this, ground samples (0.5 g) were weighed in kimwipes and placed into digestion tubes. Five (5) mL of nitric acid ( $\text{HNO}_3$  67-70%) was added to the tubes, making sure that the acid washed any remaining of ground samples on the side of the tube. After that, the samples were sat undisturbed for 50 minutes then mixed using a vortex mixer before placing them in a digestion block (previously turned on and set to a temperature between 152-155°C) for 2-3 minutes to vigorously boil the samples. When a brown fume appeared, the tubes were removed from the digestion block and let cool for 10 minutes. Three (3) mL of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was added to the samples before placing them back to the digestion block for 2 hours and 45 minutes. Samples were then taken out from the digestion block and allowed to cool before transferring the digested samples to 15 mL centrifuge tubes, completing to a volume of 12.5 mL with deionized water. The final solution was filtered using Whatman No. 1 filter paper and transferred into 10-mL tubes for ICP-OES analysis. Blank and reference samples were integrated in each batch of digestion for quality assurance.

### **3.2.5. Data Analysis**

R project (R Core Team, 2022) was used for statistical analysis through the integrated development environment (IDE) R Studio. Analysis of variance (ANOVA) was run through with the corresponding function in native R. Tukey's HSD test was performed using the agricolae package in R (Mendiburu F, 2021). Source and rate were treated as fixed effects in each year for corn and soybean yield and soil, leaf, grain, and seed S content. Non-linear regression analysis was done to estimate critical soil S and optimal S fertilizer rates using Excel and PROC NLIN in SAS. An Alpha of 0.1 was used for significance in all the analyses.

### 3.3. Results

#### 3.3.1. Climatic Conditions

The average monthly precipitation and temperature in 2019 and 2020 are presented in Figure 3.1. and 3.2., respectively. The highest average monthly precipitation was recorded in October 2020 (Figure 3.1.) with 252 mm; similar amount was recorded in April 2019 (252) and May 2019 (246 mm). Given that the S application in 2019 was done in the month with the second highest average monthly precipitation (May), a negative effect could have occurred to both corn and soybean yield due to S leaching. Overall, the year 2020 accumulated more precipitation compared to 2019. The average monthly temperature from May to September for both years was between 30°C and 31°C (Figure 3.2.).

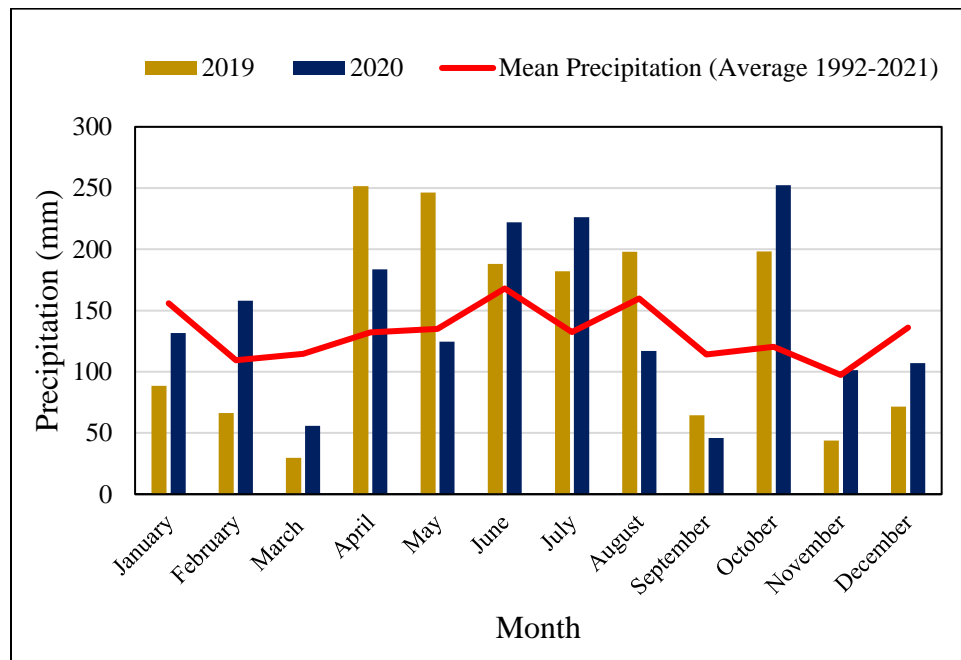


Figure 3.1. Average monthly precipitation from January to December in 2019 and 2020 at the LSU AgCenter Central Research Station in Baton Rouge, LA.

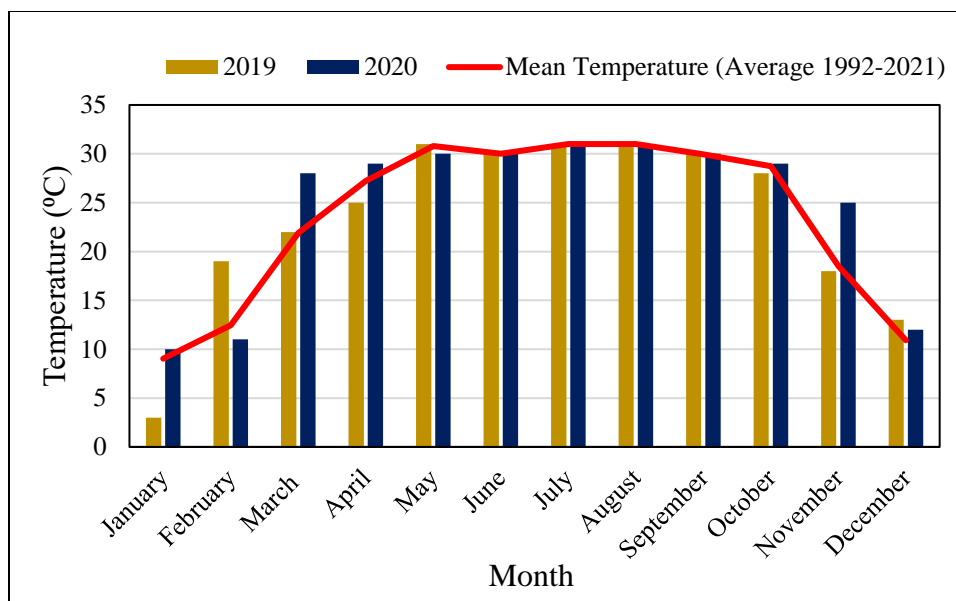


Figure 3.2. Average monthly temperature from January to December in 2019 and 2020 at the LSU AgCenter Central Research Station in Baton Rouge, LA.

### 3.3.2. Analysis of Variance on All Measure Variables.

The effect of S source, rate, and their interaction on yield, leaf S content, and soil S at 30 DAF and at harvest in 2019 and 2020 is shown in Table 3.3 and 3.4. There was a significant source effect on corn yield in 2019 and soybean yield in 2020. Both corn and soybean yield did not respond to S rate nor to source x rate interaction. For both years and crops, there was a significant effect of S rate on leaf and soil S. There were significant source effects recorded; these include soil S at harvest of corn and soybean yield in 2019, soil S 30 DAF in corn 2020, and leaf S and soil S (30 DAF and harvest) in soybean 2019. Also, there were a few significant source and rate interaction recorded: corn leaf and soil S in 2019 and 2020.

Table 3.3. Analysis of variance for the effect of S source, rate, and their interaction on yield, leaf S content, and soil S at 30 DAF and at harvest at the LSU AgCenter Central Research Station in Baton Rouge, LA, 2019.

Crop	Treatment	Grain Yield	Leaf Sulfur Content	Soil Sulfur Midseason	Soil Sulfur Harvest
		p-value			
Corn	S Source	NS	NS	NS	0.075
	S Rate	NS	0.011	<0.0001	<0.0001
	Source x Rate	NS	0.061	NS	0.075
Soybean	S Source	<0.028	NS	NS	0.020
	S Rate	NS	0.027	<0.0001	0.0003
	Source x Rate	NS	NS	NS	NS

⌘ NS indicates no significant effect at the  $p < 0.1$ .

Table 3.4. Analysis of variance for the effect of S source, rate, and their interaction on yield, leaf S, and soil S content at 30 DAF and at harvest at the LSU AgCenter Central Research Station in Baton Rouge, LA, 2020.

Crop	Treatment	Grain Yield	Leaf Sulfur Content	Soil Sulfur Midseason	Soil Sulfur Harvest
		p-value			
Corn	S Source	NS	NS	0.035	NS
	S Rate	0.06	0.058	<0.0001	<0.0001
	Source x Rate	NS	0.038	0.036	NS
Soybean	S Source	NS	0.020	0.009	0.020
	S Rate	NS	0.056	<0.0001	<0.0001
	Source x Rate	NS	NS	NS	NS

⌘ NS indicates no significant effect at the  $p < 0.1$ .

### 3.3.3. Corn and Soybean Yield Response to Sulfur Fertilization

Soybean yield in 2019 was the only variable that responded to the treatment, i.e., source effect (Table 3.3). Thiosulfate-treated soybean attained the highest yield at 3,000 kg ha<sup>-1</sup> (Figure 2.6, Chapter 2). The yield data was not adequate to validate the current S fertilizer recommendation for field crops in Louisiana (Parvej, 2021). In this chapter, the yield data was normalized across years and sources for each crop, then regressed with S application rate (Figure 3.3). An estimate of optimal S rate was made using Cate-Nelson method (Cate and Nelson, 1971). The process was challenged by identifying the S rate where quadrant I and III yielded the lowest number of data points. The resulting graph can be interpreted either as (1) crop yield tended to decline below 23 kg ha<sup>-1</sup> application rate or that the application rate < 45 kg S ha<sup>-1</sup> tended to maximize crop yield. The linear plateau model using SAS was not able to converge nor identify a critical S rate (data not shown).

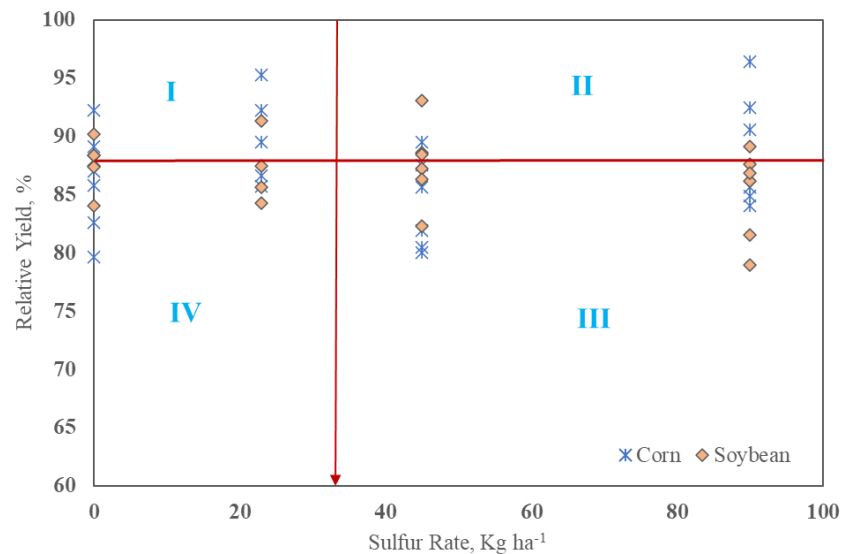


Figure 3.3. Relative yield of corn and soybean in response to S fertilization rate for both years, LSU AgCenter Central Research Station in Baton Rouge, LA.

### 3.3.4. Soil Sulfur Response to Sulfur Fertilization

The S rate impact on S content of soil was greater for samples collected at 30 DAF than those collected at harvest with coefficient of determination ( $R^2$ ) of 0.5437 and 0.1754, respectively (Figure 3.4). The relationship between soil S and S rate also showed that baseline (check or 0) soil S at harvest was slightly higher than at 30 DAF with values of 26 and 19 mg kg<sup>-1</sup>, respectively. As indicated in the ANOVA, the S rate had a more consistent effect than source (Table 3.3 and 3.4).

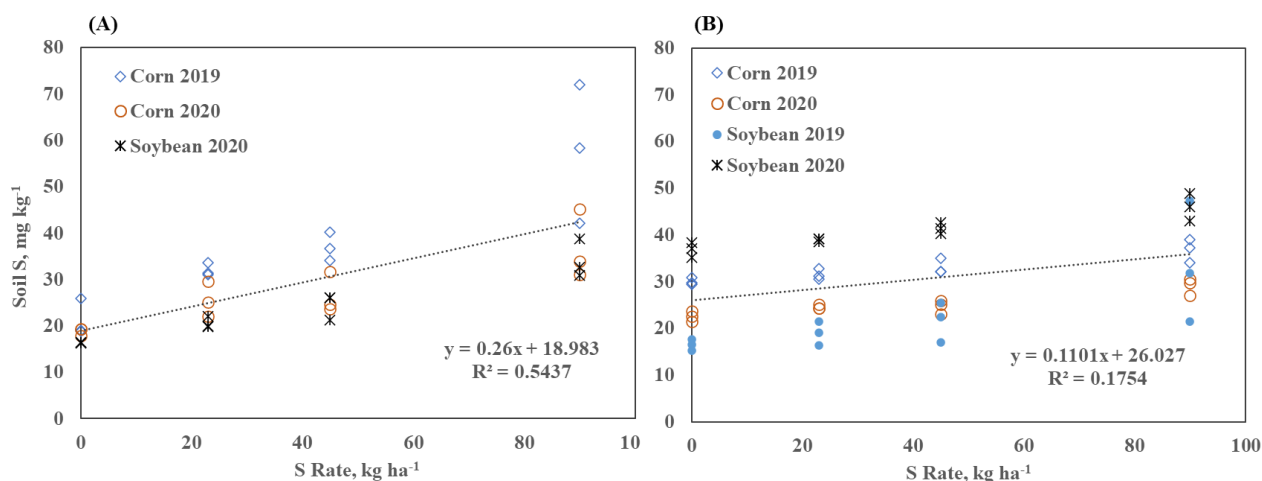


Figure 3.4. Sulfur content of soil treated with different rates of S 30 days after S application (A) and at harvest (B) of corn and soybean in 2019 and 2020, LSU AgCenter Central Research Station, Baton Rouge, LA.

### 3.3.5. Plant Sulfur Response to Sulfur Source and Rate

There was a significant rate effect and source and rate interaction effect for leaf S content in corn, whereas for soybean only the rate effect was significant (Table 3.3 and 3.4). For corn in 2019, leaf S content increased with increasing S rate with 90 kg ha<sup>-1</sup> obtaining the highest value at 0.255%; this was significantly higher than the check with leaf S content of 0.23% (Figure 3.5). In 2019, ATS-treated corn obtained the numerically highest leaf S content of 0.247% while

CaSO<sub>4</sub> + S<sup>0</sup>-treated corn obtained the lowest with 0.242% (Figure 3.6). For soybean in 2019, leaf S content also increased with increasing S rate with 90 kg ha<sup>-1</sup> obtaining the highest value at 0.291% which was significantly different from the check (0.276%) (Figure 3.7). There was a significant effect of source on soybean leaf S but only in 2020. The ATS-treated soybean obtained the highest leaf S content at 0.249%; this was significantly higher than the leaf S of soybean treated with CaSO<sub>4</sub> + S<sup>0</sup> (Figure 3.8).

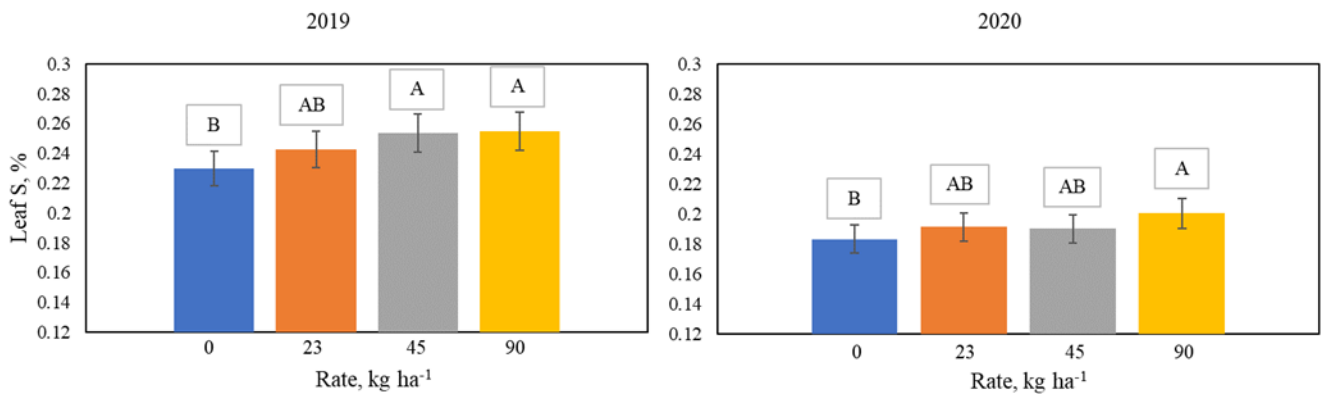


Figure 3.5. Corn leaf S content in response to S rate in 2019 and 2020, LSU AgCenter Central Research Station, Baton Rouge, LA.

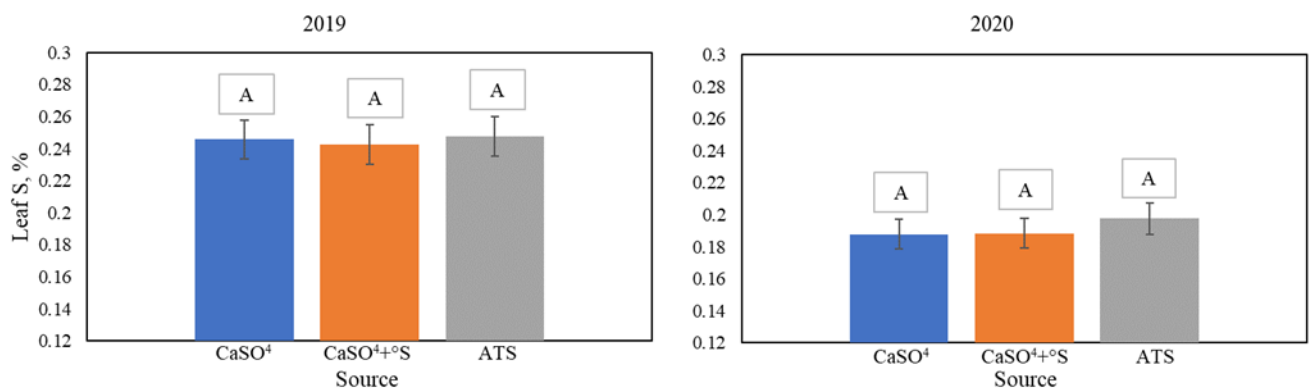


Figure 3.6. Corn leaf S content in response to S source in 2019 and 2020, LSU AgCenter Central Research Station, Baton Rouge, LA.

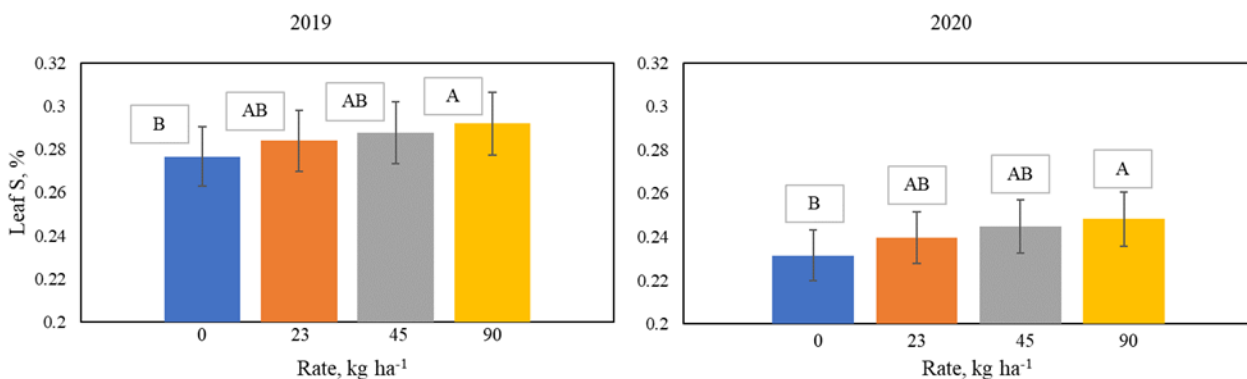


Figure 3.7. Soybean leaf S content in response to S rate in 2019 and 2020, LSU AgCenter Central Research Station, Baton Rouge, LA.

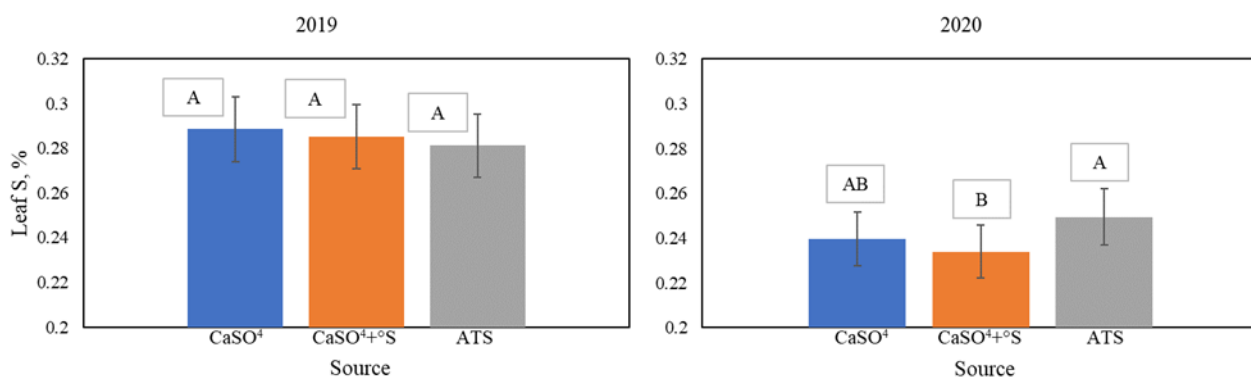


Figure 3.8. Soybean leaf S concentration in response to S source in 2019 and 2020, LSU AgCenter Central Research Station, Baton Rouge, LA.

After averaging the data across S sources, soil S and leaf S of soybean and corn for both years were regressed (Figure 3.9). There was a weak positive relationship ( $r^2 = 0.145$ ,  $p$ -value = 0.075) between these two variables. The linear plateau analysis revealed that the critical soil S was estimated at 40 mg kg<sup>-1</sup>. Beyond this soil S level, there was no statistically significant increase in leaf S that occurred. In the previous chapter, the results on ANOVA for grain/seed removal rate were reported. The grain/seed S removal rate was significantly affected by source in 2019 (Table 2.3). On the other hand, rate only affected corn grain S removal rate in 2020 (Table 2.4). The grain removal rate was pooled across source, the resulting average grain/seed S



removal rates were regressed with soil S content (Figure 3.10). Compared with leaf S, grain/seed S removal rates had a stronger relationship with soil S ( $r^2=0.373$ ,  $p<0.001$ ). The linear plateau fitted in the data points came back with an estimated optimal soil S level at 52 mg kg<sup>-1</sup>.

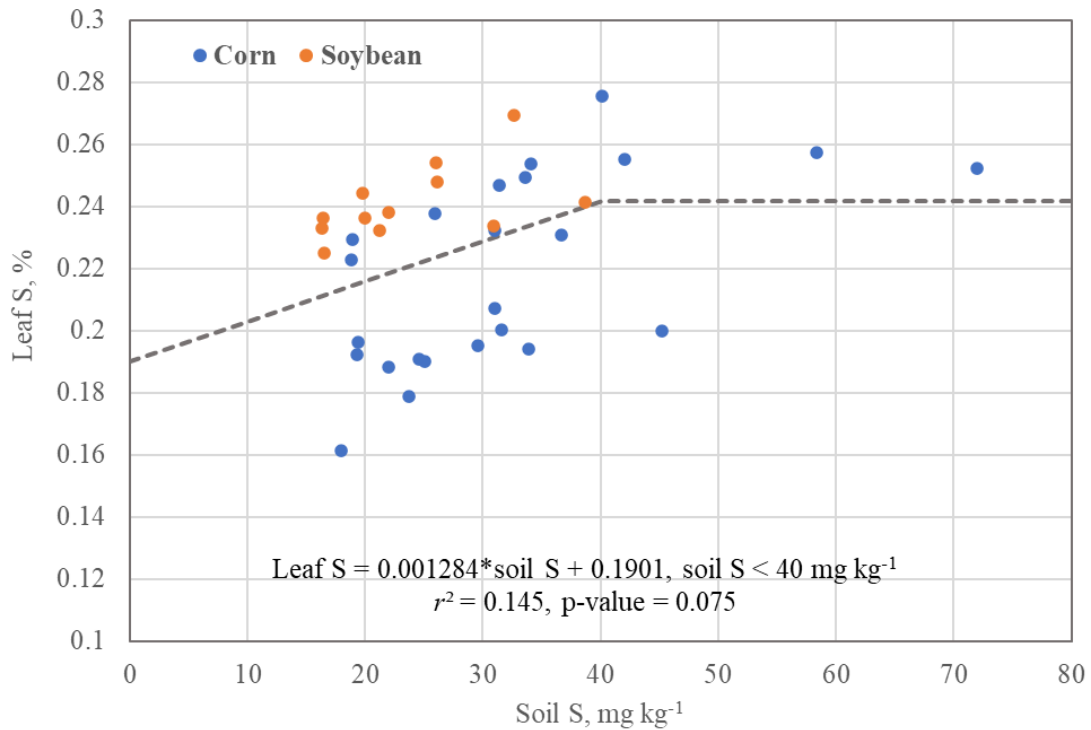


Figure 3.9. Non-linear regression analysis between soil S and leaf S content for corn and soybean in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA. Data was averaged across S source with n = 36.

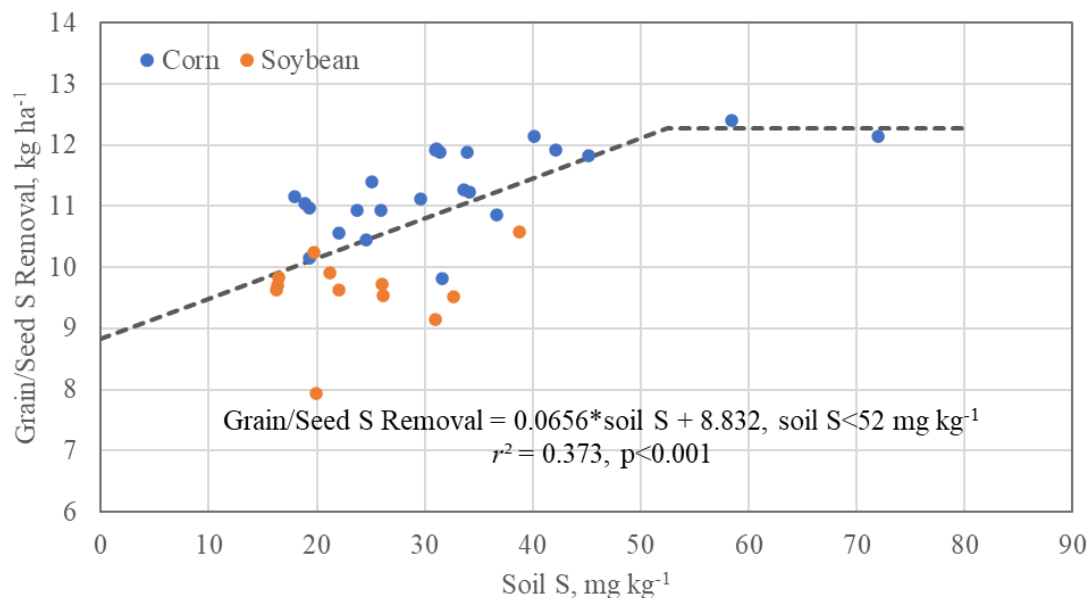


Figure 3.10. Non-linear regression analysis between soil S and grain/seed removal rate for corn and soybean in 2019 and 2020, LSU AgCenter Central Research Station in Baton Rouge, LA. Data was averaged across S source with n = 35.

### 3.4. Discussion

With two years of study, the data generated was not adequate to conduct a validation of the current S fertilizer recommendation in Louisiana soils using yield as a response variable. The actual yield was transformed to relative yield to be able to combine the corn and soybean data hence increasing the number of observations. The Cate-Nelson method (Cate and Nelson, 1971) estimated that the optimal S rate ranged between 23 and 45 kg ha<sup>-1</sup> for achieving the maximum plant S uptake. The weather interference especially during the crop establishment and fertilization period might have played a role in not achieving an acceptable level of confidence on the estimated optimal S rate based on relative yield. The above-average rainfall received in May for both years may have brought negative impacts either through reduction in yield or interference on treatment effects. For example, in 2019 late-plating, the occurrence of high

rainfall in May might have compromised the emergence and seedling survival whereas there could be losses of applied fertilizer, particularly the mobile ones like S, that subsequently reduced its impact to both corn and soybean for both years. The lower correlation of soil S and S rate in samples taken at harvest compared to 30 DAF indicates that the S rate impact was reduced perhaps due to water-facilitated movement of S via leaching and runoff. Nevertheless, increasing S rate clearly increased soil S and consequently, leaf S content.

The elevated level of soil S content due to S fertilization did not result in significant increase in yield. Previous studies reported similar outcomes. Only 62 % of the experimental sites showed positive response to S fertilization despite those fine to coarse textured soils were testing low for S (Sawyer et al., 2011). The economic optimum S application rate was estimated at 18 kg ha<sup>-1</sup> for fine-textured soils and 26 kg ha<sup>-1</sup> for coarse-textured soils. Similar to Sawyer et al. (2011), Rehm (2005) reported that yield response to S application was not seen in 100% of the sites evaluated. Sulfur deficiencies are commonly observed in coarse textured soils with low organic matter content but does not indicate certainty in terms of crop yield response to S fertilization. On the other hand, several studies showed that S response in crops were documented on soils that do not possess these properties (Feyh and Lamond, 1992; Hoeft et al., 1985; Randall et al., 1981).

Rate had greater and more consistent impact on soil S than S source. Sampling time also imposed variation on soil S response to S rate. There was a stronger association between soil S content and S rate at 30 DAF than at harvest and the baseline (check) soil S level was higher at harvest (26 mg kg<sup>-1</sup>) than at 30 DAF (19 mg kg<sup>-1</sup>). The movement of S in the soil had possibly taken place with the average monthly cumulative rain from April to June being at 200 mm; this was sufficient to diffuse the applied S in each plot. Similar result was reported by Kovar (2021)

from a pot study he conducted using three sources (ammonium sulfate, ammonium thiosulfate, and S-enhanced mono-ammonium phosphate) applied at rates of 0, 22, 34, and 45 kg S ha<sup>-1</sup>. After four weeks of incubation soil S increased with increasing application rate while source had no effect on the amount of S extracted. On the other hand, the mobility of S in the soil was also demonstrated by a study conducted by Cannon (2021). Here, soil sampled deeper than 15 cm had higher soil S content particularly in plots treated with higher S rates.

The ANOVA confirmed that rate effect on leaf S was more evident than source for both crops and years. With improvement in soil S content due to S fertilization, it was expected that corn and soybean S uptake will also improve. The non-linear regression between soil S and leaf S showed rather a weak positive relationship yielding a  $R^2 = 0.145$  ( $p < 0.1$ ). The linear plateau fitted in the data points was able to estimate the critical soil S at 40 mg kg<sup>-1</sup>. Similar analysis was conducted using the grain/seed S removal rate as response variable with the outcome having a stronger relation with soil S and a higher estimate of optimal soil S at 52 mg kg<sup>-1</sup>. Both estimates were way above the current critical S level for Louisiana soils set at 12 mg kg<sup>-1</sup> (Parvej, 2021). The lack of direct increase in yield yet S uptake was increased with S fertilization suggest luxury consumption of S (Rehm, 2005). Others may argue that the lack of yield response can be partly attributed to below-average growing conditions in 2019 and 2020 that potentially masked the positive effect of S fertilization on S uptake by corn and soybean. Also, the elevated level of soil S may have not been sustained within the critical period of growth and development of corn and soybean. Sulfate-S is mobile and can be leached out from the soil under excessive rainfall, driving the SO<sub>4</sub>-S below the root zone (Zimmerman, 2016).

### **3.5. Conclusions**

For both crops and years, S rate had stronger and more consistent effect than source for all measured parameters. An estimate of optimal S rate using Cate-Nelson method showed that yield tended to decline with application rate below 23 kg S ha<sup>-1</sup> and tended to maximize at an application rate 45 kg S ha<sup>-1</sup>. Sulfur rate impact on S content of soil was greater for samples collected at 30 DAF than those collected at harvest. Using leaf S and grain/seed S removal rate as response variables to soil S, the linear plateau model estimated the critical soil S at 40 and 52 mg kg<sup>-1</sup> for achieving maximum crop S uptake. The absence of yield response despite S uptake was improved suggested a luxury consumption of S and/or masking effect of below-average weather condition (particularly rainfall) during the critical growth stages of soybean and corn for both years. Field response studies like this should be long-term and well-replicated in space to build robust database.

## Chapter 4. Conclusions

Cases of sulfur (S) deficiency in crops are increasing due to low S deposits on the soil from the atmosphere and high crop S removal rates. Significant reduction in S emissions from industries have contributed to this problem, as well as decreased use in organic fertilizers and use of high yielding cultivars. Thus, S has become an important component of fertilization programs in many crop production systems. This study was established in 2019 at the LSU AgCenter Central Research Station in Baton Rouge, LA to (a) evaluate the effect of S different sources applied at increasing rates on corn and soybean yield under rotation system, (b) evaluate the relationship between S fertilizer rates, soil S level, yield, and plant S content, and (c) determine and validate optimal S fertilizer rates based on these measured variables in corn-soybean rotation system in Louisiana.

The outcome of the first study indicated that the response of corn and soybean to S source and rate was sporadic. Source had a significant effect on soybean yield in 2019 with thiosulfate treatment obtaining the highest yield. Sulfur source had a significant effect on seed S removal rate with thiosulfate-treated soybean obtaining the highest average S removal. The overall result of this study was not able to validate the current recommendation for application of S fertilizer. The outcome of the second study indicated that an estimate of optimal S rate using Cate-Nelson method showed that yield tended to decline with application rate below 23 kg S ha<sup>-1</sup> and maximize at an application rate not exceeding 45 kg S ha<sup>-1</sup>. Also, for both crops and years, S rate had greater and more consistent effect than S source for all measured parameters. The estimated critical soil S was 40 and 52 mg kg<sup>-1</sup> based on fitted linear plateau model using leaf S and grain/seed S removal rate as response variables to soil S. These values were above the current critical S for Louisiana soils at 12 mg kg<sup>-1</sup>. The lack of yield response despite the improvement

in soil S content, subsequently, corn and soybean S uptake possibly was an indication of either luxury consumption of S and/or weather interference during the critical crop growth stages that masked the effects of S source and rate. Overall, the outcome of this study did not generate strong data to suggest any changes should be made on the current recommendation for S fertilization for soybean and corn production in Louisiana. Studies like this should be long-term and well-replicated in space (location) to build a robust database and to increase confidence on the outcomes and recommendations.

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## **Vita**

Diego Manuel Mayorga Valladares was born in Leon, Nicaragua, in March of 1995. He attended the Pan-American Agricultural School, Zamorano in Honduras, and received his Bachelor of Science in Agricultural Sciences and Production in December 2015. After graduating he worked in family business managing sugarcane farms and in Monte Rosa sugar mill of Pantaleon group for almost two years in biological control and production of liquid biofertilizers. In March of 2019 he came to Louisiana State University as an intern at the School of Plant, Environmental, and Soil Sciences. He started his master's program in August 2019 under the guidance of Dr. Brenda Tubana working on finding optimal sulfur sources and application rates on corn and soybean production systems in Louisiana. He plans to receive his masters in May 2023.