# CORN GRAIN YIELD RESPONSE TO SULFUR FERTILIZATION IN INDIANA

by

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Dedicated to my family

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#### ABSTRACT

Reduction in sulfur deposition from power plant emissions has resulted in lower amounts of soil sulfur and, perhaps, in inadequate sulfur availability for corn. The objective of this study was to determine if corn (Zea mays L.) grain yield was responsive to S fertilization in Indiana and what soil and cropping system factors contributed to the likelihood of a response. Field scale experiments were conducted at 28 sites from 2017 to 2020, the majority in corn-soybean (Glycine max (L.) Merr) rotation. In-season measurements included soil sulfate-S concentration and soil texture from 0 to 60 cm in 20 cm increments, plant nutrient concentration in the whole plant at V3-V7, in the earleaf, and in the grain. Additional measurements were 1,000 kernel dry weight, total kernel rows per ear, and kernels per row. Sulfur treatment rates ranged from 0 to 34 kg S ha<sup>-1</sup> as ammonium thiosulfate, and were applied as starter, sidedress, and both combined. Fertilizer S increased grain yield by 0.2 to 3.0 Mg ha<sup>-1</sup> at 10 of 28 Indiana site-years, approximately a 36% frequency of response. When a response to S fertilizer occurred, the lowest sidedress rate examined in that site-year, which ranged from 8 to 17 kg S ha<sup>-1</sup>, was enough to maximize grain yield. On soils with 26 to 31 g kg<sup>-1</sup> OM, S fertilization increased yield 0.2 to 0.3 Mg ha<sup>-1</sup> at 2 of 10 site-years. Response to S fertilization at 8 of 10 site-years with soils with lower OM, 10 to 25 g kg<sup>-1</sup>, had higher yield increases ranging from 0.7 to 3.0 Mg ha<sup>-1</sup>. Grain yield responses occurred in both coarse- and fine-textured soils and were consistent and large at 2 sites. Sulfate-S concentration in the soil and S concentration in the whole plant (V4-V7) were not good indicators of response to S fertilization. For the majority of the site-years where grain yield increased with S fertilization, the grain S concentration, earleaf S concentration, and earleaf N:S were respectively <0.9 g kg<sup>-1</sup>, <1.8 g kg<sup>-1</sup>, and >15:1 without S treatment. These parameters improved with the addition of S but some site-years with these values did not have a yield response. These earleaf S and N:S 'critical values' may serve as reference for potentially S responsive sites, but more observations are necessary to validate these critical levels. Sites with higher basal values (without fertilizer treatment) for earleaf and grain S concentration and lower earleaf N:S still showed increased tissue S concentration upon S fertilizer application, albeit with no increase in grain yield. We encourage farmers to consider S fertilization at rates ranging from 8 to 17 kg S ha<sup>-1</sup> applied at sidedress. this recommendation for fields showing S deficiency symptoms or where R1 earleaf S concentration and N:S are below 1.8 g kg<sup>-1</sup> and above 15:1, respectively.

#### CHAPTER 1. LITERATURE REVIEW

#### **1.1 Sulfur dynamics**

#### **1.1.1 Total soil sulfur**

Sulfur in the soil exists in both organic and inorganic forms, with organic S being the dominant form. Oxidation states of both organic and inorganic S compounds range from -2 to +6, in order from the most reduced to most oxidized sulfide>disulfide>thiol>thiophene> sulfoxide> sulfonate>sulfate (Huffman et al., 1991). In soils, organic S is found in proportion to organic C and N, and C:N:S ratios varies with vegetation and soil properties (Eriksen, 2008; Solomon et al., 2009; Tabatabai & Bremner, 1972). In the Great Plains of North America, the C:N:S ratios ranged from 52:5:1 to 84:8:1 in native grassland and in cultivated soils slightly lower, ranging from 32:4:1 to 72:7:1 (Wang et al., 2006). In New Zealand, C:N:S in young well-drained soil averaged 79:9:1, lower than 100:8:1 in waterlogged gley soils (Ghani et al., 1991, 1992). Sulfur is a constituent element of soil organic matter (OM), therefore OM is a source of S for plants and microorganism. In addition, cultivation of soils and well-drained conditions reduces the C:N:S ratios.

Total S in the soil is usually greater in fine-textured soils (loam, silt loam, and silty clay loam) than in coarse-textured soils (loamy sand, sandy loam, sandy clay loam). In Iowa, total S ranged from 174 to 580 mg S kg<sup>-1</sup> in soils with loam, silt loam, and silty clay loam textures, and was greater than 79 mg kg<sup>-1</sup> total S measured in a loamy sand soil (Neptune et al., 1975; Tabatabai & Bremner, 1972). Total S in tropical soils with clay texture ranged from 209 to 398 mg S kg<sup>-1</sup>, whereas loamy sand, sandy loam, and sandy clay loam soils ranged from 43 to 72 mg S kg<sup>-1</sup> (Neptune et al., 1975). Regardless of the climate, coarse-textured soils usually have a lower total S concentration than fine-textured soils, increasing the likelihood of S being in short supply for plants in coarse-textured soils.

In a temperate climate, total S varied with differences in vegetation, decreasing in this order: grassland (190 to 405 mg kg<sup>-1</sup>)>transitional zone forest-grassland (105 to 285 mg kg<sup>-1</sup>)>forest (106 to 170 mg kg<sup>-1</sup>) (Bettany et al., 1973). In contrast, total S in a tropical climate were highest in forest soils (~273 mg kg<sup>-1</sup>), then derived savanna soils (~183 mg kg<sup>-1</sup>), and lowest in savanna soils (~69 mg kg<sup>-1</sup>) (Kang et al., 1981). Forest soils in temperate climates and savanna

soils in tropical climates have a reduced pool of S compared to soils in temperate grassland and tropical forest soils.

Tillage of previously undisturbed soils caused total S to decline over time, unless periodic addition of S-enriched material were made in amounts that compensated for S leaving the system (Blum et al., 2013; Kirchmann et al., 1996; Solomon et al., 2009; Spratt, 1997). Sulfur levels in the soil eventually stabilized with time, although this process took 10 to 40 years, depending on initial OM levels and weather conditions (Knights et al., 2000; Solomon et al., 2009). To substantially increase soil organic S over the long-term required a concomitant application of organic C. In a 153-year experiment in the United Kingdom, farmyard manure was applied at 35 Mg ha<sup>-1</sup> yr<sup>-1</sup> (7 Mg ha<sup>-1</sup> dry matter,  $\sim$  33 and 5 kg S ha<sup>-1</sup> organic and inorganic S, respectively), and resulted in total soil S of 521 mg S kg<sup>-1</sup> compared to total soil S of 194 mg S kg<sup>-1</sup> for the inorganic S treatment (82 kg S ha<sup>-1</sup> the first 124 years, ~34 kg ha<sup>-1</sup> the following 18 years, and 17 kg ha<sup>-1</sup> the last 11 years) (Knights et al., 2001). Total S in the soil that received fertilizer S had only 23 mg S kg<sup>-1</sup> more than the soil that received no manure or fertilizer S for 153 years. In a shorter study conducted over 35 years in Sweden, an Entisol was fertilized with ammonium sulfate (91 kg S ha<sup>-1</sup> yr<sup>-1</sup>) and 1 of 4 organic amendments (total S in kg ha<sup>-1</sup> yr<sup>-1</sup>): sewage sludge (67), animal manure (18), green manure (11), and peat (5) (Kirchmann et al., 1996). Soils to which sewage sludge and animal manure were applied had total S of 440 and 310 mg S kg<sup>-1</sup>, respectively, compared to 210 mg kg<sup>-1</sup> for the soil receiving inorganic fertilizer and 230 mg S kg<sup>-1</sup> for the untreated soil (Kirchmann et al., 1996). Sulfur fertilizers do not usually increase total S in the soil unless S is added together with C, which increases total S in the soil in the long-term. Addition of organic materials to agricultural fields would help maintain or increase S levels in the soil.

#### 1.1.2 Soil organic sulfur

Soil organic S is the most common form of S in the soil accounting for 90 to 96% of the total S in Brazilian soils (Costa, 2020), 93 to 98% in Japanese soils (Tanikawa et al., 2014), and 91 to 99% in Canadian and United States soils (Tabatabai & Bremner, 1972; Wang et al., 2006). Plant, animal, and microbial tissues are important sources of organic S compounds in the soil and are classified in two major groups: organic sulfate (S bonded to C through O (S-O-C)) and C-bonded S (Blum et al., 2013; Schoenau & Malhi, 2008). While C-bonded S is more stable,

organic sulfate is more labile and easily converted to inorganic sulfate via extracellular enzymatic hydrolyzation (McGill & Cole, 1981; McLaren et al., 1985). This rapid process makes organic sulfate an important source of immediate S for plants compared to C-bonded S that is more stable.

Carbon-bonded S is the dominant form of organic S in organic soils and exist in reduced fraction in mineral soils (Ghani et al., 1992; Neptune et al., 1975; Stanko-Golden & Fitzgerald, 1991; Xia et al., 1998). Field measurements from organic soils in Canada (Lowe & DeLong, 1963) and the United States (David et al., 1982; Landers et al., 1983) found that C-bonded S accounted for approximately 47-58% and >69% of the total S, respectively in these locations. Other studies found that the C-bonded sulfur fraction in mineral soils from Canada (Lowe & DeLong, 1963) and Ghana (Acquaye & Kang, 1987) was lower when compared to their fraction in organic soils and accounted for ~12-35% of total S. Organic soils and mineral soils with high organic matter concentration have a vast pool of S in the form of C-bonded S compared to mineral soils with low organic matter in which this form of S is less prevalent.

Previous studies reported that C-bonded S from microbial biomass S represented a small fraction of the total organic S, <3% (Saggar et al., 1981; Maynard et al., 1983). However, the rapid cycling of microbial biomass makes it a sink and a source of S to be accounted for when considering plant-available S. Soil from a native grassland and a cultivated field, both with negligible amounts of microbial S at the beginning of the experiments, where incubated at constant temperature and moisture (Gupta & Germida, 1989). In native grassland, wetting of the soil increased microbial S and reached a maximum of 11 mg kg<sup>-1</sup> after 7 days. Whereas in cultivated soil, the maximum microbial S was 7 mg kg<sup>-1</sup> and occurred after 14 days (Gupta & Germida, 1989). Under field conditions in cultivated soils in Oregon, microbial S was lower than measured in incubation experiments and changed with the seasons (Castellano & Dick, 1991). Microbial S increased from 2.0 mg S kg<sup>-1</sup> in March to 5.5 mg S kg<sup>-1</sup> by May (Castellano & Dick, 1991) which is equivalent to ~5 and ~12 kg ha<sup>-1</sup> of microbial S accounts for a minimal fraction of total C-bonded S, it can be an important source of S during spring and summer in temperate climates.

Ester sulfate fraction is usually low in organic soils and high in mineral and cultivated soils. Studies on organic layers of a Puerto Rican tropical forest found ester-sulfate was as low as 34% of the total S (Stanko-Golden & Fitzgerald, 1991) and even lower in Ethiopian highlands ranging from 16-26% of total S (Solomon et al., 2001). In contrast, in a cultivated soil in Oregon with no previous history of S fertilization, the ester sulfate fraction ranged 58 to 79% of the total S in the 0-15 cm depth (Castellano & Dick, 1991). A study was conducted at 19 forest sites in the US to measure S forms in organic and mineral horizons (Autry & Fitzgerald, 1990). In this study, ester sulfate was the main S fraction (43-77% of total S) in only 4 of 18 organic horizons. Ester sulfate was more common in mineral horizons, and was the dominant S form in 8 of 14 A/B horizons (20-40 cm depth), and in 7 of 17 B/C horizons (40+ cm depth) (Autry & Fitzgerald, 1990). Ester sulfate is the most common form of S in agricultural fields and mineral horizons, the least common in undisturbed soils, and can be easily mineralized to plant-available S.

Field measurements from previous researchers have shown the proportions of organic S compounds change with differences in land use and soil properties. For instance, forest soils in Ethiopia had both reduced and oxidized S in similar proportions, whereas ester sulfate was predominant in cultivated soils (Solomon et al., 2003). The proportions of S compounds in humus-extracts for forest and cultivated soils were respectively 26% and 15% sulfides-thiophenes, 15% and 3%, sulfoxides, 33% and 36% sulfonates, and 28% and 47% for sulfates (Solomon et al., 2003). Forest soils from Minnesota also had a significant portion of reduced S, and the humus extract composition was 31% sulfides-thiophenes, 7% sulfoxides, 18% sulfonates, and 44% sulfates (Xia et al., 1998). Whereas, in cultivated soils from Wisconsin, the proportion of reduced states of S was low and more highly oxidized S was present: 15%, 2%, 29%, and 54% for sulfides-thiophenes, sulfoxides, sulfonates, and sulfates, respectively (Xia et al., 1998). In forest soils, organic S compounds exist in reduced and oxidized forms of S. In contrast, oxidized S is the major fraction in cultivated soils and is therefore readily available to plants.

#### **1.1.3** Soil inorganic S

Inorganic S is much less abundant than organic S in the soil and it represents <10% of the total S in the surface layers (Costa, 2020; Knights et al., 2000; Landers et al., 1983; Solomon et al., 2003). Inorganic S is found in the form of the gases hydrogen sulfide (H<sub>2</sub>S) and sulfur oxides (SO<sub>x</sub>), and in ionic compounds such as sulfides, polysulfides, elemental S, thiosulfates, tetrathionates, polythionates, sulfites, and sulfates (Bohn et al., 1986). In aerated soils, S gases are short-lived and last just minutes before being oxidized to sulfuric acid, therefore their fraction is negligible (Bohn et al., 1986). Sulfate is the main form of inorganic S and is found in the soil

solution and adsorbed to soil colloids and is commonly labeled extractable sulfate-S (Bohn et al., 1986). The latter is the most immediately available S in the soil for plants, therefore plant growth and development rely on it.

Previous field studies have observed that the sulfate adsorbed to soil colloids and in the soil solution changes with change in land use and management. Adsorbed S and soil solution sulfate were respectively 3.3 and 15.9 mg S kg<sup>-1</sup> in a woodland, 3.0 and 7.2 mg S kg<sup>-1</sup> in a cultivated field with farmyard manure applied, and 1.7 and 3.9 mg S kg<sup>-1</sup> in a cultivated field fertilized with inorganic S (Knights et al., 2000). Cultivated soils had ~50-75% less adsorbed sulfur than forest soils (Knights et al., 2000), and higher concentration of soil solution sulfate than adsorbed S (Knights et al., 2000; Spratt, 1997). Introduction of undisturbed soils to cultivation reduced the overall soil extractable sulfate-S, however, additions of C and S in form of farmyard manure helped maintain higher levels of extractable S compared to using inorganic fertilizer alone.

In field studies of agricultural fields, soluble sulfate-S was less stable than adsorbed sulfate-S, increasing with warm temperatures due to increased OM mineralization and decreasing in winter and spring due to leaching (Castellano & Dick, 1991; Schoenau & Malhi, 2008). Other factors that increased sulfate-S in solution include application of sulfate-containing sources (e.g., manures and fertilizers), atmospheric S deposition (Castellano & Dick, Costa, 2020; 1991; Knights et al., 2000;), liming, and phosphate in the soil. The addition of liming increase hydroxyl groups that replace sulfate anion sorbed to colloids and phosphate competes for sorption sites with sulfate; in both scenarios sulfate in soil solution increases (Kamprath et al., 1956; Qian et al., 2017). Soil soluble sulfate-S concentration is usually higher during the growing season compared to winter; however, this S may or may not be enough to sustain crops growth requiring supplementation with S in some cases.

In acidic environments the sorption sites for sulfate-S are Fe and Al oxides and 1:1 silicate clays which sorb more sulfate than 2:1 clays (Bohn et al., 1986; Kamprath et al., 1956; Scott, 1976). Iron oxides sorb more sulfate than Al oxides (Scott, 1976; Johnson & Todd, 1983; Tabatabai, 1996), and at pH >6.5 where Fe and Al charges are neutralized S sorption is negligible (Tabatabai, 1996). Fe and Al, whether complexed with OM or not, hold phosphate more tightly than sulfate, greatly reducing sulfate sorption (Bohn et al., 1986; Kamprath et al., 1956). Johnson & Todd (1983) reported the highest sulfate accumulation in the B and C horizons on Ultisols and Inceptisols from the United States and Costa Rica. Another study in 12 cultivated fields in the Coastal Plain of

North Carolina, the B horizon had higher amounts of extractable sulfate ranging from 26 to 142 mg kg<sup>-1</sup> compared to the A horizon which ranged from 2 to 7 mg kg<sup>-1</sup> (Camberato & Kamprath, 1986). Sulfate in the B horizon would be sufficient to sustain crop growth if roots had access to it. For field experiments on Iowa soils between 2006 and 2010, extractable sulfate-S in the 0 to 90 cm profile ranged from ~24 to 105 mg kg<sup>-1</sup>, well above the range ~1 to 14 mg kg<sup>-1</sup> in the 15 cm surface layer (Sawyer et al., 2015). In Indiana, soils extractable sulfate-S was similar or decreased through 60 cm depth at 5 locations, while sulfate-S increased with increased depth at 1 location (Moser, 2016). Sulfur below the upper 15-20 cm of soil is a potential source for crops if accessible to roots, and it is particularly important in soils where sulfate-S accumulates in deeper soil layers - acidic subsoils with pH <6.5 containing Fe and Al oxides.

Mineral sulfates in dry environments are coprecipitated with Ca, Mg, K, and Na (Bohn et al., 1986); whereas in acidic soils with poor drainage S is reduced to sulfide and precipitates with Fe forming pyrite (Clark et al., 1961; Thode-Andersen & Jørgensen, 1989). Other mineral sulfides, such as greigite, mackinawite, and marcasite, are much less common (Bush & Sullivan, 1997, 2000), stable in anaerobic environments, but quickly oxidized under aerobic conditions (Ward et al., 2004). When sulfate and sulfite minerals are present, weathering and oxidation of these sources make S available to plants.

#### **1.1.4** Microbial oxidation and reduction

Oxidation of S is important to make S available in soils fertilized with reduced S sources (e.g., elemental S, thiosulfate), in parent materials high in sulfides, and in flooded areas (Eriksen, 2008). Microbial oxidation of reduced inorganic S (e.g., sulfide, elemental S, thiosulfate) is performed by bacteria (e.g., *Thiobacillus, Paracoccus,* and *Xanthobacter*) (Friedrich et al., 2001; Kelly et al., 1997) and archaea (Kletzin et al., 2004; Schönheit & Schäfer, 1995). Both autotrophic and heterotrophic bacteria are involved, but the latter is predominant (Lawrence & Germida, 1988). The oxidation of S produces intermediate products in the process, but in incubation conditions these intermediate products from oxidation of sulfide in acid sulfate soil materials were relatively short-lived - thiosulfate and tetrathionate were present during the first 2 and 4 days after initiation of oxidation and were not detected in the following 34 days (Ward et al., 2004). The ability of the

soil microbial community to oxidize reduced S determines the availability of S from these sources to plants.

Previous studies observed oxidation of S was positively correlated with increased rhizosphere biomass. In a controlled environment, wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) rhizosphere soil (compared to bulk soil) had 19-32% and 8-10% more thiosulfate- and sulfate-producing organisms, respectively (Grayston & Germida, 1990). Rhizosphere soil from corn had greater abundance of enzymes associated with microbial cycling of S, ~30% more sulfite oxidase and ~50% more sulfite reductase compared to bulk soil (Li et al., 2014). The plant rhizosphere increases the oxidation of S and therefore the availability of sulfate-S.

In poorly aerated or flooded soils, sulfate is reduced primarily by the bacteria, *Desulfobacterales* with some reduction by less common species, *Desulfovibrionales, Syntrophobacteraceae, and Clostridiale* (Eriksen, 2008; Sitte et al., 2010). Oxygen from sulfate is used for bacterial respiration after oxygen from hydroxyl and nitrate has been depleted (Eriksen, 2008). In two separate incubation experiments, it was observed that sulfate was reduced to H<sub>2</sub>S and immediately formed sulfide minerals in combination with Fe, Co, Ur, and heavy metals (Burton et al., 2014; Sitte et al., 2010). The maximum sulfide accumulation (reduced S) observed in laboratory conditions occurred at day 10, same time lapse for treatment with 100 mg S kg<sup>-1</sup> and without S (Connell & Patrick, 1969). Horizons A and B constantly affected by the water table had higher sulfate-reducing bacteria and mineral sulfide content compared to negligible activity in the A horizon with better aeration and less water accumulation (Sitte et al., 2010). Agricultural soils with poor aeration or temporally flooded had higher risk of S reduction and hence diminished availability of sulfate-S.

Degradation of methionine-, cystine-, and cysteine-containing substrates produces volatile S compounds that inhibited nitrification when measured in incubated soil under control conditions (Bremner & Bundy, 1974). For instance, in a closed-system incubation, volatile-S compounds introduced as gases successfully inhibited nitrification when measured on day 7. The inhibition of nitrification from volatile-S compounds decreased in the order carbon disulfide (96%)>dimethyl disulfide (42%)>methyl mercaptan (19%)>dimethyl sulfide (14%)>hydrogen sulfide (8%) (Bremner & Bundy, 1974). Carbon disulfide (2 mg kg<sup>-1</sup>) was the only gas effective through day 14, and nitrification inhibition was 56% compared to 11% with the commercial inhibitor (2 mg

kg<sup>-1</sup>). Under field conditions, volatile S-compounds are sorbed to the soil, therefore their inhibitory effects would be less likely under field conditions than in a laboratory incubation (Banwart & Bremner, 1975; Bremner & Bundy, 1974).

Agricultural soils with and without previous S fertilization were incubated in aerobic and anaerobic conditions, volatile S was <0.05% of total S after 60 days at 30°C, and only 4 of 25 soils released volatile S compounds under anaerobic conditions (Banwart & Bremner, 1976). In addition, higher volatilization occurred from soils with OM >5.7% than with OM <2%, suggesting that volatile S compounds may mainly be formed from microbial decomposition of organic matter. Concentrations of volatile S compounds sufficient to inhibit microbial activity under field conditions may be unlikely.

#### **1.1.5** Sulfur mineralization

Organic S mineralization to inorganic S through biochemical and biological processes involve bacteria and fungi (McGill & Cole, 1981). Mineralization of S in 48 forest and savanna soils during a 10-week aerobic incubation averaged 1.7%, with a maximum of 7%, (Acquaye & Kang, 1987). In prairie soils, 3 to 5% of total S was mineralized in 17 weeks (Maynard et al., 1983). Under field conditions, mineralization of S over 2 years was estimated at 1.7 to 3.1% of organic S per year in pots filled with soil from Danish agricultural fields under spring rape (*Brassica napus* L.)-ryegrass (*Lolium multiflorum* Lam.) rotation (Eriksen et al., 1995). The pots were kept exposed to field conditions and most of the roots were isolated from the soil (Eriksen et al., 1995). Under 22°C constant temperature and soil moisture, the estimate of organic S made available for 75 days was 2 to 3 kg S ha<sup>-1</sup> for each percent OM, but it varied with soil temperature and moisture (Kaiser & Vetsch, 2020). The capacity of the soil to mineralize soil OM determine the S made available from this source.

Enzymatic hydrolysis of organic sulfates is carried out by bacteria and fungi and is affected by pH, temperature, soil depth, phosphate, and inorganic sulfate. Optimum incubation pH for maximum enzymatic activity in 3 soils was 7.5, 7.0 and 5.3, respectively, suggesting enzymes may be adapted to different soil pH (Lou & Warman, 1992). Enzymatic activity was positively related with pH across soil types in long-term continuous corn – alluvial and volcanic soils, and noncultivated soils in mid and south Europe (Gianfreda et al., 2005). In volcanic soils, 35°C increased total S mineralization compared to  $20^{\circ}$ C and it was attributed to increased hydrolysis (Tanikawa et al., 2014). Enzymatic activity measured in incubation experiments at 35°C using *p*-nitrofenylsulfate as substrate, reported degradation of substrate at 50 to 65 mg kg<sup>-1</sup> soil h<sup>-1</sup> in long-term continuous corn (Gianfreda et al., 2005), 33 to 35 mg kg<sup>-1</sup> soil h<sup>-1</sup> in rotation crop sugar beet (*Beta vulgaris* L.), wheat, rye (*Secale cereale* L.), potato (*Solanum tuberosum* L.), wheat, and barley (*Hordeum vulgare* L.) (Marschner, 2003), and 5.5 and 11.5 kg<sup>-1</sup> soil h<sup>-1</sup> in an Iowa clay soil and a Michigan sandy soil, respectively (Taylor et al., 2002). Bacteria and fungi populations in cropland were higher in the surface 0-30 cm depth than deeper depths in both clay and sandy soils (Taylor et al., 2002). While bacteria were present at all depths, fungi were not found below 2.3 m. Exogenous sulfate and phosphate (due to displacement of sorbed sulfate into the soil solution) inhibited hydrolysis of ester-sulfate (Lou & Warman, 1992; Blum et al., 2013). Ester sulfates is the form of organic S most rapidly converted to sulfate-S by bacteria and fungi providing S for plants, however its mineralization is inhibited by fertilization with sulfate and phosphate.

Carbon-bonded S is desulfurized by bacteria as they consume C as energy or S as nutrient and in the process  $CO_2$  and sulfate are released as by-products, however any other C-containing substrate can be used in absence of C-bonded S to satisfy the microbial demand for energy (McGill & Cole, 1981). Time for complete mineralization of sulfonate, a C-bonded S compound, was evaluated in incubation experiments using activated sludge (Pakou et al., 2007). Five days were needed for intermediate products to mineralize into sulfate, and 7 days for 98% mineralization of the initial sulfonate (16.5 mg L<sup>-1</sup>) (Pakou et al., 2007). Release of S from OM depends on microbial demand for C, therefore favorable conditions for microbial activity would increase S made available from OM mineralization.

#### 1.1.6 Sulfur and immobilization

Mineralization of S is expected with C:S<200:1 and immobilization with C:S>400 (Eriksen, 2008). This was consistent with findings in incubation experiments (Tabatabai & Chae, 1991) with crop residues (cornstalk, soybean, and sawdust) and animal manure (chicken, hog, horse, and cow) with C:S >400 and alfalfa (*Medicago sativa* L.) with C:S<200:1 incorporated to the soil. All treatments except alfalfa had net immobilization of S over a 26-week period - net S immobilization ranged 15-53% for cornstalk, 15-47% for soybean, and 10-62% for sawdust. Mineralization from

animal-manured soil was not different from the control at pH 5.1, whereas at soil pH 6.5 S was immobilized ~8-11%, and at pH 7.6 mineralized ~27-63% relative to the check soil (Tabatabai & Chae, 1991). Immobilization of S was accentuated with the addition of cellulose which immobilized ~6.1 mg S kg<sup>-1</sup>, or in combination with sulfate ~8.9 mg S kg<sup>-1</sup>, compared to the control ~1.4 mg S kg<sup>-1</sup> (Saggar et al., 1981). Although immobilization of S is not expected with C:S ratios <200:1, it was nonetheless observed in incubation experiments in 18 of 48 soils from Ghana (Acquaye & Kang, 1987). Incorporation of crop residue and animal manure with C:S >400 can reduce availability of S for crops temporarily if planted at the same time. Although immobilization of S is not expected at lower C:S ratios, it has been shown to also occur.

Immobilization of S increased as microbial activity and biomass increased with the transition from winter to spring, due to increased temperature (Castellano & Dick, 1991; Schoenau & Malhi, 2008) and rewetting of the soil (Castellano & Dick, 1991; Gupta & Germida, 1989). Microbial activity in incubated soils showed an exponential increase for the first two weeks (Ghani et al., 1991). In another experiment, the exponential increase continued until week 4 to 6 of the incubation (Saggar et al., 1981). After the exponential phase immobilization continued to increase linearly at a slow rate. In contrast, drying periods and microbial decay mineralized S (Castellano & Dick, 1991). Immobilization of S by microbes reduces availability of S during the first few weeks after winter as the microbial population regenerates. However, S availability would increase following stabilization of the population.

#### **1.1.7** Sulfate leaching

Sulfate-S in the soil solution is susceptible to leaching. Risk of sulfate-sulfur leaching is higher for coarse-textured well-drained soils and in wet climates (Dick et al., 2008). Risk of sulfate leaching decreased in the presence of Fe and Al with little impact of OM, and removing Fe oxides from the soil had more impact on soil retention capacity of sulfate than removing Al oxides (Harward et al., 1962). In acidic conditions (pH >3.5 to 6.5) sulfate losses by leaching were influenced by anion exchange capacity of crystal edges of clays. In addition, the risk of S leaching decreased with clay type, kaolinite > illite > montmorillonite (Harward et al., 1962). The retention capacity of S in acidic soils is lessened with increased pH and coarse-textured soils are more susceptible to S loss by leaching.

Both phosphate and lime additions increase S leaching, and the magnitude is dependent on the sulfate adsorption capacity of the soil. For example, in a soil with low adsorption capacity (predominantly mica and illite clays) 67% of the initial S added was leached from the soil after 40 days of incubation (Bolan et al., 1988). When phosphate or lime was added, sulfate-S leaching increased to 75 and 85%, respectively. In contrast, in a volcanic soil with high adsorption capacity, sulfate leaching as a proportion of the total S added was <2, <4, and 7% in the control or with phosphate or lime added, respectively (Bolan et al., 1988). The greater effect of lime on increasing S leaching in both soils was related to increased pH increasing sulfur mineralization from OM, desorption capacity are more susceptible to S loss by leaching, reducing potential S for crops utilization.

Fertilizer with oxidized forms of S are more prone to be lost by leaching than sources with less oxidized S. In laboratory conditions, maximum S leaching from sulfate-S containing fertilizer was in week 2 to 4 of incubation, while little sulfate leached from elemental S (Dijksterhuis & Oenema, 1990). In a wheat field experiment, application of 52 kg S ha<sup>-1</sup> as ammonium sulfate resulted in 60 kg S ha<sup>-1</sup> leached, well above the 14 kg S ha<sup>-1</sup> lost from farmyard manure that applied 38 kg S ha<sup>-1</sup>, and the 9 kg S ha<sup>-1</sup> lost from the treatment receiving no additional S (Knights et al., 2000). Adding crop removal and atmospheric deposition of S to the equation, net sulfur balance was -3, +28, and +4 kg S ha<sup>-1</sup> with fertilizer S, manure, and the unamended soil (Knights et al., 2001). Fertilization with oxidized S provides readily available S to crops, but are also more susceptible to leaching compared to fertilization with reduced S. On the other hand, reduced S sources need to be oxidize to sulfate-S to become available to plants.

#### **1.2** Atmospheric sources of sulfur

#### **1.2.1 Biogenic sources**

Sulfur is released to the atmosphere from water bodies, land surfaces, volcanoes, and anthropogenic activities. Emissions of H<sub>2</sub>S have been detected in large water bodies such as the Black Sea in southern Europe (Yang et al., 1996) and Salton Sea in California (Reese et al., 2008). Tidal flat sediments released both SO<sub>x</sub> and H<sub>2</sub>S to the atmosphere, emissions averaged 7.1 and 28 mg S m<sup>-2</sup> h<sup>-1</sup> for muddy and sandy sites, respectively (Azad et al., 2005). Concentration of H<sub>2</sub>S in surface sediments in fall and summer ranged <0.1 to 4 mg S L<sup>-1</sup> with none detected during winter

and spring (Asaoka et al., 2018). Sulfur dioxide and sulfuric acid are the main S compounds released from water bodies and sediments to the atmosphere, and in temperate climates emissions are higher during warmer months compared to winter and early spring.

Volatile S compounds in incubated soils originated from S reduction in anoxic conditions (Farwell et al., 1979) and from microbial decomposition of S-containing amino acids methionine, cystine, and cysteine (Banwart & Bremner, 1975). Carbon sulfide and carbonyl sulfide were the main volatile S compounds detected from forest and cropland soils (Banwart & Bremner, 1975; Melillo & Steudler, 1989; Meredith et al., 2018), and the former was 2.3 times higher in light than in the dark (Meredith et al., 2018). Hydrogen sulfide emissions from soil have not been detected (Banwart & Bremner, 1976; Meredith et al., 2018), perhaps because it is immediately converted to metallic sulfides in the soil preventing volatilization (Banwart & Bremner, 1976). Volatile S compounds have been detected under laboratory conditions, but not under field conditions, hence S emissions from soil surfaces is unknown.

Emissions of H<sub>2</sub>S and SO<sub>2</sub> from volcanoes were much less than from anthropogenic activities, but this difference has narrowed as anthropogenic emissions of S continue to be reduced (Figure 1.1). The spatial distribution and altitude make volcanoes an important source of S to the atmosphere (Oppenheimer et al., 2011). Annual global emissions from continuous S degassing are estimated at 15 to 21 Tg SO<sub>2</sub> and from eruptions at 7 to 12 Tg SO<sub>2</sub> (Berresheim & Jaeschke, 1983; Andres & Kasgnoc, 1998), and emissions of H<sub>2</sub>S alone ranged from 1.4 to 35 Tg SO<sub>2</sub> year<sup>-1</sup> (Oppenheimer et al., 2011). In Nicaragua, volcanic emission was estimated to be dispersed across 1,250 km<sup>2</sup> downwind from the source (Delmelle et al., 2002). In Redoubt Volcano in Alaska 1992, a volcanic plume detected at 14 km aboveground had moved ~5,000 km away from the volcano 2.5 days after the eruption (Heffter & Stunder, 1993). Using satellite imagery, the volcano plume was detected at much further distances - 18,000 km away from the source 23 days from the eruption (Prata et al., 2007). Volcanos are the main natural source of S to the atmosphere and emissions reach long distances from the original source, at least 5,000 km.



Figure 1.1: Global sulfur dioxide emissions from anthropogenic sources: oil and gas, power plants, smelters, and volcanos. Adapted from data obtained from: <u>https://so2.gsfc.nasa.gov/measures.html (NASA, 2021).</u>

#### 1.2.2 Anthropogenic sources and global fluxes of S

Coal and other fossil-fuel combustion and smelting are the main anthropogenic sources of sulfur to the atmosphere - in excess of emissions from volcanos most of the years from 2005 to 2019 (NASA, 2021; Figure 1.1). Both H<sub>2</sub>S and SO<sub>2</sub> are volatilized, but the latter is the main source of S. Global fluxes of S have declined ~46% from 2007 through 2019 (NASA, 2021; Figure 1.1). Since 2005, S emissions from fossil-fuel burning and smelting have not change significantly, compared to emissions from power plants that declined by half during this time period (NASA, 2021; Figure 1.1).

From 2007 through 2014, China was the world largest contributor of SO<sub>2</sub> to the atmosphere (NASA, 2021; Figure 1.2). Since then, emissions from China have decreased (Klimont et al., 2013), and in 2015 Russia and India became the world largest contributors of S to the atmosphere (NASA, 2021; Figure 1.2). In contrast, emissions from the USA have consistently declined since implementation of the 1990 amendments to the Clean Air Act. For example, S emissions were 94% less in 2019 than in 1990 when emissions were as high as 16 Tg SO<sub>2</sub> (EPA, 2015). Coal combustion for power generation continues to be the main contributor of S in the USA accounting for >96% of the emissions from 1990 to the present (EPA, 2015). Since 2010 to the present, the global tendency for anthropogenic S emissions is to decrease, reducing the concentration of S in the air.



Figure 1.2: Annual sulfur dioxide emissions for India, China, Russia, and the USA, and the globe. Adapted from data obtained from: <u>https://so2.gsfc.nasa.gov/measures.html (NASA, 2021).</u>

#### **1.2.3 Sulfur deposition**

Sulfur particulates and gases are deposited back to terrestrial and aquatic ecosystems (Zannetti, 1990) by both wet and dry deposition (EPA, 2015). With wet deposition, sulfur gases react with water and other chemicals forming sulfuric acid that reach the ground with rainwater, whereas dry deposition occurs by sedimentation, diffusion, and wind turbulence (Beckett et al., 1998). In arid regions and coastal zones, dry deposition accounted for >70% of S deposition, while in other ecosystems dry deposition is 50 to 70% of total deposition (Vet et al., 2014). Dry deposition of S was the main source of deposition in winter, while dry and wet deposition were similar in magnitude in summer (Chen et al., 2020). In the USA, dry deposition was slightly greater than wet S deposition (Zhang et al., 2018). Sulfur is deposited back to the earth surface through rainfall or dry deposition, in the absence of rainfall dry deposition is predominant and vice versa.

Global S deposition in 2001 ranged from 4 to 50 kg S ha<sup>-1</sup> year<sup>-1</sup> equivalent to 91 Tg S worldwide (Vet et al., 2014), whereas in 2021 estimated S deposition ranged from 2 to 30 kg S ha<sup>-1</sup> year<sup>-1</sup> (Qiao et al., 2021). However, the 2021 data likely underestimated maximum deposition (30 kg S ha<sup>-1</sup> year<sup>-1</sup>) since it did not include data from India and Russia and these countries are the largest contributors of S to the atmosphere (Qiao et al., 2021). Sulfur deposition in the USA is historically concentrated in eastern states (Figure 1.3) and has decreased over time. For instances, S deposition in 2000 ranged from <4 to >23 kg S ha<sup>-1</sup> year<sup>-1</sup> and in 2019 from <2 to <12 kg S ha<sup>-1</sup>

year<sup>-1</sup> (EPA, 2021; Figure 1.3). In summary, during the last two decades, reduction in S emissions translated to a decline in S deposition by nearly half worldwide and in the US.



Figure 1.3: The amount of sulfur deposited on the land is much less in 2019 than in 2000. Red colors indicate high deposition and green low deposition. Adapted from images accessed at: <a href="https://gaftp.epa.gov/castnet/tdep/images/s\_tw">https://gaftp.epa.gov/castnet/tdep/images/s\_tw</a> (EPA, 2021b) (URL accessed May 2021)

Excessive deposition of S and N altered natural ecosystem functioning and caused acidification of soil and water, and weathering of cultural infrastructures (Burns et al., 2016). In a Canadian forest, sulfur and nitrogen deposition increased the rate of organic matter decomposition by stimulating microbial activity and extracellular enzyme activity (Wang et al., 2018). In contrast, elevated acidity due S and N additions, slowed organic matter decomposition in soil from coniferous and broadleaf forest in incubation experiments (Růžek et al., 2021). A study in semiarid tropical Australia found ant richness was negatively correlated with sulfur deposition levels (Hoffmann et al., 2000). In a boreal forest in Canada, long-term deposition of S and N was associated with increased number of root pathogens (Wu et al., 2021). Early in 21<sup>st</sup> century, the northeastern US showed negative effects on forest species due to acidification followed by lower basic cation concentrations (Duarte et al., 2013). Nevertheless, a study in a watershed of the Catskill Mountains of the US monitored fish communities was conducted from 1991 to 1993 and from 2012 to 2019 (Baldigo et al., 2021). This study found that fish diversity and population has continued to recover as water pH increased 0.16 to 0.34 units and sulfate concentration decreased from 60-65 µmol SO<sub>4</sub>-S L<sup>-1</sup> in 1991 to 25-30 µmol SO<sub>4</sub>-S L<sup>-1</sup> in 2019 (Baldigo et al., 2021). In a study conducted in Illinois for 22, 18, and 6 years in three agricultural tile-drained watersheds, S fertilization was not correlated with S concentration changes in the water stream (David et al., 2016). Instead, in 2 of 3 watersheds monitored, the decline in S deposition (~14 in 1994 to <5 kg

S ha<sup>-1</sup> in 2014) better explained the decline of S in the water (David et al., 2016). The positive effects of reduced S deposition have been noticeable in the water ecosystem with the reduction of S concentration and species communities recovering.

#### **1.3** Sulfur uptake by plants

Sulfur uptake is mediated by carriers in root cell membranes (Leggett & Epstein, 1956), and is translocated to the stele and root xylem through the symplastic pathway (Grossman & Takahashi, 2001). Carriers of low affinity regulate S uptake in S-sufficient conditions, where highaffinity carriers are highly expressed at low S availability (Cacco et al., 1980). In corn, high yielding hybrids had greater expression of low-affinity carriers than inbreds and this characteristic was positively correlated with more S in the roots and higher yields compared to those of inbreds (Cacco et al., 1980). In Arabidopsis, mRNA of high-affinity sulfate transporters (Sultr1; 1 and Sultr 1;2) were found in the cortex of mature parts of roots and in the epidermis and root hairs (Yoshimoto et al., 2002). Expression of Sultr1;1 was 10 times higher under low-S than in high-S and its suppression resulted in reduced accumulation of S in the roots. In contrast, expression of Sultr 1;2 was doubled under the same conditions and was unaffected by the suppression of Sultr 1;1. Although Sultr 1;1 is more responsive to limiting-S, it is less abundant that Sultr 1;2, and its function may be of importance only under very low S levels (Yoshimoto et al., 2002). Low-affinity carriers were also identified in the root cortex of Arabidopsis that increased S uptake to some extent, but not as much as Sultr1;1 and Sultr 1;2 (Maruyama-Nakashita et al., 2015). Plants obtain S mainly though roots and its uptake is regulated by low affinity carriers when S is sufficient and by high affinity carrier in S deficient conditions.

Sulfur uptake has been shown to be co-transported with H<sup>+</sup> into the root. As a result, higher S influx from nutrient solutions was observed at more acidic pH in barley (Leggett & Epstein, 1956), and rapeseed (Hawkesford et al., 1993). Studies with a wild-type legume (*Lotus japonicus* L.) found that the expression of S transporters increased in the absence of mycorrhiza (Giovannetti et al., 2014). Further research is necessary to understand the mechanism involved, but it is suggested that S in the area of root-fungus is higher and independent from S fluxes in the bulk soil or other unidentified transporters are involved (Giovannetti et al., 2014).

Sulfur uptake is regulated by plant S status (de Kok et al., 2005), however, the control mechanism is not fully understood. In canola, the increase of glutathione in the phloem exudates decreased S uptake (Lappartient & Touraine, 1996), and in *Arabidopsis* downregulated the

expression of *Sultr 1;2* responsible for S uptake (Maruyama-Nakashita et al., 2004). Cytokinin was also associated with downregulation of both *Sultr 1;1* and *Sultr 1;2* decreasing S uptake and S concentration in the roots of *Arabidopsis* (Maruyama-Nakashita et al., 2004). In contrast, another study found that overexpression of SLIM1/EIL3 genes stimulated activation of *Sultr 1;1* and *Sultr 1;2* increasing S uptake under S-limiting conditions, whereas in the mutant plant with no SLIM1 gene S uptake declined ~60% (Maruyama-Nakashita et al., 2006). Recent studies found that an epigenetic protein in the nucleus named "more sulfur accumulation1" (MSA1), controls responses under S-starvation and S-sufficient conditions in *Arabidopsis*. With S-starvation, MSA1 mutants accumulated and assimilated more S than MSA1 knockout mutants, but when S was adequate accumulation did not differ between mutants with or without the MSA1 gene (Huang et al., 2016). There is not complete understanding on how plants regulate S uptake, however, several mechanisms involving gene expressions and accumulation of S-compounds in the phloem have been associated with regulation of S uptake.

Inadequate S levels caused the root to shoot ratio to increase (Huang et al., 2016), and the plant developed longer roots and a greater number of lateral roots (Hopkins et al., 2004; Kimura et al., 2019) while shoot growth was stunted (Hopkins et al., 2004). In another study, corn was grown aeroponically under S-deprivation conditions (Hopkins et al., 2004). Corn in S-deprivation increased root to shoot ratio by 20% compared to corn with adequate S (Hopkins et al., 2004). Plants respond to S starvation conditions by increasing root length therefore accessing a greater soil volume for nutrient uptake, while the aboveground biomass is diminished compared to plants grown in S-sufficient conditions.

Sulfur dioxide is absorbed through stomates and the leaf cuticle in plants (Buchner et al., 2004; van Hove et al., 1989). However, S absorbed through leaves are small in comparison to that from root uptake, therefore leaf S absorption could not maintain plant growth in the long term. Brassica seedlings exposed to sulfur dioxide as the only source of S, grew normally for 10 days but deficiency symptoms appeared thereafter (Buchner et al., 2004). Absorption of atmospheric S in the leaf surface of bean (*Phaseolus vulgaris* L.) and poplar (*Populus euramericana* L.) was positively correlated with humidity, but was not significantly affected by temperature variation between 15 and 25°C (van Hove et al., 1989). Plant uptake of S through leaves occurs in low amounts that cannot substitute for S uptake by roots.

High concentrations of atmospheric S can damage guard cells, impairing stomate closure (Gerini et al., 1990; Winner & Mooney, 1980), increasing transpiration (Gerini et al., 1990), and decreasing photosynthesis (Dhir et al., 2001; Gerini et al., 1990). Concentrations of SO<sub>2</sub> ranging from 0.25 to 0.5 µL L<sup>-1</sup> caused severe injury in younger leaves, but less severe injury in mature leaves of tobacco (Nicotiano tabacum L.) (Craker & Starbuck, 1973). Chicory (Cichorium intybus L.) exposed to 1 µL L<sup>-1</sup> had 82% reduced number and size of leaves, compared to nonexposed plants (Dhir et al., 2001). Different levels of injury from SO<sub>2</sub> occurred among trees species established around an active volcano in Hawaii (Winner & Mooney, 1980). Where atmospheric SO<sub>2</sub> ranged from 1 to 100  $\mu$ L L<sup>-1</sup> most of the trees died, except one species that responded by closing stomates and reducing SO<sub>2</sub> absorption. Older leaves on this species were less injured than younger leaves, similar to what was observed with tobacco (Winner & Mooney, 1980). Atmospheric S ranging 0.04 to 0.1  $\mu L$  SO2  $L^{\text{-1}}$  did not cause visible injury in maize, and the maximum decline in photosynthetic rate was ~20% from plant exposure to 0.07  $\mu$ L SO<sub>2</sub> L<sup>-1</sup> with no effect from the lower or higher concentrations (Gerini et al., 1990). The reasons of the responses to one concentration and not to the others was not understood. Sulfur concentrations in the atmosphere above tolerable levels for plants are detrimental, and younger leaves are more susceptible to damage than older leaves. Severe exposure to S can cause plants to die.

#### **1.4 Sulfur interactions**

Sulfur interactions during plant uptake occurs with selenate (Se), molybdenum (Mo), and zinc (Zn). Wheat fertilized with Se and Mo, but not S, showed overexpression of S transporters and increased tissue concentrations of Mo and Se, indicating that S, Se, and Mo shared a common carrier transporter in roots (Shinmachi et al., 2010). Selenate and sulfate competition for adsorption sites was also observed in barley (Leggett & Epstein, 1956) and onion roots (*Allium cepa* L.). In the latter species, increasing the rate of Se from 1 to 8 g SeO<sub>4</sub> m<sup>-3</sup> decreased plant S uptake from 5.1 to 1.2 mg plant<sup>-1</sup> (Barak & Goldman, 1997). In barley and rice (*Oryza sativa* L.), the antagonism effect of S on Se uptake was stronger at high S concentrations, but the same effect was not observed from high Se rates on S uptake (Mikkelsen & Wan, 1990). Sulfur-Mo antagonism has been reported in the literature for soybean (Kumar & Singh, 1980), *Stanleya pinnata* (Pursh) Britton (Harris et al., 2014), and burley tobacco (Sims et al., 1979). Zinc and S are antagonistic in pepper (*Capsicum annuum* L.) (Kaya et al., 2018). Transporters for S, Se, and Mo in the root cortex

are the same causing competition for adsorption sites among this nutrients, and high concentrations of S reduces Se and Zn uptake.

Oxidation of reduced S releases acidity which influences availability of other nutrients. In pot experiments, maize grown in acid (pH 5.7), neutral (pH 7), and calcareous soil (pH 8.1) was fertilized with 50, 500, and 5,000 mg S kg<sup>-1</sup> of ground S (Hassan & Olson, 1966). At 45 days after planting, the highest S rate in the acid soil reduced pH from 5.7 to 3.1 and caused excessive increase in concentration of S (1.9 vs 16.8 g kg<sup>-1</sup>) and Mn (81 vs 1,022 mg kg<sup>-1</sup>) in the tissue, but increased P concentration (Hassan & Olson, 1966). Sulfur applied at 5,000 mg kg<sup>-1</sup> increased Ca, Mg, Mn, Zn, Fe, and Cu concentrations. However, plant dry matter decreased in all 3 soils with elemental S addition. For example, for the neutral soil, tissue concentration without and with S, respectively, was Ca (4.4 vs 19 g kg<sup>-1</sup>), Mg (3 vs 9 g kg<sup>-1</sup>), Mn (60 vs 944 mg kg<sup>-1</sup>), Zn (23 vs 91 mg kg<sup>-1</sup>), Fe (147 vs 450 mg kg<sup>-1</sup>), and Cu (7 vs 23 mg kg<sup>-1</sup>), and dry matter yield (1.5 vs 0.4 g pot<sup>-1</sup>). The lower S rate (50 mg kg<sup>-1</sup>) in calcareous soils significantly increased tissue Cu (10 without S vs 13 mg kg<sup>-1</sup> with S) at 45 days after planting. Fertilization with reduced forms of S has the potential to increase micronutrient availability, and can be beneficial in calcareous soils at rates adequate for plants.

Sulfur interaction with N and P has been previously observed. Fertilization of wheat fields with N and S increased N use efficiency (NUE) by nearly 50% due to greater recovery of N, but when N was limiting there was no effect of S on NUE (Salvagiotti et al., 2009). Soybean sowed in sand and fertilized with both P (80 mg kg<sup>-1</sup> soil) and S (80 mg kg<sup>-1</sup> soil), resulted in maximum S uptake of 48 mg pot<sup>-1</sup> (Kumar & Singh, 1980). The S uptake from applying both nutrients was greater compared to the uptake from P alone (20 mg pot<sup>-1</sup>) or from the control (9 mg pot<sup>-1</sup>) (Kumar & Singh, 1980). Ensuring an adequate supply of S for plants improves the efficiency of N use which might result in reduced N input requirements and nutrient losses from agricultural fields.

#### **1.5** Sulfur assimilation

Plants' assimilation pathways have been studied mainly in *Arabidopsis*, but findings are likely applicable for other plants species. Sulfur assimilation is facilitated mainly by low-affinity transporters expressed in all plant tissue, but mostly in leaves. These transporters in *Arabidopsis* were classified in 4 groups and a similar classification applies to transporters in other plants. The first group correspond to the *Sultr1*-type carriers previously discussed in nutrient uptake by roots, the second group are the *Sultr2*-type carriers accumulated in both root and leaf tissue and enable

S transport from root to leaves (Takahashi et al., 2000). Group 3 or *Sultr3*-type carriers have not shown a direct connection with S assimilation, but did appear to increase S-uptake by of *Sultr1;2* (Kataoka et al., 2004). Group 4 integrated for *Sultr4*-type carriers mediate S translocation from vacuoles to cytoplasm (Kataoka, et al., 2004b). The only high-affinity carrier identified to have a role in S transport is *Sultr1;3*, responsible for beginning the loading process from the companion cells to the phloem (Yoshimoto et al., 2003). Assimilation processes of S in plants are facilitated by transporters of low affinity found in all plant tissue, but mainly in roots and leaves.

Sulfur assimilation takes place in the mitochondria (Haas et al., 2008), chloroplast (Lunn et al., 1990; Saito et al., 1994), and cytosol (Lunn et al., 1990). Assimilation begins with the activation of sulfate into adenosine phosphosulfate (APS) a process catalyzed by ATP sulfurylase (Hawkesford, 2000). APS is then reduced to sulfide by APS reductase and sulfide reductase, and finally incorporated into cysteine (cys) in a sequential process catalyzed by serine acetyl-transferase and OAS (thiol) lyase (Hawkesford, 2000). Accumulation of cys inhibits the activity of serine acetyl-transferase autoregulating the incorporation of S into amino-acid cys (Kredich & Tomkins, 1966). During assimilation of S, sulfate is reduced to amino acid cys through several reactions catalyzed by enzymes, and accumulation of cys inhibits the incorporation process of sulfate.

Amino acid cys is the precursor of methionine (met), glutathione, proteins, and other essential S compounds for plant metabolism (Droux, 2004). Proteins containing S have a myriad of functions in plant growth and survival. Cys-containing proteins function as electron donor, metal-binding, and protein catalyst; where met-containing proteins of hydrophobic nature, play a role in protein binding (Droux, 2004). Methionine by itself is essential for cell division in apical meristems (Ravanel et al., 1998), for building proteins, and as a substrate for derivates of this amino-acid (Droux, 2004). Glutathione functions as antioxidant (Wise & Naylor, 1987), enzyme cofactor (Yadav et al., 2005), and is essential for root growth. Addition of S for growing pepper (Kaya et al., 2018) and lettuce (Freitas et al., 2019), increased activity of antioxidative enzymes, e.g. peroxidase and catalase, both associated with reduction of Zn-stress and membrane damage, and stimulation of root and shoot growth. Likewise, toxicity caused by excess Mn was reduced with increasing S, due to enhanced antioxidant mechanism of response and reduction in Mn translocation from roots to shoots (Sheng et al., 2016). Sulfur in the plant is essential for protein formation, adequate growth development, and tolerance development to oxidative stress.

#### **1.6 Grain yield and S fertilization**

#### 1.6.1 Corn

Based on previous corn field experiments, a hectare producing 22 to 25 Mg ha<sup>-1</sup> of aboveground biomass contains from 24 to 28 kg S and around half of that S (13 to 16 kg) is removed with the grain (Bender et al., 2013). In addition, one megagram of grain dry mass contains 0.9 to 1.2 kg S (Bender et al., 2013; Kim et al., 2013). Sulfur removal is expected to increase with increased yield. Corn grain yield has increased annually on average ~0.11 Mg ha<sup>-1</sup> (Nielsen, 2020), which equates to increased grain S removal of ~0.0935 kg S ha<sup>-1</sup> yr<sup>-1</sup> (assuming grain at 1.0 g S kg<sup>-1</sup> grain dry matter). In Iowa, S fertilization of corn in sandy soils increased grain yield around 1.7 Mg ha<sup>-1</sup>, which was greater than the grain yield response (~0.9 Mg ha<sup>-1</sup>) to S fertilization in fine-textured soils (Sawyer et al., 2015). Grain yield increases from S fertilizer in the Corn Belt Region ranged from 0.8 to 3.2 Mg ha<sup>-1</sup> in Illinois (Fernández et al., 2012; Hoeft & Fox, 1986), 0.4 to 0.9 Mg ha<sup>-1</sup> in Ohio (Chen et al., 2008), 0.3 to 2.4 Mg ha<sup>-1</sup> in Iowa (Sawyer et al., 2009; 2015); 0.3 to 0.9 Mg ha<sup>-1</sup> in Minnesota (Rehm, 2005; Kim et al., 2013), and ~0.5 Mg ha<sup>-1</sup> in Kansas (Husa & Ruiz Diaz, 2020). These responses were achieved from 7 to 34 kg S ha<sup>-1</sup>. With an increased removal of S from the field and increase in corn response to S, it is important to monitor for crop response to S and ensure an adequate supply of S to achieve maximum yields.

Sulfur deprivation not only limits grain yield, but it is also detrimental to grain quality. Corn grain growth in S-limited conditions decreased the concentration of cysteine and methionine 25-30%, free amino acids were 3-fold higher, and glutelin (a S-rich protein) was decreased 36-71% compared to plants supplied with S (Baudet et al., 1986). This study is the only one available showing negative effects of S on corn grain amino acids and protein as most of the studies have focused on corn grain yield since corn is not a high protein crop. Soybean and wheat quality have been more frequently affected by S deficient conditions, reducing protein content in soybean (Borja Reis et al., 2021; Salvagiotti et al., 2012; Sexton et al., 1998) and wheat (Guerrini et al., 2020; Li et al; 2013).

Critical dilution curves of S in corn determined that S rates ranging from 5 to 13 kg S ha<sup>-1</sup> were needed to achieve optimum yield (Bullock & Goodroad, 1989; O'Leary & Rehm, 1990; Rehm, 1993; Weil & Mughogho, 2000). Biennial S rates for maximum yield response were recommended in Iowa for fine-textured (19 kg S ha<sup>-1</sup>) and coarse-textured soils (28 kg S ha<sup>-1</sup>)

(Sawyer et al., 2015). Application of S is recommended early in the season and there was no apparent benefit from split applications of S (Bullock & Goodroad, 1989; Kurbondski et al., 2019; Rehm, 1993).

Supply of N and S are essential to ensure adequate levels of these nutrients in the plant and achieve maximum yield. In Minnesota, the addition of S increased earleaf N concentration by 3% when N was limiting in the soil, likewise N fertilization without S increased S concentration in the tissue by 9% (Sutradhar et al., 2017). In long-term unfertilized fields in Malawi, deficient in N and S, corn grain yield was unaffected by S fertilization and adding N fertilizer alone accentuated S-deficiency symptoms in the plant, but N and S combined increased yield (Weil & Mughogho, 2000). Adequate supply of both nutrients N and S are necessary to achieve potential maximum yield and an efficient use of N and S fertilizer by plants.

Different research studies have used plant tissue S at different stages to identify crop responses to S. Some studies found plant tissue responses to S to be a good indicator of yield response, and others did not. Field experiments in Argentina found S concentration in the 6<sup>th</sup> leaf sampled at the V6 stage correctly predicted grain yield in 3 of 5 situations (Carciochi, et al., 2019b). Tissue S at the V6 stage was responsive to S at 5 of 8 site-years, and ranged from 1.3 to 2.4 g kg<sup>-1</sup> without S and 1.6 to 2.6 g kg<sup>-1</sup> with S. Three of 5 leaf responsive sites also had increased yield with S fertilization. Where leaf S was unaffected by S fertilization the leaf ranged from 2.2 to 2.6 g S kg<sup>-1</sup>. In another experiment, tissue S concentration at the V10 stage was considered deficient at 1.2 g kg<sup>-1</sup> and increased in proportion to increased S rate, resulting in increased grain yield (Bullock & Goodroad, 1989). Nitrogen to S ratio was not better at predicting yield responses (R<sup>2</sup>=0.76) than S concentration (R<sup>2</sup>=0.93) when measured in the 12<sup>th</sup> leaf at the V12 stage at 8 site-years (Carciochi et al., 2019b). The N:S ratio narrowed as the plant went from V6 (16.7:1) to V12 (15.1:1) (Carciochi et al., 2019b; Pagani & Echeverría, 2011). Where grain yield response to S fertilization occurred, tissue S concentration at V6 and V12 also responded. However, tissue S responses to S fertilizer also occurred at sites where grain yield was unresponsive.

Earleaf S concentration at R1 has also been used to diagnose corn response to S. In two field experiments, earleaf S concentration and grain yield were responsive to S fertilization (Bullock & Goodroad, 1989). Earleaf S averaged 1.9 without treatment and increased to 2.1 g kg<sup>-1</sup> with S. In another ten experimental sites, the earleaf S responses to S fertilization predicted yield response half of times (O'Leary & Rehm, 1990). At 3 sites where earleaf and grain yield were

responsive to S fertilization, earleaf S ranged 1.6 to 2.2 g kg<sup>-1</sup> without S and increased ~0.3 g kg<sup>-1</sup> with S fertilization. At the other 3 sites where no yield increase with S fertilization occurred, earleaf S increased 0.2 to 0.5 g kg<sup>-1</sup> with S above 2.4 to 2.5 g kg<sup>-1</sup> without S (O'Leary & Rehm, 1990). Very low levels of earleaf S were reported for corn in 14 on-farm trials in Malawi, earleaf S site means ~0.9 to 1.5 g kg<sup>-1</sup>, where grain yield responded to S fertilization in all but 2 trials (Weil & Mughogho, 2000). In this study earleaf N:S (R<sup>2</sup>=0.41) was a better indicator of yield response than tissue S concentration. In contrast, earleaf S (ranging from 1.3 to 1.9 g kg<sup>-1</sup>) showed no correlation with grain yield response across 47 site-years. (Sawyer et al., 2015). Increase of S concentration in the earleaf with S fertilization does not always result in grain yield increases, but is may be useful to monitor for possible S deficiency. In some field studies, optimum yield responses were achieved with earleaf S concentration in the range 2.0 to 2.3 g S kg<sup>-1</sup> (Bullock & Goodroad, 1989; Rehm, 1993; Sawyer et al., 2015; Sutradhar et al., 2017), within the sufficiency range for S, 1.5 to 6.0 g S kg<sup>-1</sup> (Campbell, 2013). Nevertheless, this range for S sufficiency does not fit every situation.

Nitrogen to S ratio (N:S) assess both N and S status, and a suggested critical threshold in the tissue for optimum yield is N:S<18:1 (Campbell, 2013). A wide array of N:S in the earleaf has been reported for optimum yield responses ranging from 12:1 to 18:1 (Carciochi et al., 2020; Kim et al., 2013; Stecker et al., 1995; Sutradhar et al., 2017). Nitrogen to S ratio should be analyzed together with N and S tissue concentration to ensure the sufficiency of those two nutrients.

Grain S concentration increases from fertilizer S have been observed at both grain yield responsive and nonresponsive sites. For example, in Minnesota, all 3 of 3 site-years increased grain S from S fertilization, but only 2 site-years increased grain yield (Kim et al., 2013). Grain S concentration without S ranged from 0.5 to 0.9 g S kg<sup>-1</sup> and increased to 0.9 to 1.0 g S kg<sup>-1</sup> with 28 kg S ha<sup>-1</sup>. Grain yield response to S fertilization also occurred without grain S responses to S. In field studies at 2 of 4 site-years, grain S was unresponsive to 33 kg S ha<sup>-1</sup> but grain yield increased (Chen et al., 2008). The grain S concentration ranged 0.9 to 1.1 g kg<sup>-1</sup> at all site-years. In another study conducted at 47 site-years, treatments with S fertilizer increased grain S and grain yield at 6 and 3 site-years, respectively (Stecker et al., 1995). Where grain yield increased with S fertilization the grain S concentration was unresponsive. At all site-years, the grain S ranged from 0.8 to 1.1 g S kg<sup>-1</sup> (Stecker et al., 1995). Not many studies have reported grain S concentration as affected by S fertilization but based on the few studies available, sites where grain S and grain

yield responded to fertilizer S, grain S concentration without S fertilization was  $\leq 0.9$  g kg<sup>-1</sup>, and grain yield responses were not common above that grain S concentration threshold.

#### 1.6.2 Soybean

In soybean total biomass accumulation is ~9.5 Mg ha<sup>-1</sup> and uptake 17 to 20 kg S ha<sup>-1</sup> (Bender et al., 2015). The grain removes over one third of that biomass ~3.5 Mg ha<sup>-1</sup> and contains ~11 kg S. Indiana has reported soybean yield increases of ~94 kg ha<sup>-1</sup> with 27 kg S ha<sup>-1</sup>, averaged over 65 locations (Joern, 2020). However, higher yield responses ranging from 0.2 to 0.8 have been reported from application of 17 to 28 kg S ha<sup>-1</sup> (Casteel, 2018). Responses in Minnesota were less frequent occurring at 1 of 4 site-years and with OM <20 g kg<sup>-1</sup> (Kaiser & Kim, 2013). The yield increase was 0.3 Mg ha<sup>-1</sup> from 28 kg S ha<sup>-1</sup> over 1.7 Mg ha<sup>-1</sup> without S. A review study for 8 states in the US emphasized that soil OM was an important determinant for yield responses to S (Borja Reis et al., 2021). Grain yield increased ~1.9% from fertilizer S, in soils with OM ranging from 25 to 32 g kg<sup>-1</sup>. At sites with soil OM below 25 g kg<sup>-1</sup>, grain yield was unresponsive to S fertilization. In Argentina, increases in soybean grain occurred at 11 of 20 site-years and ranged from 0.3 to 0.8 Mg ha<sup>-1</sup> above 2.0 to 4.5 Mg ha<sup>-1</sup> without S (Salvagiotti et al., 2012). In the last decade, more states in the Corn Belt and in Argentina have observed soybean yield responses to S, and responses were more common in soils low in OM <32 g kg<sup>-1</sup>. Approximately 3.1 kg S is removed from the field for each megagram of soybean grain dry matter.

In field-scale experiments with soybean, tissue responses to S occurred more often than grain yield responses. While 4 of 4 site-years increased tissue S concentration from fertilizer S at V5 stage, only one site-year was grain yield responsive (Kaiser & Kim, 2013). The grain yield responsive and unresponsive sites were in the same range for tissue S, 2.7 to 3.1 g S kg<sup>-1</sup> without S and increased to 2.8 to 3.4 g S kg<sup>-1</sup> with S. Sulfur concentration was analyzed in trifoliate leaves with petioles between R1-R3 stage at 14 site-years, tissue S concentration ranged 1.6 to 3.6 g kg<sup>-1</sup> without S fertilizer (Divito et al., 2015). At 10 site-years, the tissue S concentration increased S 0.3 to 0.9 g kg<sup>-1</sup> with the addition of S compared to zero-S and only half of these sites increased grain yield with S fertilization. At 3 of the 10 site-years where yield increased with added S, but leaf S did not, leaf S ranged from 2.6 to 2.8 g S kg<sup>-1</sup>. Although soybean tissue S responds to S

fertilization when grain yield is not responsive to S, yield responses are most likely to occur when tissue S at R1-R3 plant stage is  $\leq 2.6$  g kg<sup>-1</sup> and N:S is  $\geq 14:1$ .

Soybean seed S concentration may be better for assessing S status in soybean than plant tissue S concentration (Panthee et al., 2006). Suggested critical values for grain-S concentration range from 2.0 to 2.3 g S kg<sup>-1</sup> (Hitsuda et al., 2005; Kaiser & Kim, 2013). When seed-S concentration was <1.5 g S kg<sup>-1</sup>, plant tissue showed S-deficiency symptoms and yield diminished 40% (Hitsuda et al., 2004, 2005). Nitrogen to S ratio suggested as adequate for soybean grain is ~22:1 N:S (Salvagiotti et al., 2012; Sexton et al., 1998) and sufficiency for plant tissue ranged from 12 to 15:1 (Stewart & Porter, 1969).

In soybean production an adequate S supply is a determinant for seed quality. The amount of protein in soybean grain range 330 to 351 g kg<sup>-1</sup> (Kaiser & Kim, 2013), and 1.0-1.6% of the seed dry weight correspond to S-containing amino-acids (Panthee et al., 2006). In severe S-deficient conditions, S-rich proteins declined as much as 30% and S-poor proteins increased 15-40%, resulting in grain with lower quality (Sexton et al., 1998). In field studies conducted in 8 US Corn Belt states, S fertilization increased seed protein 0.3% averaged over 72 field studies (Borja Reis et al., 2021). It has been widely observed that S-containing amino acids, and grain S concentration are the most responsive parameters to S fertilization in soils limited in S, and inadequate S reduces grain protein (Borja Reis et al., 2021; Salvagiotti et al., 2012; Sexton et al., 1998).

#### 1.6.3 Wheat

Sufficient supply of S for wheat ensures optimum grain yield and grain quality. Wheat grain yield with S fertilization occurs in a wide range from 2.3 to 9 Mg ha<sup>-1</sup>, greatly differing among cultivars and environments (Guerrini et al., 2020; Salvagiotti et al., 2009; Thomason et al., 2007). Fields showing high grain yield responses to fertilizer S in combinations with N, increased grain yield 3-fold compared to no S and no N fertilizer (Zörb et al., 2009). Grain N:S ratios at these sites were >32:1 without S and decreased to 22:1 with addition of S. Sites showing moderate responses to S produced 11-14% more grain than without S (Tao et al., 2018). Wheat grain yield is severely reduced with inadequate supply of S during crop development.
Wheat grain quality, hence baking properties, depends on adequate supply of S. In field experiments with 3 old varieties known for their poor baking qualities, fertilizer S increased grain gluten (12%) and glutenin (4 to 6%), which improved flour elasticity, desirable for better baking properties (Guerrini et al., 2020). Sulfur is also important for starch quality, which in fact represents a large fraction of grain dry weight, 60-70% (Li et al., 2013). B-type starch, preferable for better pasta quality, was highly sensitive to S availability, where A-type starch was unaffected by S fertilization (Li et al., 2013). The latter starch-synthesis started 3 days before flowering, where the former 10 days post-flowering; this physiological mechanism was associated with higher grain quality and starch synthesis from high S rates early in the season and late-season applications (Li et al., 2013; Zörb et al., 2009). Adequate S in the grain was crucial for reducing free asparagine, a precursor of carcinogen acrylamide, free asparagine in the flour went from 4.8 g kg<sup>-1</sup> at low-S to 0.2 g kg<sup>-1</sup> at sufficient-S (Granvogl et al., 2007). The quality of wheat flour is diminished when S is not sufficient, further affecting baking properties and increasing the risk of asparagine accumulation.

Wheat tolerance to oxidative stress seem to improve with adequate supply of S. The effect of S on wheat response to heat stress was evaluated in pots experiments (Tao et al., 2018). At 20 days from flowering, plants were exposed to heat stress for 5 hours each day for 2 days. In pots fertilized with S, reduction in grain yield and starch concentration from heat stress was 25 to 50% less than losses in unfertilized pots. The positive effect of S on enhancing wheat tolerance to heat requires more research for a better understanding of the extent to which S is involved and the effects in other crops.

#### **1.6.4** Fertilizer sources of sulfur

Inorganic sulfur fertilizer sources are categorized in four groups: elemental S-containing fertilizers, sulfate-containing fertilizers, the combination of elemental S and sulfate, and liquid fertilizers. Organic sources of S include crop residue, and solid and liquid manure. Fertilizers containing reduced states of S or organic sulfur must be oxidized or mineralized, respectively, before sulfate can be utilized by plants.

Elemental S is the most concentrated source of S, 50 to 100% S. However, the oxidation of S to sulfate can be slow ranging from 0.07 to 0.45  $\mu$ m per day depending on oxidation rate and

particle size (McCaskill & Blair, 1989). Complete oxidation of 0.075 to 0.15 mm particle sizes was estimated at 400 days or more and varied with environmental conditions (Boswell & Friesen, 1993). A grazing field in Australia fertilized with agricultural grade elemental S, fortified superphosphate with elemental S, and single superphosphate, showed that 99% of the S from the latter source was released after 72 days, where the fortified superphosphate and elemental S took a year to release 54 and 23%, respectively (McCaskill & Blair, 1989). A canola field fertilized with micronized elemental S (60 kg S ha<sup>-1</sup>) produced maximum seed yield compared to S-bentonite (120 kg S ha<sup>-1</sup>) that resulted in little yield increase (Karamanos & Janzen, 1991). Elemental S was more effective providing S during the second year in perennial crops like alfalfa, the effectiveness of this source in providing S during the first year was 20% compared to gypsum, and increased with each cutting from 12 to 52% (Fox et al., 1964). In terms of application method, better outcomes were observed from mixing elemental S with the soil, when flour S was applied in band it was not accessible for the crop unless accompanied with lime (Fox et al., 1964). Authors inferred that lime neutralized acidity that deprived roots and oxidizing-microorganism from accessing the nutrient in the absence of lime. The relatively slow oxidation of elemental S to sulfate, requires application ahead of plating and small particle size for readily available S for crops.

Thiosulfate must be oxidized to sulfate-S before being available to plants. In incubation of four different cultivated soils, thiosulfate oxidized to tetrathionate 3 to 7 days after addition (Barbosa et al., 1998). The final step of oxidation from tetrathionate to sulfate took 20 to 30 days usually. Similar observations from another incubation experiment with two soils, where 56-70% of the S applied as thiosulfate was oxidized to sulfate-S after 24 days (Janzen & Bettany, 1986). Most of the initial thiosulfate oxidized to sulfate-S at 74 days. The oxidation of thiosulfate releases acidity, thiosulfate equivalent to 300 mg S kg<sup>-1</sup> reduced soil pH by 0.05 to 0.15 units 30 days from incubation, compared to unamended soil.

Thiosulfates inhibit nitrification and urea hydrolysis. Ammonium thiosulfate at rates  $\geq 2,500 \text{ mg kg}^{-1}$  significantly reduced urea hydrolysis, comparable to the effect from 1 mg kg<sup>-1</sup> of a commercial inhibitor (McCarty et al., 1990). Inhibition effects were also observed with lower S rates. Sulfur at 32 and 320 mg kg<sup>-1</sup> as sodium thiosulfate reduced nitrification  $\geq 55\%$  and  $\geq 82\%$ , respectively (Goos, 1985). Under field conditions, corn fertilized with urea ammonium nitrate and ammonium thiosulfate yielded significantly more grain than urea ammonium nitrate plus single superphosphate (Graziano & Parente, 1996). The authors hypothesized that it may be an effect of

thiosulfate inhibiting nitrification and urea hydrolysis, therefore reducing the risk of N leaching. Thiosulfate successfully inhibited nitrification and urea hydrolysis under controlled conditions. However, S rates used are significantly higher than those used in agricultural fields, therefore the inhibition effect from thiosulfate under fields conditions may be unlikely depending on the method of fertilizer application.

Sulfate-containing fertilizers are available in solid and liquid forms. The sources commonly used in crop production and their average sulfur concentration are ammonium sulfate (24%), single superphosphate (11 to 17% S), and potassium sulfate (17 to 18% S) (de Kok et al., 2005). Ammonium sulfate provides readily available S and N, however this fertilizer produces acidity from nitrification of ammonium to nitrate, and this acidification was reported to be 1.4 to 2 times higher than that produced from ammonium nitrate or urea, as measured with different techniques (Chien et al., 2008). Hence, this source is preferable for soils with pH above adequate for the crop of interest. Acidification is expected to be lower with increased clay content and organic matter (Chien et al., 2008). Another source is single superphosphate, it was estimated to release  $\geq$ 99% of the sulfate in 72 days, where release was slower from superphosphate fortifiedwith elemental S (45% S), only half of the total S was release after one year of application (McCaskill & Blair, 1989). Potassium sulfate, providing P and S, has been used as an alternative to potassium chloride in crops sensitive to chloride, e. g., tobacco, turf grass, and some legumes (de Kok et al., 2005). Sulfate-containing fertilizers are most commonly used for annual crops as they provide readily available S and can be applied in combination with other macronutrient fertilizer sources.

Gypsum (17 to 24% S) is less commonly used as a source of S because of its relative slow release of sulfate compared to other sulfate sources. In controlled conditions, ryegrass fertilized with gypsum yielded 9 g pot<sup>-1</sup> of dry matter in the first cut, and increased dry matter was higher for the second and third cut. This indicates a relatively low release of S from this source (Dijksterhuis & Oenema, 1990). Under control experiments in cultivation vessels with soil, soil sulfate availability from different S fertilizer decreased in the order urea+ammonium sulfate>ammonium sulfate>gypsum (Dijksterhuis & Oenema, 1990). Gypsum provides both Ca and S, S from this source remain longer in the soil compared to sulfate-containing fertilizer but its use required a planned application to ensure release of S on time to satisfy crop demand.

# **1.6.5** Soil testing for sulfur

Extractants and determination methods has been proposed to measure S in the soil available for plant uptake. The extractants available measure inorganic S, fractions of organic S, and total S. The ideal extractant would include S in soil solution and adsorbed S according to Warman & Sampson (1992), but Blair et al. (1991) emphasized the importance of considering a fraction of organic S as it would be potentially available for plants.

Inorganic S solution extractions include water, CaCl<sub>2</sub>, KH<sub>2</sub>PO<sub>4</sub>, Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>, NH<sub>4</sub>OAc, Bray<sup>-1</sup>, Mehlich-3, and Morgan solution. Extractants containing P were more effective in acidic soils with pH  $\leq$ 6.5 compared to CaCl<sub>2</sub> because P displaced adsorbed S from soil colloids that CaCl<sub>2</sub> did not (Ketterings et al., 2011; Warman & Sampson, 1992). However, CaCl<sub>2</sub> was a better extractant than P-containing extractants when soil pH >6.5, where adsorbed S was negligible (Ketterings et al., 2011). Phosphorus-containing extractants, NH<sub>4</sub>OAc, Mehlich-3, and Morgan solution extract inorganic S, but also more fractions of organic S than CaCl<sub>2</sub> (Ketterings et al., 2011; Zhao & McGrath, 1994). There is not ideal extractant for all soils, one would be more suitable than another depending on soil pH and soil OM.

Several techniques to measure S in extractants have been developed, and among them are reduction, turbidimetric spectrometry, chromatography, and inductively coupled plasma atomic emission spectrometry.

Determination of S by the reduction method is similar to that described for total S, where S in the extraction solution is reduced with hydriodic acid (Johnson & Nishita, 1952). Although this method is considered accurate to detect very small amount of S in extracts, the complexity of the methodology is not feasible for S determination (Warman & Sampson, 1992). For the turbidimetric method, sulfate in solution needs to be precipitated with Ba for measurement of turbidity by spectrophotometry. Given than OM interferes with sulfate and Ba precipitation, it can be removed with activated C (Ketterings et al., 2011). However, this method had been found inconsistent and offer challenges for replicability (Ketterings et al., 2011). The anion exchange chromatography method has been comparable to results obtained by the reduction method, and allows determination of other nutrients (N, P, F, Cl) simultaneously (Warman & Sampson, 1992). Another method largely used in laboratories nowadays, is the inductively coupled plasma atomic emission spectrometry (ICP-AES), it uses plasma at very high temperatures that atomized organic and inorganic S forms, these atoms fluoresce emitting a spectral line that can be determine through

spectrometry. The atomization process occurs in an acid environment to prevent precipitation of metals, and the efficiency of this process relies on maintaining a consistent mean acidity (McQuaker et al., 1979). The limitation of the ICP-AES is the detection of small amounts of the element, for instances the limit of detection for S is 30  $\mu$ g L<sup>-1</sup> (Manning & Grow, 1997). Nevertheless, the ICP-AES it has the capacity to measure nearly 70 elements including S and is widely used for nutrient analysis (Manning & Grow, 1997).

The performance of techniques to test for S-availability in the soil has been evaluated under field conditions for crops. Corn grain yield (soil pH 4.1 to 6.7) was better related with sulfate-S extracted with KH<sub>2</sub>PO<sub>4</sub> (R<sup>2</sup>=0.71, 0.79), Ca(H<sub>2</sub>PO<sub>4</sub>) (R<sup>2</sup>=0.75 and 0.76), and heat-soluble S (R<sup>2</sup>=0.79 and 0.76) than CaCl<sub>2</sub>, most likely because of the acidic nature of the soils (Kang et al., 1981). Sulfur extractions with KH<sub>2</sub>PO<sub>4</sub> measured by ion chromatography (R<sup>2</sup>=0.92) and ICP-AES (0.85) were better related to spring wheat S uptake than sulfate measure by ion chromatography and ICP-AES after extraction with Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> (R<sup>2</sup>=0.85, 0.81), water (R<sup>2</sup>=0.82, 0.84), or CaCl<sub>2</sub> (R<sup>2</sup>=0.78, 0.82) (Zhao & McGrath, 1994). The first R-squared correspond to ion chromatography and the second to ICP-AES. In field experiments both techniques to measure nutrients in extractants, ion chromatography and ICP-AES correlated with yield responses, and the extractants containing P are desirable in acidic soils.

In alfalfa fields with soil pH ranging 6.3 to 7.2, the best correlation of available-S with crop growth was obtained with CaCl<sub>2</sub> extractions compared to KH<sub>2</sub>PO<sub>4</sub>, Ca(H<sub>2</sub>PO<sub>4</sub>), NH<sub>4</sub>OAc, Mehlich-3, and Morgan solution; however, the authors indicate this extractant may not be suitable for acid soils with pH <6.5, because of its limited performance extracting adsorbed S (Ketterings et al., 2011). Quantification of S with ICP-AEM measured more S than the turbidimetric measurement of sulfate in most soil samples, which is preferable because ICP-AEM measures total S in the sample rather than inorganic S only with the turbidimetric method (Ketterings et al., 2011).

Sulfur availability in the soil depends mainly on soil OM concentration when atmospheric S deposition is not enough to meet the crop demand for S. Sulfur from soil OM becomes available after mineralization. The latter process is dependent on temperature and moisture conditions. Soil testing for S is not a good estimate of S potentially made available for plants. The best indicator of S status is likely the plant itself.

# CHAPTER 2. CORN YIELD RESPONSE TO SULFUR FERTILER IN INDIANA

# 2.1 Introduction

The primary sources of S for corn include S in soil OM and atmospheric S deposition. Since the Clean Air Act in 1990 (EPA, 2020), emission of S to the atmosphere has diminished gradually, resulting in reduced S deposition from the atmosphere over time (Nopmongcol et al., 2019; Vet et al., 2014). Historically, eastern states in the US including states in the Corn Belt had the highest levels of atmospheric S deposition. Consequently, this region has had a substantial reduction in S deposition within the last three decades. The deposition of S in this region ranged from 1 to 4 kg S ha<sup>-1</sup> in 2019, much less than 15 to 25 kg S ha<sup>-1</sup> in 2000, and 24 to 38 kg S ha<sup>-1</sup> in 1990 (EPA, 2021).

Organic matter in soils is the largest source of S, accounting for 90 to 99% of the total S content (Costa, 2020; Tabatabai & Bremner, 1972; Wang et al., 2006). However, the fraction of S mineralized and made available for plants is small. In temperate soils, the average annual rate of organic S mineralized ranged from 1.7 to 3.1%. These estimates were taken from a two-year experiment under field conditions, using soil from Danish agricultural fields kept in pots and cultivated with spring rape (*Brassica napus* L.)-ryegrass (*Lolium multiflorum* Lam.) rotation (Eriksen et al., 1995). The rate of S mineralization varies seasonally, as observed in cultivated soils in Oregon, where S mineralization rate almost doubled in spring as soils warmed up and throughout summer, compared to S mineralization rate in winter (Castellano & Dick, 1991). The amount of S mineralized from soil OM may or may not be enough to provide for the crop.

Sulfur is a constituent of amino acids methionine and cysteine and involved in other facets of plant metabolism (Droux, 2004; Ravanel et al., 1998). Based on previous corn field experiments, a hectare of above-ground biomass takes up from 24 to 28 kg S and one megagram of grain contains 0.9 to 1.2 kg S (Bender et al., 2013; Kim et al., 2013). A grain yield of 10 Mg ha<sup>-1</sup> removes 10 kg S ha<sup>-1</sup> and 1.0 g S kg<sup>-1</sup> grain dry matter. Nevertheless, S removal from the soil by corn would increase as yield increases. On average, corn grain yield increased annually ~0.11 Mg ha<sup>-1</sup> (Nielsen, 2020) equivalent to increased grain S removal of ~0.0935 kg S ha<sup>-1</sup> yr<sup>-1</sup> assuming grain at 1.0 g S

kg<sup>-1</sup> grain dry matter. Because grain yield continues to increase, S demand increases as well, resulting in a longer-term issues if S fertilization is not considered.

In Indiana, Powers (1923) reported S in the soil was enough to supply crops, later Bertramson et al. (1950) concluded annual S deposition from the atmosphere by rainfall was sufficient to supply the needs of corn as there was no response to S fertilization. Few S response trials were conducted in Indiana prior to 2015, showing positive response of S in wheat (Camberato & Casteel, 2010). Crop response to S fertilization in research trials conducted in the Corn Belt in the latter 20<sup>th</sup> century through 2000 was non-existent (Sawyer & Barker, 2002), or infrequent (Sawyer et al., 2015). In the following 20 years, crop response to S fertilization in Indiana and the Corn Belt was more frequent and also occurred on heavier-textured and higher OM soils: Indiana (wheat; Camberato & Casteel, 2010 and soybean; Casteel et al., 2018), Illinois (corn; Fernández et al., 2012), Ohio (corn; Chen et al., 2008), Iowa (alfalfa and corn; Sawyer et al., 2009; alfalfa, corn, and soybean; Sawyer et al., 2015), Kansas (corn; Husa & Ruiz Diaz, 2020), and Minnesota (corn; Rehm, 2005). With an apparent increase in S deficiency symptoms in Indiana, it is necessary to test for corn crop response to S fertilization and, if required, update S recommendations to farmers.

The objective of this study was to determine if corn grain yield was responsive to S fertilization at sites in Indiana and evaluate soil characteristics and soil and plant analysis as indicators of S deficiency.

# 2.2 Materials and methods

#### 2.2.1 Experimental Sites

During the 2017 to 2020 growing seasons, 28 field experiments were conducted at Davis-Purdue Agricultural Center (DPAC), Northeast-Purdue Agricultural Center (NEPAC), Pinney-Purdue Agricultural Center (PPAC), Southeast-Purdue Agricultural Center (SEPAC), Southwest-Purdue Agricultural Center (SWPAC), Throckmorton-Purdue Agricultural Center (TPAC), and on farmers' fields: Blackford, Henry, Shelby(1), and Shelby(2). Location, field size, and soil series information are shown in Table 2.1.

# 2.2.2 Soil sampling & analysis

Three to six composite soil samples (12 to 15 cores per composite) were collected before treatment application from 0 to 20 cm depth in 2017 and 2018, from 0 to 60 cm in 20-cm increments in 2019 and 2020, and from 0 to 40 cm in 20-cm increments at SWPAC18. The sampling method in the field was a diagonal or zig-zag pattern through a replication or area of soil. In 2019 soil sampling was from each replicate, except for Rice19 where 3 composite samples represented the field and at Shelby(1)19 where sampling areas were based on low, medium, and high NDVI indices derived from satellite imagery from previous seasons (Morales, 2020). In 2020 samples were located according to predominant soil types derived from Web Soil Survey (Soil Survey Staff, 2020). Blackford17-20, Henry20, Shelby(2)19, and Shelby(1)18 were not soil sampled before sidedress, and farmers provided prior information for pH and OM for two sites: Henry20 and Shelby(1)18. Blackford20, Shelby(1)19, and Shelby(2)19 were sampled the following spring after harvest, but sulfate-S measurements were not included in the dataset used to explain yield. Sampling at Blackford20 was based on soil type, whereas both Shelby(1) and Shelby(2) were sampled according to level of yield response to S fertilizer.

Samples were air-dried, ground and analyzed by A&L Great Lakes Laboratories (Fort Wayne, IN) for pH (1:1 soil:water), organic matter determined by loss on ignition at 360 °C and essential nutrients besides N (Mehlich-3 extractions with nutrients quantified by inductively coupled plasma spectroscopy). For inorganic N determination, ten grams of soil and 50 ml of KCl solution were shook for 1 hr and then gravity filtered through Whatman 1 filter paper. Ammonium, nitrite, and nitrate in the extract were determined with a SEAL AQ2 discrete analyzer (method 353.2, revision 2.0). Soil texture was determined using the Bouyoucus hydrometer and sodium hexametaphosphate was the dispersing agent; 40 seconds and 2 hours reading corresponded to sand and silt content, respectively, and the remaining particles in suspensions were attributed to clay (Wintermyer & Kinter, 1955).

#### 2.2.3 Crop management & experimental design

All fields used in this study were non-irrigated and at most site-years soybean was the previous crop, except at SWPAC where melons (Family: *Cucurbitaceae*) were the prior crop. The majority of the trials were managed no-till, except DPAC and Herrmann (strip-tillage), and

Blackford and TPAC (chisel-plowed). Average air temperature and accumulated precipitation (Table 2.2) were obtained from the nearest weather station to each site (Midwestern Regional Climate Center, http://mrcc.isws.illinois.edu/).

Corn hybrids (104- to 114-day comparative relative maturity), seeding rate (67,000 to 86.000 seeds ha<sup>-2</sup>), and planting, date varied by site-year (Table A-1, A-2). Non-treatment fertilizers applied prior to, or at planting, are shown in Table A-3. Weeds and diseases were controlled with pesticides as needed.

Sulfur treatments were applied in starter and/or sidedress fertilizer applications in 2017 and 2018, but only in sidedress applications in 2019 and 2020 (Table 2.3). Sulfur rates ranged from 0 to 34 kg S ha<sup>-1</sup> as ammonium thiosulfate (ATS) (12-0-0-26) in combination with ammonium polyphosphate (10-34-0) and/or urea ammonium nitrate (UAN) (28-0-0) as a starter fertilizer or with UAN at sidedress. Starter fertilizer was injected 5 cm beside and 5 cm below the seed placement at planting, except Rice19 where fertilizer was applied on the soil surface 5 cm to the side of the seed. Sidedress treatments were injected midway between the corn rows approximately 10-cm deep, between growth stages V3 and V7 (Abendroth et al., 2011). The exception being Shelby(1)18 where N alone or in combination with S was applied at V3 stage or split between V3 and V12 stage. Total N rate was the same for all treatments within a site-year and was based on the recommended economic or agronomic optimum N rate (Camberato & Nielsen, 2019) ranging from 197 to 295 kg N ha<sup>-1</sup>. Boron was a secondary treatment in some experiments at rates ranging from 0.4 to 2.2 kg B ha<sup>-1</sup> applied as Solubor (Southern Agricultural insecticides, Inc).

Treatments were arranged in a randomized complete block design with 3 to 6 treatments and 3 to 6 replicates, except Blackford20 and Henry20 that each had 2 treatments and 12 and 5 replicates, respectively. Most site-years had 9-m wide plots (12 rows of 76-cm wide), except Blackford, Henry, and Shelby(1) (12-m wide plots – 16 rows of 76-cm wide) and Shelby(2) (12-m wide plots – 24 rows of 50-cm wide). The length of individual plots ranged from 100 to 350 m long.

#### 2.2.4 Plant sampling & analysis

For most sites in 2019 and 2020 (except Blackford19 and Henry20), three to six aboveground plant samples (15 to 30 plants per sample) were collected before sidedress (V3-V7 stage) following the same criteria as soil sampling for spatial distribution and for total samples per field. Plant samples were not collected prior to sidedress in 2017 and 2018. Samples were dried in a forced air dryer at 60°C for 3 to 4 days, then ground to pass a 1-mm mesh screen. Plant population was estimated at the time of plant sampling prior to sidedress or before harvest in some cases. Plants were counted in 2 adjacent rows, each 7.6 m long, in one sample area per replicate.

For nutrient analysis and dry biomass, composited earleaf samples (15 to 20 leaves) were randomly collected at R1 from the middle rows of each plot, excluding 18 to 22 m at the beginning and end of each plot, for all site-years (except DPAC17, Rice17, Blackford17-19, and Henry20). Samples were dried with forced-air at 60°C for 4 to 6 days until they achieved constant weight. Average leaf dry weight was determined and then leaves were ground to pass a 1-mm mesh screen.

At post-physiological maturity, 12 to 16 ears plot<sup>-1</sup> were collected at random from rows immediately adjacent to the harvested rows. The number of kernel rows per ear and kernels per row were determined, excluding kernels that did not fully develop. Ears were shelled (Agriculex Inc., Ontario, Canada), oven-dried at 60°C until constant weight, and 1000 kernel weight determined with an Old Mill seed counter with packager (International Marketing and Design Co., San Antonio, TX). A 500 ml kernel sample measured in a beaker was ground to a fine powder with a Blixer® 3 Series D grinder (Robot Coupe U.S.A., Inc., Ridgeland, MS) for nutrient analysis.

Plant samples from 2017-2019 were analyzed by A&L Great Lakes Laboratories (Fort Wayne, IN) and those from 2020 were analyzed by SureTech Laboratories (Indianapolis, IN). Both laboratories measured total N by the Dumas Method (method AOAC 990.03 on an Elementary Rapid-N Cube). Tissue digestion for mineral analyses differed between the laboratories. At A&L 0.2 g of tissue was digested with 2 ml of nitric acid and 1 ml peroxide (SW846-3051A), whereas at SureTech 0.1 g of tissue was digested with 5 ml nitric acid (AOAC 2017.02). In both laboratories, nutrients were quantified with a Thermo iCAP 6500 (method AOAC 985.01).

# 2.2.5 Grain yield estimation

At all but one site (SWPAC) yield was estimated with a commercial combine equipped with a calibrated GPS-enabled yield monitor from the middle 6 or 8-rows dependent on the site. At the Shelby(2) site (50 cm row spacing), the harvest width was 12 rows. Harvested grain weight at SWPAC was determined with a calibrated weigh wagon and grain yield estimated using that weight, the estimated grain moisture content, and the measured area of each plot. Grain moisture content at harvest was estimated using integrated moisture sensors in the combines or (SWPAC) with a calibrated portable grain moisture meter. Yield monitor data processing and cleaning involved removing data points from atypical areas showing poor plant development not related to treatment (excessively wet or dry field areas, animal damage, etc.) and from 23 to 25 m at each end of the plots. Remaining data points per plot were averaged to a single number for yield and moisture. Grain yield was reported at 15% grain moisture content.

Grain yield at three site-years (Blackford20 and Shelby(1)18,19) were calculated for the whole field and for areas within the field. Yield at Blackford10 was determined according to soil type map (Soil Survey Staff, 2020). Yield for both site-years at Shelby(1) was classified as more responsive and less responsive area to S fertilizer based on aerial images prior to harvest.

#### 2.2.6 Statistical analysis

The response of parameters of interest to S fertilizer was determined by analysis of variance, least significant difference procedure (LSD), and regression analysis in R studio (RStudio Team, 2020). When treatment effects were significant, single-degree-of-freedom contrasts were used to identify differences between sulfur rates. For statistical analysis of yield response to sulfur rates, each site-year was treated independently, and alpha  $\leq 0.10$  was considered significant for all the analysis.

# 2.3 Results

#### 2.3.1 Weather data

In April through July and October 2017, accumulated precipitation was slightly higher than the 30-year average at most sites (Table 2.2). August and September 2017 had less precipitation than the 30-year average. April, September, and October 2017 were warmer months relative to the 30-year average (Table 2.2), whereas May to August temperatures were near average.

The beginning of the 2018 growing season was dry and cool at most sites, with April and May precipitation less than the 30-year average (Table 2.2). Precipitation in June was greater than average while July precipitation was near average, except at SWPAC and Rice that received less precipitation than the 30-year average. In August and September, several sites received nearly

twice the average precipitation, except SEPAC(1)and SWPAC in August, and NEPAC and Rice in September. Air temperatures in April were well below average at all sites, where May and September were warmer months. In June through August and October, air temperature was near 30-year average (Table 2.2).

In April and May 2019, accumulated precipitation was well above the 30-year average at all sites (Table 2.2). In June through August, precipitation was near average at most sites, except some that received more or less rainfall relative to average. SEPAC, SWPAC, and NEPAC in June, July, and August respectively, received less rainfall than average, whereas SEPAC(1,2)and Shelby(2) in July, and Rice in both July and August received more rainfall than average. Air temperature was near average through the growing season at all sites relative to the 30-year averages (Table 2.2).

The 2020 growing season was dry compared to the 30-year average, excluding October that accumulated more precipitation that average (Table 2.2). Air temperature from April to October has little deviation from average at most sites, except Shelby(2) that had higher temperature in April through July (Table 2.2).

# 2.3.2 Corn grain yield and moisture

Grain yield was responsive to S fertilization at 10 of 28 site-years. At all site-years that responded to S fertilization, the increase in grain yield was similar across all S sidedress rates (8 to 34 kg S ha<sup>-1</sup>) (Table 2.4-2.7). The larger yield increases, 0.7 to 3.0 Mg ha<sup>-1</sup>, occurred at Blackford20, Rice17-19, Shelby(1)18-19, Shelby(2)19, and SWPAC18. Smaller increases, 0.2 to 0.3 Mg ha<sup>-1</sup>, occurred at Henry20, and SEPAC(1)18 (Table 2.5, 2.7). Fertilization with 6 kg S ha<sup>-1</sup> decreased grain yield 0.2 and 0.6 Mg ha<sup>-1</sup> below the yields of the control at SEPAC(1)20 and the average of the higher S rates at NEPAC20, respectively (Table 2.7).

Different responses within a field were identified at three site-years (Blackford20 and Shelby(1)18,19) (Table 2.6, 2.7). Shelby(1)18 grain yield increases was 1 Mg ha<sup>-1</sup> above the grain yield mean 12.7 Mg ha<sup>-1</sup> without S averaged for the whole field. In the same site, the most responsive area with S yielded 2 Mg ha<sup>-1</sup> more than 11.7 Mg ha<sup>-1</sup> without S. Grain yield increases at Blackford20 differed with soil type. Bono was unresponsive to S fertilization, Whitaker and Martinsville increased yield 0.9 and 1.5 Mg ha<sup>-1</sup>, respectively, and Saranac decreased yield 1.7 mg ha<sup>-1</sup> in treatment with S.

Grain yield was unaffected by S in the starter fertilizer at 7 of 8 sites (Table 2.4, 2.5). The exception was SEPAC(1)18 where 6 kg ha<sup>-1</sup> starter S increased yield 0.3 Mg ha<sup>-1</sup> above 15.2 Mg ha<sup>-1</sup> without S, and was equivalent to grain yield with sidedress or split application of 17 and 28 kg S ha<sup>-1</sup>.

Starter N, starter S, and sidedress S were evaluated at 3 sites in 2017. At Rice17, no starter, N-only starter, and N starter plus 4 kg S ha<sup>-1</sup> treatments produced similar yield (~13.8 Mg ha<sup>-1</sup>). All yielded ~0.8 Mg ha<sup>-1</sup> less than any treatment receiving sidedress S (Table 2.4). At DPAC17, N starter with or without 6 kg S ha<sup>-1</sup> decreased yield compared to the no starter treatment (Table 2.4). If S was applied at sidedress to the N starter treatments at this site-year, yield was equivalent to the no starter treatments. At Blackford17, starter N at 37 kg N ha<sup>-1</sup> without S reduced yield (~0.7 Mg ha<sup>-1</sup>) with or without sidedress S, compared to the no N starter and low N plus S treatments (Table 2.4). When 5 kg S ha<sup>-1</sup> was included with 37 kg N ha<sup>-1</sup>, yield was equivalent to the no N and low N starter treatments, with no difference in yield between these starter treatments with sidedress N.

Numbers of rows per ear and/or seeds per row were affected by S fertilization at 3 of 15 sites (Table A-4, A-5). Rice19 had 46 more seeds per ear<sup>-</sup> with 22 kg S ha<sup>-1</sup> compared to 516 seeds per ear without S fertilization. SWPAC19 produced 41 more seeds per ear with 8 kg S ha<sup>-1</sup> compared to 561 seeds per ear without S, and greater S rates were similar to the control. DPAC20 was the only site-year where all S rates increased the number of rows per ear by an average of one compared to zero-S (Table A-4, A-5).

Sulfur fertilization decreased grain moisture at 7 of 28 site-years and increased grain moisture at two site-years (Table 2.4-2.7). Moisture was decreased 1 to 7 g kg<sup>-1</sup>, equivalent to ~0.5-4.0%, below grain moisture without S at Blackford17,18, Shelby(1)18, Shelby(2)19, SWPAC18, and Rice19 (Table 2.4-2.7). The only sites with increased moisture from fertilizer S were DPAC20 and Blackford20 where grain moisture increased ~2 and 5 g kg<sup>-1</sup> over the zero S mean, respectively (Table 2.5). For most sites, grain moisture was decreased by all S rates equally, the exception being SWPAC18 where splitting 34 kg S ha<sup>-1</sup> between planting and sidedress reduced grain moisture, but there was no effect from the same rate applied only at sidedress. While these S effects on moisture were detected, the practical significance of such small effects is minor.

Fertilization with 0.4 to 1 kg B ha<sup>-1</sup> decreased grain moisture at Blackford19 (195 vs 198 g kg<sup>-1</sup>) and increased moisture at DPAC20 (175 vs 171 g kg<sup>-1</sup>) (Table 2.5, 2.7). At 10 of 12 other sites there was no effect of B on grain moisture.

#### 2.3.3 Grain yield response to sulfur fertilization versus predicted sulfur response

Grain yield responses to fertilizer S from this work were compared to a model (Ohio State University, 2021) that predicts the likelihood of crop response to fertilizer S based on estimates of atmospheric S wet deposition, organic S mineralization, S leaching, and low (15 kg S ha<sup>-1</sup>; e.g., corn) and high (30 kg S ha<sup>-1</sup>; e.g., alfalfa) crop S requirement (Kost et al., 2008). The model was used to categorize both responsive (10 site-years) and nonresponsive (18 site-years) site-years from this study into 1 of 3 categories: response to S is most likely (category 1), response to S will usually occur (category 2), and response to S is likely based on crop species and site conditions (category 3) (Table 2.8). No sites were in category 4, where crop response is unlikely but might occur under certain conditions, nor category 5, where crop response is unlikely under any circumstances. Eight of 10 site-years with real yield increases from fertilizer S were also predicted to respond to S according to the model (7 site-years fell into category 2 and 1 site-year into category 1), the other 2 sites were predicted to respond with crops of high S demand like alfalfa, but less likely to occur with a low S demand crop like corn. Among the 18 nonresponsive sites, eight were expected to increase yield with S (3 sites were category 1 and 5 sites were category 2), and 10 sites were in category 3 where response was dependent on crop species and site conditions. Sites like Shelby(1)19 and Blackford20 had zones of high and low or no yield response within the field, however the model's prediction of S response in those zones did not differ (Table 2.8).

# 2.3.4 Grain nutrient concentration

Sulfur concentration in the grain averaged 0.7 to 1.0 g kg<sup>-1</sup> without S at the 15 site-years where it was determined (Table 2.6, 2.7), and fertilizer S increased grain S concentration ( $p \le 0.10$ ) at 8 site-years. For 6 of 8 site-years (NEPAC20, SEPAC(1)19, Shelby(1)19, (Shelby(2)19, SWPAC19, and TPAC20) increases in grain S concentration were similar with all S rates and ranged from 0.1 to 0.2 g kg<sup>-1</sup>, ~11-29% above the zero S means which averaged 0.7 to 0.9 g S kg<sup>-1</sup>. Rice19 grain S concentration increased with increased S rate, it went from 0.7 mg S kg<sup>-1</sup> without

S fertilization to a maximum grain S of 1.1 g kg<sup>-1</sup> with 34 kg S ha<sup>-1</sup>. At Rice20, grain S increased with increased S at rates ranging 11 to 22 kg S ha<sup>-1</sup>, and no effect from 6 kg S ha<sup>-1</sup>. At this site, grain S was 0.8 mg S kg<sup>-1</sup> without S, and the maximum increase was 0.3 g kg<sup>-1</sup> with the highest S fertilization rate (22 kg S ha<sup>-1</sup>) (Table 2.6, 2.7).

Grain N concentration was not often responsive to fertilizer S and site-years ranged from 11.3 to 13.1 g kg<sup>-1</sup> without S (Table 2.6, 2.7). Grain N responded to increased S at Shelby(2)19 and Rice20. At Shelby(2)19, grain N with S increased ~0.5 g kg<sup>-1</sup> above 11.6 g kg<sup>-1</sup> without S and the response was similar at both rates, 11 and 22 kg S ha<sup>-1</sup> (Table 2.6). At Rice20, grain N concentration averaged 11.8 g kg<sup>-1</sup> without S and increased 0.1 to 0.5 g kg<sup>-1</sup> with increased S rate (Table 2.9).

Grain N:S without S fertilization ranged from 12.0:1 to 19.2:1 across all site-years (Table 2.6, 2.7). Most site-years had grain N:S $\leq$ 14:1 without S, other than Rice19,20, and Shelby(1,2)19 where N:S ranged from 14.5:1 to 19.2:1. Grain N:S concentration decreased with addition of fertilizer S at 8 of 15 site-years (Table 2.6, 2.7). In half the site-years increasing S rate decreased grain N:S proportionally (NEPAC19, Rice19,20, and Shelby(2)19), but in the other half of site-years all rates of S gave a similar decrease in grain N:S ratio (NEPAC20, SEPAC(1)19, Shelby(1)19, and SWPAC19). For sites with proportional grain N:S decreases with increased S rate, the decrease in grain N:S ranged from 2.3 to 6.9 units, a 17-36% decline in N:S ratio below that of the zero S treatment. On the other hand, at sites with similar grain N:S response at all S rates the decrease in grain N:S with S fertilization ranged from 1.5 to 2.0 units, a 10-14% decline in N:S ratio below the zero S treatment.

Fertilizer S affected the concentration of other nutrients in the grain in addition to N and S in some site-years (Table A-4, A-5). At SEPAC(1)19, fertilizer S increased grain P (0.3 to 0.6 g kg<sup>-1</sup>), K (0.3 g kg<sup>-1</sup>), and Zn (0.2 to 0.4 mg kg<sup>-1</sup>), above the grain zero S means of 2.1 g P kg<sup>-1</sup>, 3.3 g K kg<sup>-1</sup>, and 14 g Zn kg<sup>-1</sup>. Fertilizer S at TPAC19 decreased grain Zn (2.0 mg kg<sup>-1</sup>) and Fe (2 to 4 mg kg<sup>-1</sup>) below the zero S means of 20 mg Zn kg<sup>-1</sup> and 17 mg Fe kg<sup>-1</sup>. Grain Zn at NEPAC20 was decreased 2 mg Zn kg<sup>-1</sup> by S fertilization below 23 mg Zn kg<sup>-1</sup> without S.

Fertilizer B did not usually affect grain nutrient concentrations. The exceptions were Rice20 and NEPAC20 where 1 kg B ha<sup>-1</sup> decreased grain S concentration from 1.1 g S kg<sup>-1</sup> to 1.0 g S kg<sup>-1</sup>, and at Shelby(1)19 where grain N concentration was increased from 11.0 g N kg<sup>-1</sup> with zero B to 11.7 g N kg<sup>-1</sup> with 0.4 kg B ha<sup>-1</sup> (Table 2.6, 2.7).

# 2.3.5 Earleaf dry matter and nutrient concentrations at silking

Earleaf dry matter at R1 was unaffected by S fertilization at 16 of 18 site-years in 2019 and 2020 (except TPAC19 and SEPAC(1)20) (Table A-6, A-8). Earleaf dry matter at TPAC19 was increased 0.2 g leaf<sup>-1</sup> with 17, 25, or 34 kg S ha<sup>-1</sup> from 4.4 g leaf<sup>-1</sup> with zero S. In contrast, SEPAC(1)20 earleaf weight was decreased from 5.1 g leaf<sup>-1</sup> without S to 4.7 g leaf<sup>-1</sup> with 6 kg S ha<sup>-1</sup>. Earleaf weight from the highest S rates were not different than that without S.

Even without fertilization, all site-years had earleaf S concentration (Table 2.5-2.7) within the sufficiency range (1.5 to 2.2 g S kg<sup>-1</sup>; Campbell, 2013). However, 7 site-years that were grain yield responsive to S fertilization had earleaf S without S fertilization of 1.5 or 1.6 g S kg<sup>-1</sup>, at the lower end of the sufficiency range (Blackford20, Rice18,19, SWPAC18, Shelby(1)18,19, and Shelby(2)19).

Earleaf S concentration at R1 increased with the addition of fertilizer S at 12 of 22 site-years (Table 2.5-2.7). In about half the site-years, increasing S rate increased leaf S concentration proportionally (Simpon18, Rice18,19, DPAC19, SWPAC19, and NEPAC19,20), but in the other half of site-years all rates of S gave a similar increase in leaf S concentration (NEPAC18, Shelby(1,2)19, TPAC19, Blackford20, and Rice20). In both types of response, the increases in earleaf S concentration with S fertilization ranged from 0.2 to 0.4 g S kg<sup>-1</sup>, approximately a 10-25% increase in concentration above the zero S treatment. The lowest sidedress S rate tested at the site-years ranged from 11 or 17 kg S ha<sup>-1</sup> and the highest S rate at those sites was 34 kg S ha<sup>-1</sup>.

Nitrogen concentration in the earleaf at R1 ranged from 28 to 33 g kg<sup>-1</sup> at most site-years (Table 2.5-2.7), within the sufficiency range of 28 to 40 g kg<sup>-1</sup> (Campbell, 2013). The exceptions were SWPAC18, NEPAC18,20, Rice18,19, and Blackford20 where earleaf N ranged from 24.4 to 27.9 g kg<sup>-1</sup>.

Earleaf N concentration at R1 was less affected by S fertilization than earleaf S (Table 2.5-2.7). Sulfur fertilization affected earleaf N at 7 of 22 site-years (Blackford20, NEPAC20, Rice20, SEPAC(1)18, Shelby(2)19, SWPAC19, and TPAC19). Earleaf N usually increased 1.0 to 1.8 g kg<sup>-1</sup>, equivalent to 3-6% above the leaf N concentration without S, except at one site (Shelby(2)19) where increases in leaf N were much higher and ranged from 3.8 to 4.5 g kg<sup>-1</sup> or ~14-16%. Earleaf N was increased similarly by all S rates at most sites (Table 2.5, 2.7). TPAC19 leaf N where leaf N responded to S rates of 25 and 34 kg ha<sup>-1</sup>, but not to lower rates (Table 2.6).

The exception was SEPAC(1)18, it was the only site where earleaf N decreased with increased S rate ranging from 0 to 24 kg ha<sup>-1</sup> at sidedress, as determined by regression analysis (Table 2.9).

Earleaf N:S without S fertilization ranged from 14.8 to 16:1 well below the critical level of 18:1 (Campbell, 2013) at 14 of 22 site-years (DPAC18-20, NEPAC18,19, Rice20, SEPAC(1)18-20, SEPAC(2)19, Shelby(1)18, SWPAC20, TPAC19,20) (Table 2.5-2.7), whereas at 8 of 22 site-years N:S ranged from >16:1 to 18.4:1 (Blackford20, NEPAC20, Rice18,19, Shelby(1,2)19, and SWPAC18,19) (Table 2.5-2.7). Four site years had earleaf N:S near or at the critical level; Shelby(1)19 (18.4:1), Shelby(2) (17.5:1), SWPAC19 (17.3:1), and Rice19 (17:1) (Table 2.6). At 10 of 22 site-years, fertilizer S decreased leaf N:S 0.8 to 2.5 units, about 3-15% below the N:S ratio without S. At nearly half the site-years, earleaf N:S decreased with increased S rate (NEPAC19, Rice18, SEPAC(1)19, and SWPAC19) (Table 2.6). At two other site-years (Rice19 and DPAC19) leaf N:S plateaued with increasing S rate at 18 and 27 kg S ha<sup>-1</sup>, respectively. For the remaining site-years (Blackford20, TPAC19, and Shelby(1,2)19), earleaf N:S was decreased about the same amount at all S rates ranging from 6 to 34 kg S ha<sup>-1</sup> (Table 2.6-2.7).

Concentrations of P, K, and Ca in the earleaf were at sufficient levels (Campbell, 2013) for all site-years, including those where S fertilizer rate affected the concentration of a nutrient (Table A-6-A-8). The site-year means for the zero S treatment ranged from 2.9 to 4.7 g P kg<sup>-1</sup>, 3.3 to 6.1 g Ca kg<sup>-1</sup>, and 18.8 to 30.1 g K kg<sup>-1</sup>. For most site-years, R1 earleaf concentrations of Mg were at sufficient levels (1.5 to 6.0 g Mg kg<sup>-1</sup>) and site-year means ranged from 2.0 to 4.0 g Mg kg<sup>-1</sup>; except for SWPAC18 (1.1 g Mg kg<sup>-1</sup>) for all treatments and Shelby(2)19 (1.4 g Mg kg<sup>-1</sup>) without S application only (Table A-6-A-8).

For most site-years, concentrations of P, K, Ca, and Mg in the earleaf at R1 were unaffected by S treatment (Table A-6-A-8) although there were some exceptions. At Shelby(1)18, 22 to 34 kg S ha<sup>-1</sup> lessened leaf P concentration 0.6 and 0.3 g P kg<sup>-1</sup>, respectively, below 3.7 g P kg<sup>-1</sup> without S (Table A-6). Earleaf K increased with increased S at SEPAC(2)19 (2.8 increase above 21.2 g K kg<sup>-1</sup> without S), Shelby(1)19 (1.5 increase above 19.3 g K kg<sup>-1</sup> without S), and Blackford20 (0.7 increase above 21.1 g K kg<sup>-1</sup> without S) (Table A-7, A-8). Earleaf Ca decreased with fertilizer S at Rice19 (0.3 decrease below 4.0 g Ca kg<sup>-1</sup> without S) and SEPAC(2)19 (0.8 decrease below 5.9 g Ca kg<sup>-1</sup> without S), and was unaffected elsewhere. Earleaf Mg concentration increased with fertilizer S at Shelby(2)19 (0.2 increase above 1.4 g Mg kg<sup>-1</sup> without S) (Table A-7), but there were no effects at other site-years. Earleaf Mn, Fe, and Cu were not deficient at any site-year (Table A-6-A-8). Fertilization with S increased earleaf concentrations of Mn, Fe, and Cu at several site-years (Table A-6-A-8). Earleaf Mn concentration was increased 23 and 17 mg kg<sup>-1</sup> with 11 and 22 kg S ha<sup>-1</sup> above 60 mg Mn kg<sup>-1</sup> without S at Shelby(2)19. Fertilizer S increased earleaf Fe concentration 3 to 10 mg Fe kg<sup>-1</sup> at Rice19, 12 mg Fe kg<sup>-1</sup> at Shelby(2)19, and 11 to 17 mg Fe kg<sup>-1</sup> at Shelby(1)19. Earleaf Fe without S fertilization was 69, 81, and 71 mg kg<sup>-1</sup>, respectively, for the 3 site-years. Sulfur fertilization increased earleaf Cu 2 mg Cu kg<sup>-1</sup> at Shelby(2)19 and 3 mg Cu kg<sup>-1</sup> at Shelby(1)19; above Cu concentrations without S fertilization of 9 and 10 mg kg<sup>-1</sup>, respectively, for the two site-years.

#### 2.3.6 Earleaf nutrient concentrations at silking as affected by boron fertilization

The effects of fertilizer B were evaluated at 12 sites in 2019 and 2020. At 4 site-years earleaf B increased with B fertilization: NEPAC20, SEPAC(1)20, Shelby(1)19, and SWPAC20 (Table A-7, A-8). Fertilizer B increased leaf B by 1 or 2 mg kg<sup>-1</sup> over the zero S site-year means which ranged from 5 to 8 mg B kg<sup>-1</sup>. At most site-years, earleaf-B concentration was adequate,  $\geq$ 4 mg B kg<sup>-1</sup> (Campbell, 2013), with the exception of NEPAC18, Rice18, SEPAC(1)18, Shelby(1)18, Shelby(2)19, and SEPAC(1)19 (Table A-6-A-8). None of these sites with leaf B concentration <4 mg kg<sup>-1</sup> were B response trials.

Fertilization with B affected earleaf concentrations of S, N, and/or N:S at only 2 of 12 siteyears (Table 2.6, 2.7). SWPAC20 decreased earleaf S by 0.1 g kg<sup>-1</sup> below the zero B mean 2.1 g S kg<sup>-1</sup>. The same site also decreased earleaf N 0.5 g kg<sup>-1</sup> below 31.7 g kg<sup>-1</sup> without B. Addition of B increased earleaf N from 28.4 without B to 30.2 and 31.8 g kg<sup>-1</sup> with 1 or 2 kg B ha<sup>-1</sup>, respectively, and increased N:S from 14.3 without B to 15.8:1 at SEPAC(2)19 from both B rates.

#### 2.3.7 Plant tissue nutrient levels at V3-V7

Plant tissue nutrients were determined prior to the sidedress applications of S treatments at V3 to V7 growth stages at 14 of 18 sites in 2019 and 2020. Tissue S concentration at all sites prior to treatment application (Table 2.10) was considered sufficient (>1.5 g S kg<sup>-1</sup>), including site-years where soil SO<sub>4</sub>-S concentration were below the critical level of 8 mg kg<sup>-1</sup> (Table 2.11). Nitrogen to S ratios equal to or greater than 18:1 are not considered adequate for corn (Campbell, 2013).

Seven of the 14 site-years had N:S≥18:1 - DPAC19-20, NEPAC20, RICE19-20, SEPAC(1)20, and SWPAC20 (Table 2.10).

Nutrient concentrations in the whole plant at V3-V7 (Table 2.10), other than S, were sufficient (Table A-9) in most site-years. Tissue macronutrient concentrations were just below critical levels at Shelby(1)19 at V4 (29.4 vs 30.0 g N kg<sup>-1</sup>), NEPAC19 and Shelby(1)19 at V4 (2.9 vs 3.0 g P kg<sup>-1</sup>), and SWPAC20 at V3 (2.5 vs 3.0 g P kg<sup>-1</sup>). Boron was the only micronutrient that was below critical levels (<5 mg kg<sup>-1</sup>) at V4 at DPAC19 (4 mg B kg<sup>-1</sup>), V4 at NEPAC19 (3 mg B kg<sup>-1</sup>), V6 SEPAC(1)19&19b (3 and 2 mg B kg<sup>-1</sup>), and V4 at Shelby(1)19 (4 mg B kg<sup>-1</sup>) (Table 2.10).

# 2.3.8 Soil physical and chemical characteristics

Soil series, US taxonomy classification, and drainage class for all 28 sites are shown in Table 2.1. Mollisols and Alfisols were predominant soil orders at 15 and 10 sites, respectively. Ultisols and Inceptisols occurred at 3 (SEPAC(1)18-20) and 1 (SWPAC20) site, respectively. Wisconsin till was the parent material at NEPAC, DPAC, and Blackford, and Wisconsin outwash for SWPAC18 and Henry20. SWPAC19-20 parent material was recent alluvium, and Shelby(1) parent material was loess over till. SEPAC was the only site with Illinoian till (SEPAC(1)19), outwash (SEPAC(1)18-20), or both (SEPAC(2)19) as parent materials. Most of the sites were very poorly-or somewhat poorly-drained (DPAC17-20, Rice17-20, SWPAC20, Blackford17-20, Henry20, and Shelby(1)18-19), and fewer were moderately well- to well-drained (NEPAC18-20, SEPAC(1)18-20, Shelby(2)19, and SWPAC18-19). TPAC19-20 had soils that were somewhat poorly-, moderately well- and well-drained dependent on position in the landscape.

Soil texture at 15 of 28 site-years was fine-textured (clay, silty clay, silty clay loam, clay loam, silt loam, and loam) and 8 sites were coarse-textured (loamy sand, sandy loam, and sandy clay loam) (Table 2.11, A-10). NRCS textural classes for soil series at 5 site-years where soil texture was not directly measured were SiC (Blackford17), SiL (Blackford18&19 and Henry20), and SiCL (Shelby(1)18).

Soil organic matter (OM) and pH was determined for 26 of 28 site-years (Table 2.11, A-10). Soil OM in the upper 20 cm ranged from 10 to 49 g kg<sup>-1</sup> across all sites, and 13 sites had OM  $\leq$ 25 g kg<sup>-1</sup>. Three of 7 sites sampled in 2020 had different levels of OM among soil series within the same field: DPAC20, Rice20, and Blackford20. The lowest and highest OM levels were 28 and 35 g OM kg<sup>-1</sup> at DPAC20, 19 and 27 g OM kg<sup>-1</sup> at Rice, and 21 and 49 g OM kg<sup>-1</sup> at Blackford20. Soil OM decreased slightly with depth at most sites, except SWPAC20 which showed a steeper decline than other site-years.

Soil pH in the upper 20 cm ranged from 5.8 to 7.1 pH. Soil pH decreased with depth from 20 to 60 cm for most site-years, except DPAC, NEPAC, and Shelby(1) where pH increased with depth (Table 2.11, A-10).

# 2.3.9 Soil sulfate-sulfur

Mehlich-3-extractable soil sulfate-sulfur concentration (SO<sub>4</sub>-S) was determined for 20 of 28 sites (Table 2.11, A-10). At Rice18, NEPAC18, SWPAC18, DPAC19, SWPAC19, Rice20, and TPAC20; the SO<sub>4</sub>-S concentration in the upper 20 cm of soil was 4 to 8 mg SO<sub>4</sub>-S kg<sup>-1</sup> (Table 2.11), values below the suggested as a critical level for sufficiency (Culman et al., 2020). At the other 14 sites SO<sub>4</sub>-S ranged from 9 to 12 mg kg<sup>-1</sup> in the upper 20-cm soil. Sulfate-S was greater at DPAC20 and SEPAC(1)19,20 in the 20-40-cm and 40-60-cm depths, than in the upper 20-cm (Table 2.11). At NEPAC19, Rice19,20, Shelby(1)19, and SWPAC19,20, SO<sub>4</sub>-S decreased with increased depth. For all the other sites, SO<sub>4</sub>-S was similar throughout the 0-60-cm depth. SO<sub>4</sub>-S weighted profile mean (WPM) was calculated at 16 sites to create a single SO<sub>4</sub>-S concentration to represent the entire 0-60-cm soil depth (Table 2.11). The WPM for most site-years ranged from 4.9 to 8.5 mg SO<sub>4</sub>-S kg<sup>-1</sup>, whereas SEPAC(1)19 and SEPAC(1)20 had much greater WPM (16.4 and 13.9 mg SO<sub>4</sub>-S kg<sup>-1</sup>, respectively) primarily due to higher SO<sub>4</sub>-S in the 20-60-cm soil depth (Table 2.11). Rice20 was the only site where the WPM of one soil type (Gilford, 3.5 mg SO<sub>4</sub>-S kg<sup>-</sup> <sup>1</sup>) was substantially lower than another soil type in the same field (Maumee, 6.9 mg SO<sub>4</sub>-S kg<sup>-1</sup>). Interestingly, the loamy sand Maumee soil with less OM and less clay content had the higher WPM compared to the sandy loam Gilford (Table 2.11). The Maumee was slightly more acidic and had higher extractable-Fe in the 0-20-cm and 20-40-cm soil depths than the Gilford (182 vs 161 in 0-20 cm and 124 vs 100 mg kg<sup>-1</sup> in 20-40-cm), which may have resulted in greater sorption of SO<sub>4</sub>-S in the Gilford.



Figure 2.2: Relationship between Mehlich-3 extractable SO4-S concentration in the soil and soil pH at three depths (a) 0-20 cm, (b) 20-40 cm, and (c) 40-60 cm at ACRE, Blackford, DPAC, NEPAC, Rice, Shelby(1), Shelby(2), SWPAC, and TPAC in Indiana in 2015, 2019, and 2020. A solid line represents a linear or linear-plateau regression model fitted to the data ( $p \le 0.10$ ), with adjusted R squared (R sq.) or residual standard error (RSE) accordingly. 2015 data from Moser (2016).



Figure 2.1: Relationship between Mehlich-3 extractable SO4-S concentration in the soil and soil pH at three depths (a) 0-20 cm, (b) 20-40 cm, and (c) 40-60 cm at SEPAC in 2015, 2019, and 2020. Solid line represents a linear or linear-plateau regression model fitted to the data ( $p \le 0.10$ ), with adjusted R squared (R sq.) or residual standard error (RSE) accordingly. 2015 data from Moser (2016).

A SO<sub>4</sub>-S weighted profile mean (WPM) was calculated at 16 sites to create a single SO<sub>4</sub>-S concentration to represent the entire 0-60-cm soil depth (Table 2.11). The WPM for most site-years ranged from 4.9 to 8.5 mg SO<sub>4</sub>-S kg<sup>-1</sup>, whereas SEPAC(1)19 and SEPAC(1)20 had much greater WPM (16.4 and 13.9 mg SO<sub>4</sub>-S kg<sup>-1</sup>, respectively) primarily due to higher SO<sub>4</sub>-S in the 20-60-cm soil depth (Table 2.11). Rice20 was the only site where the WPM of one soil type (Gilford, 3.5 mg SO<sub>4</sub>-S kg<sup>-1</sup>) was substantially lower than another soil type in the same field (Maumee, 6.9 mg SO<sub>4</sub>-S kg<sup>-1</sup>). Interestingly, the loamy sand Maumee soil with less OM and less clay content had the higher WPM compared to the sandy loam Gilford (Table 2.11). The Maumee was slightly more acidic and had higher extractable-Fe in the 0-20-cm and 20-40-cm soil depths than the

Gilford (182 vs 161 in 0-20 cm and 124 vs 100 mg kg<sup>-1</sup> in 20-40-cm), which may have resulted in greater sorption of SO<sub>4</sub>-S in the Gilford.

Sufficiency ranges for Mehlich-3 extractable P, K, Ca, and Mg in the upper 20-cm soil (Culman et al., 2020) are shown in Table A-9. At SEPAC(1)19 and SEPAC(2)19, Mehlich-3 extractable K was below critical levels for K (Table A-11), however, this site received 0-0-49.8 (N-P-K) before planting each year and tissue K was at sufficient levels in early-growth tissue (V3-V7) (Table 2.9) and at R1 (Table 2.6). For all other sites in 2019 and 2020, soil P, K, Ca, and Mg met criteria for adequacy (Table A-9).

# 2.4 Discussion

# 2.4.1 Grain yield and moisture

Treatments with fertilizer S yielded 0.2 to 3.0 Mg ha<sup>-1</sup> more grain than treatments without S at 10 of 28 Indiana site-years, a 36% frequency of response (Blackford20, Henry20, Rice17-19, SEPAC(1)18, Shelby(1)18,19, Shelby(2)19, and SWPAC18). When a response to S fertilizer occurred, the maximum yield response occurred at the lowest sidedress rate examined in in each particular site-year (ranging from 8 to 17 kg S ha<sup>-1</sup>). The increases in grain yield from S fertilization occurred in several areas of Indiana with no history of S deficiency, suggesting that areas with low inputs of S (soil sulfate-S, S from OM, atmospheric S, incidental S) require S fertilization. Grain yield responses to S fertilization has been also observed in other states in the Corn Belt.

Experiments in Illinois conducted in the late 1970's, found that 6% of 82 experimental siteyears responded to S fertilization (Hoeft & Fox, 1986). In the same state in 2009 to 2011, fertilization with 34 kg S ha<sup>-1</sup> increased yield 1.3 and 3.2 Mg ha<sup>-1</sup> in 2 of 27 on-farm trials, a 7% response rate (Fernández et al., 2012). However, a higher response rate (~31%) occurred in 16 small-plot experiments with an average yield increase of ~0.8 Mg ha<sup>-1</sup> with 27 kg S ha<sup>-1</sup>. In Ohio, adding 33 kg S ha<sup>-1</sup> increased grain yield 0.4 to 0.9 Mg ha<sup>-1</sup> in 2 of 4 site-years from 2002 to 2005 (Chen et al., 2008).

Corn grain yield in Iowa was unresponsive to fertilizer S in six experiments from 2000 to 2001 (Sawyer & Barker, 2002). In later studies conducted from 2006 to 2008, grain yield was increased 0.8 to 2.4 Mg ha<sup>-1</sup> with S fertilization in ~69% of 51 site-years (Sawyer et al., 2009). Additional studies conducted at 30 sites in 2009 and 2011 to 2013, had a 37% frequency of response to fertilizer S, and yield was increased 0.3 to 2.0 Mg ha<sup>-1</sup> (Sawyer et al., 2015). Current

S recommendations for maximizing corn and soybean yield in Iowa are 19 and 28 kg S ha<sup>-1</sup> for fine- and coarse-textured soils, respectively. The authors suggested that S-containing fertilizers should be applied only before corn in a corn-soybean rotation, or every other year in a corn-corn rotation (Sawyer et al., 2009).

In Minnesota in 1999 to 2001, 6.7 kg S ha<sup>-1</sup>increased corn grain yield 0.3 to 0.9 Mg ha<sup>-1</sup> compared to the control at 5 of 6 sites (Rehm, 2005). Higher S rates (13 and 20 kg S ha<sup>-1</sup>) did not increase yield further (Rehm, 2005). Experiments in 2008 and 2009 also showed response to S in Minnesota at 2 of 4 sites, where a S rate of 24 kg S ha<sup>-1</sup> increased yield 1.1 and 1.8 Mg ha<sup>-1</sup> more compared to a no S control (Kim et al., 2013). In a recent study in Kansas in 2019, corn grain increased ~0.5 Mg ha<sup>-1</sup> with the lowest S rate tested, 34 kg S ha<sup>-1</sup> (Husa & Ruiz Diaz, 2020).

Our study did not find starter S applied at 4 to 6 kg S ha<sup>-1</sup> to have an effect on grain yield in 8 of 9 site-years. This contrasts with results from Minnesota, where 6.7 kg S ha<sup>-1</sup> starter S increased corn grain yield at 5 of 6 site-years (Rehm, 2005). However, those fields were planted late in April where in our study fields were planted in May and June. Early planting with cold temperatures, increases the chances for corn to respond to S fertilization.

Six kg S ha<sup>-1</sup>, compared to the zero S treatment or S rates >6 kg S ha<sup>-1</sup>, decreased grain yield 0.2 and 0.3 Mg ha<sup>-1</sup> at SEPAC(1)20 and NEPAC20, respectively. At SEPAC(1)20, earleaf dry matter at silking was also decreased (0.4 g below 5.1 g without S) by 6 kg S ha<sup>-1</sup> compared to the zero S treatment or higher rates of S, but this did not occur at NEPAC20. Negative yield responses were also observed at 4 of 47 site-years in Missouri where yield decreases ranged from 0.6 to 1.6 Mg ha<sup>-1</sup> averaged over S rates of 17, 34, and 67 kg S ha<sup>-1</sup> (Stecker et al., 1995). Corn and soybean yield were decreased with S fertilization in Ohio at 2 of 27 and 1 of 13 sites, respectively (Fleuridor et al., n.d.). Few details were given on those experiments. Grain yield decreases with the lower S rate only were not expected in our study and we cannot explain these responses.

Sulfur fertilization decreased moisture in the grain in the range of 1 to 7 g kg<sup>-1</sup> at 7 of 28 site-years. At 4 site-years where grain moisture was decreased by S fertilization, yield was increased (Rice19, Shelby(1)18, Shelby(2)19, and SWPAC18) and at 3 site-years grain moisture decreased, but yield was unaffected (Blackford17,18, and DPAC17). Similar decreases in grain moisture occurred at all S rates within each site-year. Only two other studies were found that evaluated effects of S on grain moisture and neither documented any S effects. Grain yield increased, but grain moisture was unaffected by fertilizer S in 2 experiments in Georgia (Bullock

& Goodroad, 1989). Neither yield nor grain moisture responded to S in Minnesota (Kurbondski et al., 2019). In some fields from our study, when sampling earleaves it appeared that plants with S silked slightly earlier than plants without S, but quantification of the differences were not made. Drier grain would likely be a result of earlier silking. In irrigated field experiments in India, ear development without S began 49 days after emergence, whereas fertilization with 20 and 50 kg S ha<sup>-1</sup> shortened this period to 45 and 42 days, respectively (Kumar & Bohra, 2014).

In contrast to the 7 site-years where S fertilization decreased grain moisture; S fertilization increased grain moisture in 2 of 4 soil types at Blackford 20 where grain yield also increased with S. In the other two soil types, grain moisture was unaffected by fertilizer S, and grain yield was unaffected in one soil type and reduced with fertilizer S in the other. Drought stress was distinctive and pronounced in the two soil types where S fertilization increased grain yield, and less noticeable in the two soil types where yield did not increase with the addition of S. Perhaps plants with S had better root systems and were able to get more water which resulted in later senescence.

# 2.4.2 Grain S, N and N:S

Fertilizer S increased grain S concentration at all site-years where grain yield was increased by S fertilization and grain was sampled (Rice19, and Shelby(1,2)19) (Figure 2.3). Grain S also increased at 5 of 12 site-years where grain yield was unaffected by S fertilization (NEPAC20, Rice20, SEPAC(1)19, SWPAC19, and TPAC19) (Figure 2.3). At most sites, grain S increases were similar with all S rates except at two site-years (Rice19,20) where grain S increased with increased S rate. Where grain yield was responsive to S (Rice19, and Shelby(1,2)19), grain S increased 0.1 to 0.5 g kg<sup>-1</sup> with S fertilization above 0.7 to 0.8 g kg<sup>-1</sup> without S. Additionally, grain N:S narrowed ~1:1 to 7:1 below 19:1 to 14.5:1 range without S. For the 12 grain yield unresponsive site-years, grain S concentration ranged from 0.9 to 1.1 g S kg<sup>-1</sup>. At nearly half of these sites (NEPAC20, Rice20, SEPAC(1)19, SWPAC19, and TPAC19), S fertilization increased grain S and decreased N:S. Other researchers found grain S increased with S fertilizer when S concentration was <0.9 g S kg<sup>-1</sup>, yield responses occurred less often with greater grain S concentrations, and grain S response was not always accompanied by a yield response. In an example from Minnesota with 3 site-years, grain S (Kim et al., 2013). Grain S increased with fertilizer S in soils with OM ranging from 0 to >40 g kg<sup>-1</sup>. Grain yield increased at 2 of 3 site-years with grain S 0.5 to 0.7 g kg<sup>-1</sup> without S. The other site was grain yield unresponsive to S fertilization and grain S was ~0.9 g kg<sup>-1</sup>. Studies in Ohio showed in 3 of 4 site-years where grain yield responded to S fertilization grain S was responsive at 1 site-year and it increased from ~1.0 without S to ~1.1 g kg<sup>-1</sup> with 33 kg S ha<sup>-1</sup>; grain S at the other sites ranged ~0.9 to 1.1 g kg<sup>-1</sup> without S (Chen et al., 2008). Grain S concentration at yield responsive sites in our studies was always <0.9 g S kg<sup>-1</sup>. Perhaps, grain S will be a good indicator of grain yield responses to S fertilization, but the number of responsive sites we sampled was limited.



Figure 2.3: Grain and earleaf S responses to S fertilization at 15 grain yield responsive and nonresponsive site-years, and B) earleaf S responses to fertilizer S at 5 grain yield responsive site-years with only earleaf S data and no grain S data.

An infrequent increase in grain N concentration with S fertilization was expected since R1 earleaf N was considered deficient at only 6 of 22 site-years (SWPAC18, NEPAC18,20, Rice18,19,

and Blackford20) and grain N was unresponsive to S at those site-years. At another 2 site-years (Rice20 and Shelby(2)19), S fertilization increased N concentration in the grain. At these site-years, leaf N was sufficient ( $\geq$ 28 g kg<sup>-1</sup>), although at Shelby(2)19 leaf N was at the lower threshold value for sufficiency (28 g N kg<sup>-1</sup> without S). At the remaining 14 of 22 site-years where N was adequate in the earleaf tissue, grain N concentration was unaffected by S fertilization. In contrast, in experiments in Minnesota earleaf N increased and grain N was unaffected by S fertilizer applications (Sutradhar et al., 2017). In Ohio studies, grain N increased in response to S fertilization at 1 of 3 site-years, but earleaf N was not measured (Chen et al., 2008). In Argentina, grain N was not responsive to S at any of the 24 site-years, and when N concentration was measured in the stover it was unresponsive as well (Carciochi et al., 2020; Salvagiotti et al., 2017).

Total seed rows ear<sup>-1</sup>, seed number ear<sup>-1</sup>, and 1,000 seed weight responded infrequently to S fertilization. Two site-years had 41 and 46 more kernels ear<sup>-1</sup> with S fertilization but only at specific S rates. In addition, just one of two had a yield increase. At another site-year, ears had one more single row with S. In 19 site-years of S response trials in Argentina, grain number explained 63% of the yield response to S, 3,565 seeds m<sup>-2</sup> with S vs 3,391 seeds m<sup>-2</sup> without S (Salvagiotti et al., 2017). Increased grain weight with S, 286 mg with S vs 279 mg without S, explained 20% of the yield response to S in this same study. Considering that our study was conducted in large fields. It was challenging to sample a field thoroughly enough to adequately avoid the effects of natural spatial variability among plants for these yield components.

# 2.4.3 Earleaf dry mass and nutrients

Earleaf dry matter at R1 was unaffected by S fertilization rate at all but 2 of 16 site-years (except TPAC19 and SEPAC(1)20). Earleaf dry matter at TPAC19 was 0.2 g greater with 17, 25, or 34 kg S ha<sup>-1</sup> than 4.4 g leaf<sup>-1</sup> without S, but grain yield was unresponsive at this site-year. At SEPAC(1)20 the 6 kg S ha<sup>-1</sup> treatment decreased earleaf dry matter 0.4 g leaf<sup>-1</sup> from 5.1 g leaf<sup>-1</sup> for the zero S treatment. At this site-year, grain yield was also reduced at this S rate. There are no other reports in the literature concerning S fertilization effects on corn earleaf dry matter, but there are reports of the effects of S fertilization on total biomass per area or dry matter per plant. Compared to zero S aboveground dry matter at one site 1.1 Mg ha<sup>-1</sup> at V6 and a second site 17.0 Mg ha<sup>-1</sup> at R1, 32 kg S ha<sup>-1</sup> increased aboveground dry matter ~0.2 Mg ha<sup>-1</sup> at V6 and 1.4 Mg ha<sup>-1</sup>

at R1 in 2 of 6 S response studies (Carciochi, et al., 2019). Both sites increased grain yield ~1.0 to 0.9 Mg ha<sup>-1</sup> above 14.0 Mg ha<sup>-1</sup> at site 1 and 10.7 Mg ha<sup>-1</sup> at site 2 without S. The increase in R1 dry matter at site 2 was ~8% and was proportional to the yield increase, where V6 dry mass increase ~16% much higher than ~8% yield increase (Carciochi, et al., 2019). Similarly, 25 kg S ha<sup>-1</sup> increased dry matter 7 g plant<sup>-1</sup> above the zero S mean which averaged 40 g plant<sup>-1</sup> at an undefined growth stage in a two year-study (Kumar & Bohra, 2014). For most site-years in our study, earleaf weight at R1 was unaffected by fertilizer S and so was not a good predictor of yield response to S fertilization.

Earleaf S concentration at R1 increased at all 3 site-years (Rice19, and Shelby(1,2)19) where grain yield and grain S increased with fertilizer S (Figure 2.3). At these site-years, earleaf S ranged from 1.5 to 1.6 g kg<sup>-1</sup> without S and from 2.0 to 2.1 g kg<sup>-1</sup> with S. There were 5 site-years where grain yield responded to S fertilization, but no grain was sampled. At 3 of those 6 site-years, earleaf S ranged from 1.5 to 1.7 g kg<sup>-1</sup> without S and from 1.8 to 2.2 g kg<sup>-1</sup> with S fertilization (Blackford20, Simpon18, Rice18). At the remaining 2 locations (SEPAC(1)18 and SWPAC18), earleaf S was unresponsive and ranged from 1.6 to 2.1 g kg<sup>-1</sup> without S. In field experiments, the earleaf S concentration at R1 for optimum yield responses ranged from 2.0 to 2.3 g S kg<sup>-1</sup> (Bullock & Goodroad, 1989; Rehm, 1993; Sawyer et al., 2015; Sutradhar et al., 2017), within the sufficiency range for S, 1.5 to 6 g S kg<sup>-1</sup> (Campbell, 2013).

Earleaf S concentration increased with S fertilization at 8 of 17 site-years where grain yield was unresponsive to S fertilization, and at 4 of the 8 site-years where S concentration in the grain also increased with S fertilization (Figure 2.3). Leaf S concentration ranged from 1.5 to 2.1 g S kg<sup>-1</sup> without S, and increased with increased S rate at most site-years, and a minority (3 of 8 site years) observed similar increases in leaf S from all S rates. Previous studies have observed earleaf S response with no yield increases. In Michigan, earleaf S concentration increased at 1 of 8 site-years from 1.7 without S to 1.9 with S (0 to 25 kg S ha<sup>-1</sup>), but yield was unaffected at this site (Steinke et al., 2015). In Iowa, earleaf S increased with S fertilization, but there was no yield gain at 7 of 10 site-years (Sawyer & Barker, 2002). Leaf S ranged from 1.3 to 1.8 g kg<sup>-1</sup> without S and increased 0.1 to 0.4 g kg<sup>-1</sup> with S. Field experiments in Wisconsin found that earleaf R1 S concentration increased 0.2 to 2.5 g kg<sup>-1</sup> with the addition of S fertilizer above the 1.9 to 2.4 g kg<sup>-1</sup> with zero S at 5 of 16 site-years (Buckley & Wolkowski, 2012). Although earleaf S concentration increased with S fertilization, none of the sites had yield increases. In Brazil at 2 field experiments,

treatments with S increased earleaf S 0.7 and 0.2 g kg<sup>-1</sup> above 1.2 and 1.8 g kg<sup>-1</sup> without S, respectively, and only one increased yield from 8.9 Mg ha<sup>-1</sup> without S to 10.3 with S (Crusciol et al., 2019).

Sulfur concentration in the most recently collared leaf at V6 and/or at V12 was evaluated as diagnostic criteria for plant S status and to predict yield response to fertilizer S (Carciochi, et al., 2019b). Leaf S concentration at V6 ( $R^2$ = 0.93) was more accurate than at V12 ( $R^2$ = 0.83) for identifying grain yield responsiveness to S fertilization. The critical S concentration threshold was 2.1 g kg<sup>-1</sup> at V6 and 1.65 g kg<sup>-1</sup> at V12. Whole plant S concentration measured at V12 and 10-15 days pre-silking did not relate to yield response to S, but tissue S measured 10-15 days post silking predicted yield response with 78% accuracy (Pagani & Echeverría, 2011). Earleaf S concentration was useful to assess S status in the plant however it did not identify responsive from unresponsive sites successfully. In our study most site-years where grain yield increased, earleaf S ranged from 1.5 to 1.7 g kg<sup>-1</sup> without S fertilization. Earleaf S should be accompanied with other parameters like grain S concentration, the latter was less responsive to S than the earleaf at sites where yield was unresponsive.

Earleaf N responded to fertilizer S at 7 of 22 site-years. Two site-years (Blackford20 and Shelby(2)19) increased grain yield with S fertilization and at the other 5 site-years, grain yield was unaffected by S (NEPAC20, Rice20, SEPAC(1)18, SWPAC19, and TPAC19). Only 1 site-year (Blackford20) was earleaf N deficient. Leaf N increased similarly with all S rates at 4 site-years. While at the other 3 site-years, the leaf N increased with increased S rate, but the magnitude of the increase was small. Researchers in Minnesota found fertilizer S increased earleaf N only when N was limiting in the soil (Sutradhar et al., 2017). In contrast, in Argentinian soils S fertilization (0, 15, and 32 kg S ha<sup>-1</sup>) without N did not increase N concentration in the shoot at R6 (Carciochi et al., 2020). Earleaf N responses to S fertilization have had little relation with yield responses, when earleaf N concentration increased with S fertilization, it was usually at sites where leaf N was deficient or near the lower threshold for sufficiency (28 kg N kg<sup>-1</sup>).

For treatments with S, the earleaf N:S ratio narrowed 0.6 to 2.0 at 9 of 22 site-years. Similar decreases in N:S were obtained with all S rates. Earleaf N:S ratio usually decreased at site-years where earleaf S was responsive to fertilizer S, except at TPAC20 where there were not statistical differences in earleaf S or N. At site-years where grain yield responded to S fertilization and earleaves were sampled, the N:S ratio without S ranged from 15:1 to 18:1. Decrease in N:S with

increased tissue S was reported previously (Carciochi, et al., 2019; Pagani & Echeverría, 2011; Steinke et al., 2015; Sutradhar et al., 2017). When N:S ratio was evaluated at the  $12^{th}$  leaf of V12 corn as a tool to predict corn yield response to S fertilization, its accuracy ( $R^2$ =0.76) was no better than the S concentration in the same leaf ( $R^2$ =0.93) (Carciochi et al., 2019b). In addition, using both N:S and S did not improve predictions. The N:S threshold for maximum yield was 15:1. Differences in critical N:S ratio at different plant stages was suggested as a limitation for yield predictions. The critical N:S ratio narrowed from 16.7:1 at V6 to 15.1:1 at V12 (Carciochi et al., 2019; Pagani & Echeverría, 2011). In our study, earleaf N:S at R1 did not distinguish grain yield responsive from nonresponsive sites to S fertilization, nevertheless site-years where grain yield increased leaf N:S ranged from 15 to 18:1 without S. Nitrogen to S ratio helped as indicator of N and S balance in the plant, however it should be used together with tissue S and tissue N to ensure their individual adequacy.

# 2.4.4 Plant tissue nutrient levels at V3-V7

Plant tissue S and N status at V3-V7 plant stage were considered adequate (1.5 to 4.0 g kg<sup>-1</sup> for S and 30 to 40 g kg<sup>-1</sup> for N) at the 13 of 14 site-years sampled in 2019 and 2020. The exception was Shelby(1)19 which was considered N-deficient (29 g kg<sup>-1</sup>) at V4 stage, but was adequate when measured at R1. Another site-year (Rice19) was adequate for N and S in the whole tissue and in the earleaf at R1, but was yield responsive. Whole tissue S at early stages was not measured at other yield responsive sites. However, plant tissue S status had significant changes from V3-V7 stage to earleaf, making this parameter inaccurate to predict yield responses.

The N:S ratio in V3-V7 plant tissue was a poor predictor of grain yield response to S. For 7 site-years with N:S equal to or above the critical threshold of 18:1 at V3-V7, these ratios improved with S fertilization to below the critical threshold in the earleaf at R1 for all treatments including the zero S. Based on the N:S threshold one site was categorized adequate (Shelby(1)19) and another was considered inadequate (Rice19), but both had increased grain yield with S fertilizer.

Plants at several site-years showed symptoms of S deficiency, and after sidedress plants without S appeared shorter (although not measured). However, yield was unaffected by S fertilization at most of these sites. Sidedress N and longer root development under S-limiting

conditions, may have contributed plants to access enough S late in the season to achieve maximum yield and be unaffected from fertilizer S at sidedress.

# 2.4.5 Soil physical and chemical characteristics

Soil sulfate-S concentration was measured at 18 of 28 site-years. At 4 of 18 site-years SO<sub>4</sub>-S was  $\leq 8$  mg S kg at 0-20 cm, but only at one (Rice19) was grain yield increased with fertilizer S. At the other 14 site-years SO<sub>4</sub>-S ranged from 8 to 12 mg kg<sup>-1</sup> in the upper 20 cm soil. At deeper depths, soil SO<sub>4</sub>-S increased at 3 site-years, decreased at 6, and remained similar everywhere else. At most sites, the relationship between soil pH, OM, texture, and extractable SO<sub>4</sub>-S was poor, with these parameters explaining very little of the variation in extractable SO<sub>4</sub>-S at all three sampling depths. In a study in Argentina, soil SO<sub>4</sub>-S concentration from 0 to 20 and 0 to 60 cm depth at planting and V6 plant stage, soil organic matter (SOM), SOM:clay ratio, and SOM:clay+silt were used to predict grain yield responses to S (Carciochi et al., 2016). All models successfully predicted yield responses to S with 62 to 70 % of accuracy. However, the best predictions were with soil sulfate-S concentration from 0 to 60 cm at planting (R<sup>2</sup>=0.68) and the V6 stage (R<sup>2</sup>=0.70). The critical values where 40 and 59 kg S ha<sup>-1</sup> above which no yield respond is expected. No further improvement in the model occurred when edaphic factors were included. In our study, soil SO<sub>4</sub>-S concentration from 0 to 60 cm measured in 20cm increments, did not predict yield responses. In some cases, it helped to explain the presence of absence of response to S fertilization

Soil S availability represented by a weighted profile means (WPM), represents the relative contribution of each layer from 0 to 60 cm in 20 cm increments to soil SO<sub>4</sub>-S availability (Probert & Jones, 1977). For sorghum, a WMP of 13.8 was suggested as the critical level below which yield was lessened (Hue and Cope, 1987). This assessment may be a better estimator of SO<sub>4</sub>-S availability for plant utilization and was evaluated in our study as a means to predict grain yield responses to S fertilization. WPM ranged from 3.5 to 13.9 mg kg<sup>-1</sup>, but did not correlate with corn yield response to S fertilizer. For example, at some nonresponsive sites the WPM was lower in value than at sites that responded to S fertilization. In a S study in Coastal Plain soils in North Carolina, WPM of sulfate-S for the A- and B-horizon ranged 4.2 to 31.7 mg kg<sup>-1</sup>, and none of the sites had a grain yield response to fertilizer S (Camberato, 1982). Sulfate-S in the soil alone does not ensure plant access. Factors to consider for plant uptake are root development, soil moisture at

levels that allow mass flow of nutrients, and presence of layers obstructing root development (Graveel et al., 2002; Nibau et al., 2008). Taking into consideration WMP with soil physical properties may be necessary to better predict crop response to S.

Grain yield response to S fertilization was more frequent in coarse-textured soils (loamy sand, sandy loam, and sandy clay loam) than in fine-textured soils (clay, silty clay, silty clay loam, clay loam, silt loam, and loam). Grain yield increased with S fertilization at 5 of 8 site-years (~63%) with coarse-textured soils, while 5 of 20 site-years with fine-textured soils (25%) showed yield response to S. When grouping grain yield increases by soil type, yield increased on coarse-textured soils 0.3 to 1.4 Mg ha<sup>-1</sup> and on fine-textured soils 0.2 to 3.0 Mg ha<sup>-1</sup>. Therefore, the magnitude of response was similar across both textural divisions. Soil pH did not show a relationship with yield response to S fertilization as pH at responsive sites ranged from 5.8 to 7.0 in the 0-20 cm depth, essentially the entire range of pH seen in this study. Research in Iowa found fertilizer S increased yield in both coarse-textured (64% frequency) and loam and clay loam textured soils (62% frequency) (Sawyer et al., 2015). In Minnesota, the addition of starter S increased corn grain yield at 5 of 6 site-years, 3 sites were sandy soils, one loam and one clay loam soil (Rehm, 2005). Yield increases were similar among soil textures in agreement with my research. In Argentina at 15 sites ranging in soil texture from sandy loam to clay loam, 3 of 7 coarse-textured soils had grain yield increases with S, but no responses occurred in the other textures (Carciochi et al., 2016). In other studies in Michigan and Ohio with loam to clay loam soils, the frequency of response was 50 to 60%, nearly double that observed in my research for fine-textured soils (Chen et al., 2008; Kim et al., 2013). Although grain yield responses to S fertilization were more frequent on coarse-textured than on fine-textured soils, S deficiency occurred in both.

The lowest sidedress S rate tested (8 to 17 kg ha<sup>-1</sup>) at each site-year was enough to maximize grain yield, regardless of soil texture. Research in Iowa recently suggested biannual rates for maximum corn and soybean yield response for sandy soils was 28 kg S ha<sup>-1</sup> and for loam and clay loam soils was 19 kg S ha<sup>-1</sup> (Sawyer et al., 2015; Sawyer, 2020). They suggested S applications precede corn in a corn-soybean rotation or be applied every other year in continuous corn. We did not examine residual effects of fertilizer applications.

Phosphorus and zinc fertilization were incidental source of S at 15 site-years, including 6 responsive sites: Blackford20, Rice17-19, SEPAC(1)18, and Shelby(1)19. Time of application was

either the previous fall, preplant or in-furrow. In some cases, these sources contributed approximately 0.7 to 3 kg S ha<sup>-1</sup> (Table A-3).

Grain yield responses to S fertilization occurred at 8 of 12 site-years (67%) with soil OM between 10 and 25 g kg<sup>-1</sup>, but only at 2 of 8 site-years (25%) with OM between 25 and 49 g kg<sup>-1</sup>. A systemic review from 55 Brazilian no-till fields reported yield response to S in soils with low and high OM. In the low OM group ( $\leq 25$  g kg<sup>-1</sup>), 36% of 25 sites were responsive to fertilizer S while in the high OM group (>25 g kg<sup>-1</sup>), 23% of 30 sites responded to S fertilization (Pias et al., 2019). In S trials in corn, soybean, and alfalfa fields in Iowa, OM did not distinguish yield responsive from nonresponsive sites (Sawyer, et al., 2012; 2015). However, soils with OM <35 g  $kg^{-1}$  had greater yield increases than higher soil OM (>3.5 to 6 g kg^{-1}). A study in Argentina found a linear-plateau correlation between soil OM and grain yield response to S, where the critical value was 60 g OM kg<sup>-1</sup>, but yield predictions from the model were not highly reliable ( $R^2=0.41$ ) according to the authors (Carciochi et al., 2016). Soil OM predicted yield responses for 4 fields in Minnesota ( $R^2=0.57$ ), and grain yield increases from S fertilization occurred in soils with OM ranging from 7 to 39 g kg<sup>-1</sup> OM (Kim et al., 2013). This in agreement with Kaiser & Kim (2013), who reported soybean yield response to S fertilization in soil with OM ranging from 1 to 5 g kg<sup>-1</sup>. However, they noted that the absence of higher OM values, may put in question the use of this model across other locations with broader variability in OM (Kaiser & Kim, 2013).

Soil pH was not related to yield response and was weakly correlated to soil SO<sub>4</sub>-S concentration in the surface 20 cm, but was better related to soil SO<sub>4</sub>-S at deeper depths. Soil pH had a linear-plateau relation with SO<sub>4</sub>-S concentration at 20 to 40 and 40 to 60 cm depth at SEPAC, and at 40 to 60 cm everywhere else. The value where sulfate-S began to accumulate was pH 6.3 at 20 to 40 cm at SEPAC(1,2) and 6.2 at 40 to 60 cm at all site-years including SEPAC. Slightly to moderately acid pH below pH 6.5 increased SO<sub>4</sub>-S availability by increasing sorption on clays and with aluminum and iron oxides (Tabatabai, 1996).

# 2.4.6 Other nutrients

Sulfur fertilization on a few occasions altered nutrient concentrations in the earleaf, besides S and N. Fertilizer S increased uptake of K and Fe (3 site-years each), Cu (2 site-years), and Mg and Mn (1 site-year each). Lower concentrations of P and Ca occurred with S fertilization (2 site-

years each). The effect of S fertilization on Zn was variable with increased uptake at 1 site-year and decreased uptake at 2 site-years.

Ammonium thiosulfate, the source of S and N in this study, creates acidity in the soil media from ammonium nitrification and thiosulfate oxidation (Zhou et al., 2009). Exchangeable Mn, Zn, and Cu increased as pH decreased from 8 to 4, and at pH >5.2 these were mostly bound to iron-oxides and organic complexes (Sims, 1986). Manganese and Fe are chemically and biologically reduced to more available forms by the oxidation of thiosulfate to sulfate (Myers & Nealson, 1988). The micronutrients Mn, Fe, Zn, and Cu interact with each other affecting their uptake and metabolism. Since we only measured earleaf dry mass and nutrient concentration, it is not possible to know if total plant uptake was affected or the changes in leaf composition were a result of differential partitioning or redistribution of the nutrients.

At 1 site where earleaf P concentration declined with fertilizer S, earleaf S without S averaged 2.0 g kg<sup>-1</sup> within the range of sufficiency. However, this site was responsive to S fertilization. Research has shown that under soil S-limiting conditions, transporters of P from roots to shoots increased therefore promoting P uptake, and increasing P concentration in above-ground biomass (Allahham et al., 2020). In *Arabidopsis thaliana*, sulfolipids and glycerophospholipids both structural lipids in the plant membrane were found exchangeable (Okazaki et al., 2009; Yu et al., 2002). Sulfur-limited conditions enhanced glycerophospholipids and P-limited conditions enhanced sulfolipid accumulation. This as a plant adaptation to balance anionic charges. Nevertheless, reduction of earleaf P from fertilizer S was not observed at other site-years.

#### 2.4.7 Boron

Fertilizer B did not have any effect on yield at 11 site-years with B rates of 0.4 to 2 kg B ha<sup>-1</sup>. However, at one site-year (SEPAC(2)19) B fertilization increased earleaf N and N:S ratio. Increased N uptake by corn with B fertilization was observed by Aref (2011), this in concomitant with adequate to high Zn levels and no response at low Zn (Aref, 2011). SEPAC(2)19 had the highest soil Zn concentration (3.1 mg kg<sup>-1</sup>) of all site-years (0.9 to 2.3 mg kg<sup>-1</sup>), thus the increase in earleaf N with B fertilization may have been related to presence of high soil Zn.

# 2.4.8 Conclusions

Corn grain yield was increased 0.2 to 3.0 Mg ha<sup>-1</sup> from sidedressing S at 10 of 28 site-years. The lowest S rate at each site-year, ranging from 8 to 17 kg S ha<sup>-1</sup>, was enough S to maximize grain yield at responsive sites. Where S was needed to optimize yield, sidedress applications alone resulted in the same grain yield response as starter plus sidedress S. Starter S alone at rates of 4 to 6 kg S ha<sup>-1</sup> did not increase grain yield at 7 of 8 site-years where it was examined.

Whole plant S concentration at V3-V7 and R1 earleaf responses to S were not a good indicator of yield responses to S. Instead, sulfur concentration in the R1 earleaf and in the grain were better correlated with yield responses. At most site-years where grain yield was increased by S fertilization, R1 earleaf S without S fertilization ranged from 1.5 to  $1.7 \text{ g S kg}^{-1}$  and grain S from 0.7 to 0.8 g S kg<sup>-1</sup>. However, these 'critical values' were based on a limited number of observations, 22 and 15 site-years for earleaf and grain tissue, respectively. Furthermore, it remains to be determined whether S deficiency diagnosed at R1 can be corrected with a subsequent S application and grain S of course would only be helpful for future crops. Increases in R1 earleaf and grain S concentration with S fertilization did not differentiate sites where grain yield was increased by S fertilization from those where grain yield was unaffected.

Both soil sulfate-S and a weighted profile mean (WPM) for soil sulfate-S, which attempts to represent S availability over a greater soil depth (0-60 cm in our case), did not differentiate responsive from non-responsive locations. Sulfate-S in the 0-20 cm ranged from 8 to 12 mg kg<sup>-1</sup> at 14 of 18 site-years and a lower range from 4 to 7 mg kg<sup>-1</sup> at the remaining locations. Sulfate-S increased with depth at nearly half of the site-years and remained the same at most of the other sites. Sulfate-S from 40-60 cm increased with increased pH and plateaued at pH ranging from 6.2 to 6.3. Soil-sulfate WPM ranged narrowly from 3.5 to 8.5 mg S kg<sup>-1</sup> at most site-years indicating little difference in sulfate-S availability among site-years.

Higher yield increases occurred with soil OM  $\leq 25$  g kg<sup>-1</sup> compared to OM above that concentration. Soil OM examined ranged from 10 to 49 g S kg<sup>-1</sup> and grain yield response to S fertilization occurred in the range of 10 to 31 g kg<sup>-1</sup>. Yield increases ranged from 0.2 to 1.5 Mg ha<sup>-1</sup> in soils with 26 to 31 g kg<sup>-1</sup> OM (2 of 10 responsive site-years) and slightly higher increases ranging from 0.7 to 3.0 Mg ha<sup>-1</sup> in soils with 10 to 25 g kg<sup>-1</sup> OM (8 of 10 responsive site-years). Grain yield responses occurred more frequent in coarse- (5 of 8 site-years; loamy sand, sandy loam, and sandy clay loam) than in fine-textured soils (5 of 20 site-years; clay loam, silty clay, silty clay

loam, silt loam, and loam), however the magnitude of those increases was relatively wide across both textural groups.

We encourage farmers to consider S fertilization at rates ranging from 8 to 17 kg S ha<sup>-1</sup>. This recommendation is for fields with one or more of the following characteristics: S deficiency symptoms, earleaf at R1 S concentration and grain S concentration have been below 1.8 g kg<sup>-1</sup> and 0.9 g kg<sup>-1</sup>, respectively, or earleaf N:S greater than 14:1.

# 2.4.9 Tables

Table 2.1: Site name and year of experiment (Site-year), coordinates (latitude, longitude), total experimental area, predominant soil series (% of the total area), soil classification (suborders and great groups), and drainage class for corn response to sulfur experiments conducted from 2017 to 2020.

Site-year	Latitude - longitude	Total	Soil series (% of the	Soil	Drainage
		area	area)	classes†	class‡
DPAC178-10	40.314°N 85.142°W	(ha) 11	Blount Glynwood (56)	٨En	SPD
DIACHAI	40.314 IN - 03.142 W	11	Dount-Orynwood (50)	лср те	
DDA C18 8-20	10 21 1°N 95 1 16°W	12	Plount Clunwood (52)	1E AEn	VFD SDD
DFAC10&20	40.314 IN - 63.140 W	12	Double-Oryllwood (33)	Аср те	
NEDA C10	41 11 CONT OF 4410W	4	Pewalilo (47)		
NEPACIY	41.110 <sup>°</sup> N - 85.441 <sup>°</sup> W	4	Kawson (55)	IE A En	MWD
	41 1150NL 05 4200W	10	Haskins (29)	АЕр	MWD
NEPAC18&20	41.115°N - 85.439°W	12	Rawson $(37)$	OH	MWD
		10	Haskins (33)	AEp	MWD
Rice17&19	41.328°N - 86.800°W	18	Gilford (80)	TE	PD
Rice18&20	41.327°N - 86.796°W	19	Maumee (58)	TE	PD
			Gilford (42)	TE	PD
SEPAC(1)18&20	39.065°N - 85.525°W	21	Ryker-Muscatatuck (97)	FP	WD
SEPAC(1)19	39.0371°N - 85.534°W	6	Nabb (72)	AF	MWD
<b>SEPAC(2)19</b>	39.044°N - 85.525°W	9	Ryker-Muscatatuck (50)	FP	WD
			Nabb (34)	AF	MWD
SWPAC18	38.745°N - 87.482°W	2	Ade (53)	LA	SED
SWPAC19	38.749°N - 87.485°W	4	Conotton (96)	TH	WD
SWPAC20	38.748°N - 87.482°W	5	Petrolia (100)	FEe	PD
TPAC19	40.268°N - 86.879°W	11	Throckmorton (40)	MOH	MWD
			Toronto-Millbrook (21)	UE	SPD
TPAC20	40.280°N - 86.863°W	36	Lauramie (33)	MH	WD
			Toronto-Millbrook (30)	UE	SPD
Blackford17	40.392°N - 85.251°W	13	Saranac (77)	FEo	VPD
Blackford18	40.382°N - 85.388°W	15	Blount- Glynwood (40)	AEp	SPD
			Pewamo (32)	TE	VPD
Blackford19	40.395°N - 85.351°W	19	Blount- Glynwood (64)	AEp	SPD
Blackford20	40.391°N - 85.258°W	30	Whitaker (41)	AEn	SPD
			Saranac (30)	FEo	VPD
			Martinsville (6)	TH	WD
			Bono (21)	TE	VPD
Shelby(1)18	39.657°N - 85.684°W	21	Brookston (57)	ТА	PD
			Crosby (43)	AEp	SPD
Shelby(1)19	39.651°N - 85.686°W	16	Brookston (63)	TĂ	PD
Shelby(2)19	39.496°N - 85.940°W	6	Martinsville (100)	TH	WD
Henry20	39.827°N - 85.442°W	10	Eldean (75)	TH	WD

<sup>†</sup> Aeric Epiaqualfs (AEp), Typic Endoaquolls (TE), Oxyaquic Hapludalfs (OH), Mollic Epiaqualfs (ME), Fragiaquic Paleudults (FP), Aquic Fragiudalfs (AF), Lamellic Argiudolls (LA), Typic Hapludalfs (TH), Fluvaquentic Endoaquepts (FEe), Mollic Oxyaquic Hapludalfs (MOH), Udollic Epiaqualfs (UE), Mollic Hapludalfs (MH), Fluvaquentic Endoaquolls (FEo), Aeric Endoaqualfs (AEn), Typic Argiaquolls (TA), Fragic Glossaqualfs (FG).

‡ Somewhat excessively drained (SED), well drained (WD), moderately well drained (MWD), somewhat poorly drained (SPD), poorly drained (PD), and very poorly drained (VPD).
Table 2.2: Average monthly air temperature and accumulated precipitation from April 1<sup>st</sup> to October 31<sup>st</sup> for sites from 2017 to 2020. Values in parentheses represent the deviation from the 30-year average based on the monthly National Center for Environmental Information (NCEI) normal of 1981 through 2010.

	Site-year	A	pril	Ν	lay	J	une	Jı	ıly	Au	ıgust	Septe	mber	Oct	ober
		°C	mm	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm
	DPAC17	13.1(+3.2)	9.8(+0.5)	15.4(-0.4)	20.0(+8.8)	) 20.3(-0.8)	15.8(+5.0)	22.1(-0.8)	13.0(+0.9)	) 20.4(-1.3)	4.1(-5.0)	19.3(+1.4)	4.2(-3.4)	14.1(+2.7)	10.1(+2.6)
	DPAC18	5.5(-4.4)	9.4(+0.2)	19.2(+3.4)	) 4.8(-6.4)	22.3(-1.2)	17.5(+6.8)	21.8(-1.0)	10.7(-1.4)	22.9(+1.2)	15.7(+6.7)	20.6(+2.7)	14.2(+6.6)	12.4(+1.0)	4.6(-2.9)
	DPAC19	10.2(+0.3)	18.6(+9.3)	16.6(+0.7)	) 13.1(+1.9)	) 21.0(-0.1)	14.0(+3.2)	24.6(+1.8)	12.7(+0.6)	) 21.9(+0.2)	5.7(-3.3)	21.0(+3.1)	4.3(-3.2)	13.1(+1.7)	8.6(+1.1)
	DPAC20	8.8(-1.2)	4.4(-4.9)	14.3(-1.6)	12.5(+1.3)	) 21.1(+0.0)	6.0(-4.8)	24.3(+1.4)	15.9(+3.8)	) 21.7(+0.0)	4.3(-4.7)	17.9(+0.0)	5.9(-1.7)	10.9(-0.4)	15.3(+7.8)
	NEPAC18	5.1(-4.1)	4.8(-4.5)	18.2(+3.2)	7.9(-3.0)	21.1(+0.8)	11.8(+0.4)	22.3(+0.1)	13.5(+3.2)	) 21.9(+0.6)	23.3(+13.5)	19.7(+2.4)	3.7(-4.4)	11.1(+0.3)	7.7(+0.1)
	NEPAC19	9.0(-0.2)	12.1(+2.8)	14.9(-0.1)	11.5(+0.5)	) 20.1(-0.3)	9.8(-1.6)	24.1(+1.8)	10.8(+0.5)	) 21.4(+0.1)	15.7(+6.0)	19.8(+2.5)	9.0(+0.9)	12.1(+1.3)	7.8(+0.2)
	NEPAC20	6.5(-2.7)	5.5(-3.8)	13.9(-1.1)	12.7(+1.8)	) 20.6(+0.2)	7.3(-4.0)	23.6(+1.4)	4.9(-5.5)	22.0(+0.7)	10.7(+0.9)	16.8(-0.5)	2.7(-5.3)	9.7(-1.1)	8.6(+1.0)
	Rice17	12.3(+1.6)	9.9(+0.7)	13.9(-2.2)	14.0(+3.9)	) 21.4(+0.4)	6.8(-3.6)	22.0(-0.5)	19.9(+8.5)	) 19.8(-2.1)	5.6(-5.6)	18.3(+0.3)	3.7(-4.9)	13.8(+1.7)	15.3(+6.1)
	Rice18	5.2(-5.6)	6.0(-3.2)	19.2(+3.1)	) 11.5(+1.3)	) 21.6(+0.6)	10.9(+0.5)	22.3(-0.2)	3.8(-7.6)	22.5(+0.6)	14.5(+3.3)	19.5(+1.4)	5.4(-2.2)	11.4(-0.7)	14.7(+5.6)
	Rice19	9.5(-1.2)	11.1(+1.9)	15.0(-1.1)	16.8(+6.7)	) 19.9(-1.2)	12.1(+1.7)	23.6(+1.1)	7.4(-4.0)	21.0(-0.9)	5.4(-5.8)	19.7(+1.6)	15.8(+7.3)	11.3(-0.8)	9.3(+0.2)
	Rice20	7.4(-3.3)	12.0(+2.8)	14.6(-1.6)	11.8(+1.7)	) 21.4(+0.4)	7.3(-3.1)	23.7(+1.2)	7.9(-3.5)	21.6(-0.3)	6.2(-4.9)	17.2(-0.9)	4.6(-4.0)	10.2(-1.8)	6.2(-2.9)
72	SEPAC(1)18	10.1(-2.7)	11.0(-0.3)	22.1(+4.5)	) 5.4(-7.4)	23.2(+0.9)	15.0(+4.1)	23.9(-0.2)	14.7(+3.3)	) 23.6(+0.2)	9.2(-1.7)	21.8(+2.2)	17.6(+9.7)	13.9(+0.5)	3.4(-5.9)
	SEPAC(1&2)19	0 13.8(+1.0)	16.9(+5.5)	19.2(+1.6)	) 15.4(+2.6)	) 21.3(-1.1)	22.9(+12.0)	24.8 (+0.7)	4.5(-7.0)	23.4(-0.1)	11.1(+0.2)	22.9(+3.4)	0.3(-7.6)	15.3(+1.8)	13.3(+4.0)
	SEPAC(1)20	11.4(-1.4)	8.3(-3.1)	16.6(-1.1)	9.2(-3.5)	22.5(+0.2)	6.4(-4.6)	25.2(+1.1)	8.9(-2.6)	22.7(-0.7)	10.3(-0.6)	19.3(-0.2)	2.6(-5.4)	13.3(-0.2)	14.7(+5.4)
	SWPAC18	9.1(-3.6)	9.3(-1.7)	23.7(+5.5)	) 5.5(-9.6)	25.4(+2.3)	18.1(+7.5)	25.3(+0.4)	5.2(-6.6)	24.7(+0.6)	7.8(-0.9)	23.3(+3.2)	22.5(+14.0)	14.5(+0.8)	3.8(-5.7)
	SWPAC19	13.5(+0.9)	18.8(+7.8)	19.5(+1.3)	) 20.1(+5.0)	) 22.8(-0.3)	11.5(+0.8)	26.1(+1.2)	17.6(+5.8)	) 24.2(+0.0)	12.8(+4.1)	23.7(+3.6)	1.7(-6.9)	14.7(+1.1)	13.9(+4.3)
	SWPAC20	11.1(-1.6)	8.5(-2.5)	16.8(-1.4)	12.1(-2.9)	24.1(+0.9)	12.8(+2.1)	26.1(+1.2)	16.5(+4.7)	) 23.7(-0.5)	7.0(-1.7)	20.2(+0.0)	0.6(-8.0)	13.7(+0.0)	14.0(+4.4)
	TPAC19	10.4(-0.3)	15.1(+6.5)	16.7(+0.1)	) 12.9(+1.1)	) 21.2(-0.6)	9.7(-1.8)	24.7(+1.3)	7.4(-3.0)	21.9(-0.5)	8.4(-1.6)	21.1(+2.3)	6.2(-0.9)	12.9(+0.8)	10.2(+3.4)
	TPAC20	8.8(-1.9)	8.3(-0.4)	15.4(-1.2)	8.3(-3.5)	22.7(+0.8)	9.9(-1.7)	24.3(+1.0)	11.3(+1.0)	) 21.8(-0.6)	7.8(-2.2)	18.4(-0.4)	4.9(-2.2)	11.5(-0.7)	6.8(-0.1)
	Blackford17	13.2(+2.8)	15.4(+6.7)	15.5(-0.7)	17.5(+6.8)	) 21.7(+0.2)	12.0(+2.0)	22.9(-0.2)	12.2(+1.0)	) 20.4(-1.6)	7.2(-3.0)	19.7(+1.4)	3.9(-4.0)	13.9(+2.1)	8.8(+1.8)
	Blackford18	6.0(-4.4)	8.3(-0.6)	20.4(+4.2)	) 4.8(-5.9)	22.8(+1.3)	16.3(+6.2)	22.9(-0.3)	10.8(-0.4)	22.9(+0.8)	19.0(+8.8)	19.7(+1.4)	23.2(+15.4)	12.6(+0.7)	9.2(+2.2)
	Blackford19	10.0(-0.4)	17.3(+8.6)	16.0(-0.2)	16.4(+5.7)	) 21.0(-0.5)	8.5(-1.5)	24.8(+1.7)	15.6(+4.4)	) 21.7(-0.3)	6.7(-3.5)	20.9(+2.6)	3.4(-4.5)	13.4(+1.6)	8.2(+1.1)
	Blackford20	8.8(-1.6)	4.6(-4.1)	15.1(-1.1)	7.6(-3.1)	22.3(+0.8)	5.8(-4.2)	24.3(+1.2)	10.5(-0.7)	22.0(-0.1)	4.3(-5.9)	18.4(+0.1)	7.8(+0.0)	10.9(-1.0)	15.0(+8.0)
	Shelby(1)18	7.5(-3.3)	13.3(+1.8)	21.0(+4.4)	) 4.8(-8.3)	22.9(+1.3)	14.2(+2.6)	23.2(+0.1)	11.7(+0.2)	) 23.1(+0.8)	17.8(+8.7)	20.9(+2.6)	15.2(+6.9)	12.8(+0.9)	2.7(-5.6)
	Shelby(1)19	10.9(+0.1)	12.2(+0.6)	17.2(+0.7)	) 10.4(-2.7)	20.9(-0.7)	15.5(+3.9)	24.6(+1.4)	13.9(+2.3)	) 22.8(+0.5)	8.0(-1.1)	21.9(+3.6)	2.1(-6.3)	14.0(+2.1)	11.9(+3.6)
	Shelby(2)19	10.7(+0.1)	15.9(+4.8)	17.0(+0.6)	) 13.4(-0.9)	19.8(-1.9)	13.8(+2.0)	23.9(+0.6)	6.1(-5.0)	21.3(-1.3)	12.6(+3.6)	20.6(+2.0)	3.0(-4.9)	13.6(+1.6)	13.8(+5.5)
	Henry20	8.6(+8.6)	4.4(+4.4)	14.3(+4.4)	) 14.8(+4.6)	) 20.7(+4.9)	7.8(-5.1)	23.4(+2.6)	11.4(-0.5)	20.8(-1.7)	10.1(-2.6)	17.3(-4.3)	1.4(-6.8)	10.7(-0.9)	13.7(+5.7)

	2017			2018			2019			2020			
Site	N1	$N_2$	<b>S</b> 1	<b>S</b> <sub>2</sub>	Nt	$S_1$	<b>S</b> <sub>2</sub>	Nt	$S_2$	<b>B</b> <sub>2</sub>	Nt	$S_2$	B <sub>2</sub>
							kg h	a <sup>-1</sup>					
DPAC		241	0	0	286	0	0	280	0		280	0	
		241	0	17	286	0	17	280	7		280	6	
	45	196	0	0	286	0	34	280	13		280	11	
	45	196	0	17	241	6	0	280	20		280	17	
	45	196	6	0	241	6	11	280	27		280	22	
	45	196	6	11	241	6	28	280	34		280	22	1
NEPAC					250	0	0	295	0		286	0	
					250	0	17	295	7		286	6	
					250	6	11	295	13		286	11	
					250	6	22	295	20		286	17	
								295	27		286	22	
								295	34		286	22	1
Rice		213	0	0	252	0	0	207	0	0.4	235	0	
		213	0	17	252	0	17	207	8	0.4	235	6	
	27	186	0	0	252	0	28	207	17	0.4	235	11	
	27	186	0	17	252	6	0	207	25	0.4	235	17	
	27	186	3	0	252	6	11	207	25	0	235	22	
	27	186	3	13	252	6	22	207	34	0.4	235	22	1
SEPAC(1)					207	0	0	234	0		234	0	
					207	0	17	234	7		234	6	
					207	0	28	234	13		234	11	
					207	6	0	234	20		234	17	
					207	6	11	234	27		234	22	
					207	6	22	234	34		234	22	1
SEPAC(2)								234	0	0			
								234	13	0			
								234	0	1.1			
								234	13	1.1			
								234	0	2.2			
								234	13	2.2			
SWPAC					202	0	0	202	0	2.2	234	0	
					202	0	34	202	8	2.2	234	11	
					197	6	0	202	17	2.2	234	22	
					197	6	28	202	25	2.2	234	22	1
								202	34	2.2			
								202	25	0			

Table 2.3: Nitrogen (N), sulfur (S) and boron (B) applied as starter (1) and/or sidedress (2) in corn sulfur response trials. Starter N (N1) applied only in 2017, starter S (S1) in 2017 and 2018, and sidedress B (B2) in 2019 to 2020. From 2018 to 2020, the total N rate (Nt) was split between starter and sidedress for all treatments.

<sup>†</sup> applied at V3 corn stage

‡ applied at V12 corn stage

		201	17			2018			2019			2020	
Site	N <sub>1</sub>	$N_2$	<b>S</b> 1	<b>S</b> <sub>2</sub>	Nt	$S_1$	<b>S</b> <sub>2</sub>	Nt	<b>S</b> 1	<b>B</b> <sub>2</sub>	Nt	<b>S</b> 1	<b>B</b> <sub>2</sub>
							- kg h	a <sup>-1</sup>					
TPAC								228	3 0	0.4	235	0	
								228	8 8	0.4	235	6	
								228	8 17	0.4	235	11	
								228	3 25	0.4	235	17	
								228	3 34	0.4	235	22	
								228	8 25	0	235	22	1
Blackford		241	0	0	268	0	0	233	<b>3</b> 0	0	228	0	
		241	0	17	261	6	0	233	8 11	0	228	17	
	37	204	0	0	268	0	17	233	8 17	0			
	37	204	0	17	261	6	11	233	8 22	0			
	37	204	4	0	261	6	28	233	3 22	0.6			
	37	204	4	12									
Shelby(1)					226	$0^{\dagger}$	$0^{\ddagger}$	210	) 0	0			
					226	0	0	210	) 11	0			
					226	22	0	210	) 17	0			
					226	11	11	210	) 22	0			
					226	34	0	210	) 22	0.4			
					226	17	17						
Shelby(2)								202	2 0				
								202	2 11				
								202	2 22				
Henry											186	0	
											188	17	

Table 2.3, *continued*:

<sup>†</sup> applied at V3 corn stage ‡ applied at V12 corn stage

Table 2.4: Corn yield response to sulfur (S) and nitrogen (N) applied at planting and/or at sidedress at sites in 2017. Means with different letters within a site indicate differences between treatments at  $p \le 0.10$  using Tukey LSD test. Mean, coefficient of variance (C.V.), p-value for the effect of S rate (Pr>F), and least significant difference (LSD 0.10) are included.

Site	Treatment†	Grain yield	Grain moisture
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>
DPAC	ON OS	12.6	170 a
	0N -17S	12.6	168 b
	45N	12.2	164 d
	45N -17S	12.8	166 c
	45N 6S	12.3	164 cd
	45N 6S -11S	12.6	166 c
	Mean	12.5	166
	C.V.	4.2	1.1
	Pr(>F)	0.35	<0.001
	LSD 0.10	ns	1.8
		Pr>F for single D.	F. contrasts of least-squares means
	ON OS vs all rates	< 0.001	<0.001
	0N 0S vs 45N	< 0.001	<0.001
	0N 0S vs 17S	< 0.001	< 0.001
	45N vs 45N 6S	0.47	0.07
	Split S vs 17S	0.59	0.67
	45N+45N6S vs Split S+17S	0.51	0.90

† Treatments: starter - sidedress rates (kg ha-1)

‡ moisture sensor failed

Site	Treatment†	Grain yield	Grain moisture
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>
Rice	ON OS	13.6 b	÷ ÷
	0N - 17S	14.6 a	
	27N - 0S	13.8 b	
	27N - 17S	14.6 a	
	27N 4S - 0S	13.7 b	
	27N 4S - 13S	14.7 a	
	Mean	14.2	
	C.V.	2.2	
	Pr(>F)	< 0.001	
	LSD 0.10	0.3	
		Pr>F for single D.F. co	ntrasts of least-squares means
	ON OS vs all rates	< 0.001	
	0N 0S vs 45N	0.22	
	0N 0S vs 15S	< 0.001	
	28N vs 28N 3S	0.85	
	Split S vs 15S	0.82	
	28N+28N 3S vs Split S+17S	< 0.001	

Table 2.4, *continued*:

† Treatments: starter - sidedress rates (kg ha<sup>-1</sup>) ‡ moisture sensor failed

Site	<b>Treatment</b> <sup>†</sup>	Grain yield	Grain moisture
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>
Blackford	0N 0S	13.2	194 a
	0N - 17S	13.3	191 ab
	37N - 0S	12.5	190 ab
	37N - 17S	12.7	183 bc
	37N 5S - 0S	13.1	183 bc
	37N 5S - 12S	13.7	176 c
	Mean	13.1	186
	C.V.	4.7	4.0
	Pr(>F)	0.13	0.03
	LSD 0.10	ns	9.2
		Pr>F for single D.	F. contrasts of least-squares means
	ON OS vs all rates	0.70	0.03
	0N 0S vs 37N	0.14	0.46
	0N 0S vs 17S	0.80	0.58
	37N vs 37N 5S	0.19	0.17
	Split S vs 17S	0.03	0.19
	37N+37N 5S vs Split S+17S	0.03	0.04

Table 2.4	continued
1 able 2.4,	commuea:

† Treatments: starter - sidedress rates (kg ha<sup>-1</sup>) ‡ moisture sensor failed

Table 2.5: Grain yield and harvest grain moisture, and earleaf nitrogen to sulfur ratio (N:S), nitrogen (N) and sulfur
(S) concentration at growth stage R1 for S treatments applied at planting and/or at sidedress in 2018. Means with
different letters within a site indicate differences between treatments at $p \le 0.10$ using a Tukey LSD test. Mean,
coefficient of variation (C.V.), p-value for the effect of S rate (Pr>F), least significant difference (LSD 0.10), and
single-degree-of-freedom contrasts are included.

		Grain	Grain		Earle	eaf				
Site	Treatment†	yield	moisture	N:S	Ν	S				
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>		g k	g <sup>-1</sup>				
DPAC	OS	13.7 ab	142	15.6	29.0	1.9 a				
	0S - 17S	13.6 ab	143	16.0	27.8	1.7 bc				
	0S - 34S	13.9 a	143	16.0	30.0	1.9 a				
	6S - 0S	13.4 b	143	16.1	27.3	1.7 c				
	6S - 11S	13.4 b	142	15.7	28.9	1.8 ab				
	6S - 28S	13.6 ab	142	15.2	28.9	1.9 a				
	Mean	13.6	143	15.8	28.6	1.8				
	C.V.	2.0	0.4	4.5	3.8	4.1				
	Pr(>F)	0.09	0.24	0.61	0.13	0.04				
	LSD 0.10	0.3	ns	ns	ns	0.1				
	Pr>F for single D.F. contrasts of least-squares means									
	OS vs all	0.48	0.13	0.58	0.51	0.24				
	OS vs all sidedress S	0.62	0.03	0.37	0.85	0.24				
	OS vs all split S	0.15	0.41	0.82	0.37	0.29				
	Split S vs sidedress S	0.05	0.04	0.19	0.98	0.16				
NEPAC	0S	15.2	180	15.9	23.9	1.5 b				
	0S - 17S	15.2	179	14.6	24.4	1.7 a				
	6S - 11S	15.5	180	15.1	26.6	1.8 a				
	6S - 22S	15.4	178	14.2	25.1	1.8 a				
	Mean	15.3	179	14.9	25.0	1.7				
	C.V.	1.7	0.8	5.5	8.3	5.7				
	Pr(>F)	0.11	0.22	0.20	0.47	0.04				
	LSD 0.10	ns	ns	ns	ns	0.2				
		Pr>	F for single D.F. cor	trasts of leas	t-squares m	neans				
	OS vs all	0.08	0.37	0.45	0.57	0.87				
	0S vs sidedress S	0.59	0.24	0.11	0.79	0.08				
	OS vs all split S	0.11	0.42	0.09	0.24	0.008				
	Split S vs sidedress S	0.39	0.08	0.26	0.40	1.00				

-		Grain	Grain		Earle	eaf		
Site	<b>Treatment</b> <sup>†</sup>	yield	moisture	N:S	N	S		
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>		g k	g <sup>-1</sup>		
Rice	OS	11.4 b	161	16.8 a	25.2	1.5		
	0S - 17S	12.8 a	159	16.1 ab	27.2	1.7		
	0S - 28S	12.8 a	160	14.3 c	25.8	1.8		
	6S - 0S	11.6 b	156	16.9 a	26.3	1.6		
	6S - 11S	12.6 a	161	15.1 bc	25.6	1.7		
	6S - 22S	12.7 a	162	14.6 c	25.9	1.8		
	Mean	12.3	160	15.6	26.0	1.7		
	C.V.	3.0	2.9	5.4	8.1	8.7		
	Pr(>F)	< 0.001	0.39	0.01	0.89	0.18		
	LSD 0.10	0.4	ns	1.3	ns	ns		
		Pr>	r>F for single D.F. contrasts of least-squares means					
	OS vs all	< 0.001	0.37	0.02	0.49	0.05		
	OS vs all sidedress S	< 0.001	0.37	0.02	0.40	0.04		
	OS vs all split S	< 0.001	0.44	0.04	0.63	0.10		
	Split S vs sidedress S	0.27	0.30	0.47	0.53	0.85		
SEPAC(1)	OS	15.2 b	193	15.1	31.8	2.1		
	0S - 17S	15.5 a	194	15.3	30.2	2.0		
	0S - 28S	15.4 ab	194	15.0	29.0	1.9		
	6S - 0S	15.5 a	193	16.1	31.6	2.0		
	6S - 11S	15.6 a	193	15.8	28.0	1.8		
	6S - 22S	15.5 a	193	14.4	28.8	2.0		
	Mean	15.4	193	15.3	29.9	2.0		
	C.V.	1.2	0.7	5.7	8.6	7.9		
	Pr(>F)	0.09	0.16	0.29	0.41	0.28		
	LSD 0.10	0.2	ns	ns	ns	ns		
		Pr>!	F for single D.F.	contrasts of	least-squar	res means		
	OS vs all	0.009	0.95	0.73	0.19	0.11		
	0S vs all sidedress S	0.05	0.22	0.96	0.25	0.20		
	OS vs 6S + split S	0.005	0.33	0.62	0.20	0.10		
	Split S vs sidedress S	0.28	0.01	0.92	0.44	0.47		

Table 2.5, *continued*:

		Grain	Grain		Earleaf	
Site	<b>Treatment</b> <sup>†</sup>	yield	moisture	N:S	Ν	S
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>		g k	g <sup>-1</sup>
SWPAC	08	9.7 b	147 a	16.1	26.2	1.6
	0S - 34S	10.7 a	148 a	15.5	25.9	1.7
	6S - 0S	9.7 b	147 a	14.9	27.3	1.8
	6S - 28S	10.7 a	144 b	15.0	29.0	1.9
-	Mean	10.2	146	15.4	27.1	1.8
	C.V.	2.8	1.1	6.5	12.0	11.0
	Pr(>F)	0.006	0.08	0.54	0.67	0.29
	LSD 0.10	0.4	0.3	ns	ns	ns
		Pr>F	for single D.F. con	trasts of leas	t-squares me	ans
	OS vs all	0.02	0.37	0.50	0.29	0.14
	0S vs 6S	0.91	1.00	0.92	0.55	0.55
	Split S vs sidedress S	0.94	0.03	0.55	0.91	0.84
Blackford	0S	11.8	168 ab			
	6S - 0S	11.8	165 d			
	0S - 17S	11.4	167 bc			
	6S - 11S	11.6	166 cd			
	6S - 28S	11.7	166 cd			
-	Mean	11.7	16.7			
	C.V.	5.0	0.7			
	Pr(>F)	0.92	0.01			
	LSD 0.10	ns	0.1			
		Pr>F	for single D.F. con	trasts of leas	t-squares me	ans
	OS vs all	0.62	0.01			
	0S vs 6S	0.98	0.005			
	Split S vs sidedress S	0.63	0.48			

Table 2.5, *continued*:

		Grain	Grain		Earlea	af
Site	Treatment†	yield	moisture	N:S	Ν	S
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>		
Shelby(1)	V <sub>3</sub> N‡	12.7 b	199 a	14.9	30.2	2.0 a
whole field	Split N	12.3 b	200 a	17.3	28.4	1.7 b
	V <sub>3</sub> N & 22S	13.7 a	193 bc	15.1	32.7	2.2 a
	Split N & 22S	13.5 a	194 b	15.0	32.9	2.2 a
	V <sub>3</sub> N & 34S	13.7 a	191 c	14.2	31.7	2.2 a
	Split N & 34S	13.7 a	191 c	14.3	30.8	2.2 a
	Mean	13.3	19.5	15.1	31.1	2.1
	C.V.	3.0	1.2	8.4	9.6	9.4
	Pr(>F)	< 0.001	< 0.001	0.13	0.48	0.04
	LSD 0.10	0.4	0.3	ns	ns	0.3
		Pr>F	for single D.F. co	ontrasts of l	east-square	s means
	V <sub>3</sub> N vs all	0.002	< 0.001	0.77	0.57	0.68
	V <sub>3</sub> N vs Split N	< 0.001	< 0.001	0.05	0.48	0.04
	V <sub>3</sub> N vs V <sub>3</sub> N&S	< 0.001	< 0.001	0.76	0.36	0.26
	V <sub>3</sub> N vs Split N&S	0.67	0.84	0.76	0.45	0.30
	V <sub>3</sub> N&S vs Split N&S	0.55	0.04	0.32	0.39	0.89
Shelby(1)	V3 N ‡	11.7 b	206 a			
Responsive area	Split 174N	10.9 c	207 a			
	V <sub>3</sub> N & 22S	13.7 a	197 bc			
	Split1 74N & 22S	14.0 a	200 b			
	V <sub>3</sub> N & 34S	13.8 a	196 c			
	Split 174N & 34S	13.3 a	198 bc			
	Mean	13.0	20.1			
	C.V.	3.9	1.5			
	Pr(>F)	< 0.001	< 0.001			
	LSD 0.10	0.6	0.3			
		Pr>F	for single D.F. co	ontrasts of l	east-square	s means
	V <sub>3</sub> N vs all	< 0.001	< 0.001			
	V <sub>3</sub> N vs Split N	0.02	0.60			
	V <sub>3</sub> N vs V <sub>3</sub> N&S	< 0.001	< 0.001			
	V <sub>3</sub> N vs Split N&S	< 0.001	< 0.001			
	V <sub>3</sub> N&S vs Split N&S	0.75	0.18			

Table 2.5, *continued*:

Table 2.6: Grain yield and harvest grain moisture, and nitrogen to sulfur ratio (N:S), nitrogen (N) and sulfur (S) concentration in both the earleaf at growth stage R1 and grain for sidedress-applied fertilizer S and boron (B) treatments in 2019. Means with different letters within a site indicate differences between treatments at  $p \le 0.10$  using Tukey LSD test. Mean, coefficient of variance (C.V.), p-value for the effect of S rate (Pr>F), and least significant difference (LSD 0.10) are included.

		Grain	Grain	]	Earleaf			Grain	
Site	<b>Treatment</b> <sup>†</sup>	yield	moisture	N:S	Ν	S	N:S	Ν	S
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	ratio	g k	g <sup>-1</sup>	ratio	g kg <sup>-1</sup>	
DPAC	0S	11.1	191	15.0	29.6	2.0 c	13.0	11.4 bc	0.9 b
	6S	11.0	193	15.1	31.0	2.1 bc	13.0	11.4 bc	0.9 b
	13S	11.0	190	14.5	30.2	2.1 bc	12.3	11.3 c	0.9 ab
	20S	11.2	189	14.4	30.0	2.1 bc	11.7	11.4 bc	1.0 a
	27S	11.1	189	14.1	31.2	2.2 bc	12.6	11.7 b	0.9 ab
	34S	11.0	190	14.2	30.6	2.2 ab	12.3	12.1 a	1.0 a
	Mean	11.1	191	14.5	30.4	2.1	12.5	11.6	0.9
	C.V.	3.9	1.4	4.4	3.4	5.2	6.7	2.7	6.1
	Pr(>F)	0.93	0.32	0.11	0.17	0.04	0.13	0.008	0.03
	LSD 0.10	ns	ns	ns	ns	0.12	ns	0.34	0.06
			Pr>F f	for single D	.F. cont	rasts of leas	t-squares me	ans	
	0S vs all S rates	0.99	0.54	0.11	0.07	0.02	0.12	0.20	0.04
	6S vs >S rates	0.67	0.03	0.02	0.35	0.18	0.06	0.27	0.02
NEPAC	0S	13.0	234	15.5 a	30.4	2.0 c	13.7 a	12.5	1.0
	6S	13.2	231	15.3 ab	31.1	2.0 bc	13.2 ab	12.9	1.0
	13S	13.2	230	15.0 b	32.1	2.1 ab	12.5 bc	12.3	1.0
	20S	13.1	230	14.9 bc	31.6	2.1 ab	12.1 bc	12.4	1.0
	27S	13.3	231	14.5 cd	31.5	2.2 a	11.9 c	12.5	1.1
	34S	13.2	231	14.4 d	31.7	2.2 a	11.4 c	12.1	1.1
	Mean	13.2	231	15.0	31.4	2.1	12.5	12.4	1.0
	C.V.	2.2	1.3	2.9	3.7	5.1	10.2	4.2	8.7
	Pr(>F)	0.64	0.30	0.002	0.24	0.009	0.04	0.19	0.17
	LSD 0.10	ns	ns	0.43	ns	0.11	12.5	ns	ns
			Pr>F f	for single D	.F. cont	rasts of leas	t-squares me	ans	
	OS vs all S rates	0.18	0.04	0.002	0.03	0.002	0.13	0.93	0.19
	6S vs >S rates	0.64	0.88	0.009	0.24	0.02	0.005	0.02	0.04

<sup>†</sup> Sidedress S and B rates (kg ha<sup>-1</sup>).

		Grain	Grain		Earleaf			Grain	
Site	<b>Treatment</b> <sup>†</sup>	yield	moisture	N:S	Ν	S	N:S	Ν	S
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	ratio	g kg <sup>.</sup>	1	ratio	g kg	-1
Rice	0S 0.4B	11.2b	192 bc	17.0 a	27.2	1.6 c	19.2 a	12.7	0.7 c
	8S 0.4B	12.1a	190 d	16.2 a	28.8	1.8 b	15.6 b	13.1	0.8 b
	17S 0.4B	12.2a	191 c	15.1 b	28.8	1.9 ab	14.3 c	12.9	0.9 b
	25S 0.4B	12.1a	193 a	15.1 b	27.1	1.8 b	12.3 d	12.8	1.0 a
	34S 0.4B	12.2a	192 bc	14.9 b	29.6	2.0 a	12.5 d	13.5	1.1 a
	25S	12.1a	193 ab	14.8 b	27.8	1.9 ab	12.3 d	13.3	1.1 a
	Mean	12.0	192	15.5	28.2	1.8	14.0	13.1	0.9
	C.V.	1.2	1.1	5.0	6.0	7.3	7.7	3.9	8.6
	Pr(>F)	0.007	< 0.001	0.001	0.17	0.004	< 0.001	0.13	< 0.001
	LSD 0.10	0.5	1.2	0.84	ns	0.15	12.1	ns	0.09
			Pr>H	<sup>7</sup> for single I	D.F. cont	rasts of least-	-squares mea	ans	
	0S vs all S rates	< 0.001	0.41	< 0.001	0.08	< 0.001	< 0.001	0.06	< 0.001
	8S vs >S rates	0.68	0.06	0.005	0.95	0.05	< 0.001	0.71	< 0.001
	0B vs 0.4B	0.71	0.45	0.56	0.53	0.35	0.97	0.13	0.44
SEPAC(1)	05	10.4	218	14.8 a	30.6	2.1	13.8 a	12.8	0.9 c
	6S	10.6	213	14.8 a	31.1	2.1	11.8 c	13.4	1.1 a
	13S	10.7	220	14.6 a	31.2	2.1	12.0 bc	: 13.2	1.1 ab
	20S	10.5	207	14.4 b	30.7	2.1	12.7 b	13.6	1.1 b
	27S	10.7	212	14.3 b	30.5	2.1	12.1 bc	: 13.3	1.1 ab
	34S	10.4	212	14.0 c	30.8	2.2	12.3 bc	: 13.6	1.1 ab
	Mear	n 10.6	214	14.5	30.8	2.1	12.5	13.3	1.1
	C.V	. 2.8	3.1	1.0	3.5	3.4	5.0	3.2	4.1
	Pr(>F)	) 0.70	0.28	< 0.001	0.97	0.41	0.03	0.35	0.003
	LSD 0.10	) ns	ns	0.22	ns	ns	9.29	ns	0.06
			Pr>F	F for single I	D.F. cont	rasts of least-	squares mea	ans	
	0S vs all S rates	0.42	0.21	0.002	0.76	0.14	0.003	0.06	< 0.001
	7S vs >S rates	0.82	0.94	< 0.001	0.66	0.31	0.27	0.84	0.17

Table 2.6, continued

		Grain	Grain		Earleaf			Grain	
Site	<b>Treatment</b> <sup>†</sup>	yield	moisture	N:S	Ν	S	N:S	N	S
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	ratio	g kg <sup>-1</sup>		ratio	g kg	-1
SEPAC(2)	0S	9.9	182	14.3 c	28.4 d	2.0	12.2	12.0	1.0
	13S	9.9	185	15.2 b	29.1 co	d 1.9	12.2	12.4	1.0
	1B	9.7	181	15.8 at	o 31.8 a	2.0	12.0	11.9	1.0
	13S 1B	10.1	181	15.6 at	o 31.0 al	b 2.0	11.7	11.9	1.0
	2B	9.4	184	15.9 a	30.2 b	c 1.9	12.2	11.9	1.0
	13S 2B	10.0	181	15.4 at	o 30.4 al	b 2.0	11.4	11.9	1.0
	Mea	n 9.8	182	15.4	30.2	2.0	11.9	12.0	1.0
	C.V	7. 4.8	2.4	4.3	4.7	6.5	5.6	3.2	4.6
	Pr(>F	<sup>c</sup> ) 0.46	0.63	0.02	0.01	0.65	0.34	0.29	0.27
	LSD 0.1	0 ns	ns	0.7	1.5	ns	ns	ns	ns
			Pr>F	for single l	D.F. contra	asts of least-	squares me	ans	
	0B 0S vs all	0.81	0.87	0.001	0.006	0.80	0.27	0.97	0.17
	0B 0S vs 12S (0B	) 0.70	0.86	0.007	0.02	0.84	0.17	0.76	0.06
	0B 0S vs B (0S)	0.28	0.90	0.001	0.003	0.78	0.63	0.74	0.69
SWPAC	0S 2B	10.4	160	17.3 ab	31.1	1.8 b	14.0 a	11.2	0.8 b
	8S 2B	10.8	161	17.4 a	31.3	1.8 b	12.0 b	11.6	1.0 a
	17S 2B	11.0	160	16.1 c	32.6	2.0 a	10.8 c	11.1	1.0 a
	25S 2B	10.7	161	16.4 c	32.2	2.0 ab	10.7 c	11.1	1.0 a
	34S 2B	10.7	160	16.0 c	32.9	2.1 a	10.6 c	11.0	1.0 a
	258	10.8	155	16.4b c	32.8	2.0 a	10.9 c	11.2	1.0 a
	Mean	10.7	160	16.6	32.2	1.9	11.5	11.2	1.0
	C.V.	3.2	1.6	3.7	5.3	6.1	5.4	5.0	4.9
	Pr(>F)	0.86	0.13	0.06	0.68	0.07	< 0.001	0.84	< 0.001
	LSD 0.10	ns	ns	0.90	ns	0.17	9.11	ns	0.07
			Pr>F	for single D	.F. contras	sts of least-so	quares mea	ns	
	0S vs all S rates	0.16	0.96	0.05	0.32	0.05	< 0.001	0.89	< 0.001
	8S vs >S rates	0.86	0.75	0.01	0.26	0.02	0.01	0.20	0.07
	0B vs 0.4B	0.80	0.02	0.96	0.74	0.74	0.91	0.78	1.00

Table 2.6, continued

		Grain	Grain		Earleaf			Grain	
Site	<b>Treatment</b> <sup>†</sup>	yield	moisture	N:S	Ν	S	N:S	Ν	S
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	ratio	g kg-1		ratio	g kg	-1
TPAC	0S 2B	14.1	211	15.8 a	29.8 bc	1.9 c	12.0	12.0	1.0
	8S 2B	13.9	210	14.8 b	30.4 ab	2.1 a	11.5	11.9	1.0
	17S 2B	14.3	210	15.0 b	29.3 c	2.0 bc	12.4	12.0	1.0
	25S 2B	13.9	211	15.2 b	30.8 a	2.0 ab	12.2	12.2	1.0
	34S 2B	14.0	211	15.0 b	31.0 a	2.1 a	12.6	13.0	1.0
	258	14.0	211	14.7 b	30.1 ab	2.1 a	12.1	12.1	1.0
	Mean	14.1	211	15.1	30.2	2.0	12.1	12.2	1.0
	C.V.	1.6	0.6	3.7	3.2	4.7	7.7	8.4	5.0
	Pr(>F)	0.21	0.36	0.03	0.04	0.01	0.51	0.40	0.24
	LSD 0.10	ns	ns	0.5	1.0	0.09	ns	ns	ns
			Pr>F t	for single D	.F. contras	ts of least-s	quares mea	ins	
	OS vs all S rates	0.37	0.44	0.004	0.17	0.003	0.73	0.63	0.72
	8S vs >S rates	0.27	0.17	0.38	0.99	0.46	0.07	0.28	0.18
	0B vs 0.4B	0.38	0.46	0.17	0.23	0.76	0.98	0.93	1.00
Shelby(2)	0S	10.3 b	166 a	17.5 a	28.0 b	1.6 b	16.5 a	11.6 b	0.7 c
	11 <b>S</b>	10.6 a	159 b	15.9 b	31.8 a	2.0 a	13.7 b	12.0 a	0.9 b
	22S	10.5 a	158 b	15.5 b	32.5 a	2.1 a	12.9 c	12.1 a	0.9 a
	Mean	10.5	161	16.3	30.8	1.9	14.2	11.9	0.8
	C.V.	1.2	1.5	3.3	4.4	4.6	4.5	3.1	4.3
	Pr(>F)	0.009	0.001	0.001	0.002	< 0.001	< 0.001	0.09	< 0.001
	LSD 0.10	0.1	0.3	0.6	1.6	0.1	0.76	0.44	0.043
			Pr>	>F for single	e D.F. cont	rasts of leas	st-squares n	neans	
	0S vs all S r	ates 0.00	3 <0.001	< 0.00	< 0.001	< 0.001	< 0.001	0.04	< 0.001
	11S vs 22	S 0.67	0.32	0.23	0.47	0.11	0.09	0.68	0.03

Table 2.6, continued

		Gra	nin	Grain		E	arleaf			Grain	
Site	<b>Treatment</b> <sup>†</sup>	yiel	d	moisture	N:S	Ν	N	S	N:S	Ν	S
		Mg	ha <sup>-1</sup>	g kg <sup>-1</sup>	ratio	-	g kg-1		ratio	g k	g <sup>-1</sup>
Shelby(1)	0S		11.5 ł	o 218							
Whole field	11S		13.1	a 215							
	17S		13.2	a 212							
	22S		12.6 a	a 217							
	22S 0.	4B	13.1 a	a 213							
		Mean	12.7	215							
		C.V.	7.1	2.6							
	I	Pr(>F)	0.01	0.21							
	LSI	O 0.10	0.8	ns							
				Pı	>F for sir	ngle I	D.F. con	trasts of leas	st-squares 1	neans	
	OS vs all S	S rates	0.002	0.21							
	11S vs >S	S rates	0.52	0.70							
	0B vs 0	.4B	0.26	0.16							
Shelby	y(1) 0S		13.1 ł	o 218	18.	4 a	28.6	1.6 b	14.5 a	11.3	0.8 c
Less respons	sive 11S		13.7 a	a 215	15.	9 b	32.2	2.0 a	13.0 b	11.4	0.9 b
	17S		13.8 a	a 212	15.	2 b	32.0	2.1 a	12.7 b	11.4	0.9 ab
	22S		13.7 a	a 217	15.	4 b	31.2	2.0 a	11.7 b	11.0	1.0 a
	22S 0.4	4B	13.9 a	a 213	15.	3 b	31.4	2.1 a	13.0 b	11.7	0.9 ab
		Mean	13.6	215	16.	1	31.1	2.0	13.0	11.4	0.9
		C.V.	6.5	2.6	3.6		6.7	5.4	9.0	2.9	6.3
	I	Pr(>F)	0.01	0.21	<0.	001	0.17	< 0.001	0.04	0.16	0.01
	LSI	O 0.10	0.8	ns	0.7		ns	0.13	1.5	ns	0.1
				Pı	>F for sin	ngle I	D.F. con	trasts of leas	st-squares i	neans	
	OS vs all S	S rates	0.001	0.21	<0.	001	0.02	< 0.001	0.005	1.00	0.001
	11S vs >S	S rates	0.50	0.70	0.1	2	0.63	0.57	0.25	0.46	0.17
	0B vs 0	.4B	0.26	0.16	0.8	1	0.89	0.74	0.13	0.02	0.23

Table 2.6, continued

			Grain	Grain		Earleaf	Î		Grai	1
Site	Treat	tment <sup>†</sup>	yield	moisture	N:S	Ν	S	N:S	Ν	S
			Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	ratio	g kg	g <sup>-1</sup>	ratio	g l	kg <sup>-1</sup>
She	elby(1)	0S	9.4 b	219						
More resp	onsive	11S	12.4 a	u 218						
		17S	12.3 a	u 216						
		22S	12.1 a	. 221						
		22S 0.4B	11.0 a	ıb 214						
			Mean	11.5	218					
			C.V.	10.5	3.5					
			Pr(>F)	0.07	0.80					
			LSD 0.10	1.8	ns					
		OS vs all	S rates	0.01	0.83					
		11S vs >	>S rates	0.44	0.95					
		0B vs	0.4B	0.29	0.29					
Blackford	1	03	S	13.1	198a					
		11	S	12.9	198a					
		17	'S	13.0	198a					
		22	S	13.1	198a					
		22S 0	.4B0	13.1	195b					
			Mean	13.0	197					
			C.V.	3.9	1.0					
			Pr(>F)	0.59	< 0.001					
			LSD 0.10	ns	1.0					
		0S vs all	S rates	0.50	0.30					
		11S vs >	>S rates	0.18	0.55					
		0B vs	0.4B	0.76	< 0.001					

Table 2.6, continued

Table 2.7: Grain yield and harvest grain moisture, and nitrogen to sulfur ratio (N:S), nitrogen (N) and sulfur (S) concentration in both the earleaf at growth stage R1 and grain for sidedress-applied fertilizer S and boron (B) treatments in 2020. Means with different letters within a site indicate differences between treatments at  $p \le 0.10$  using Tukey LSD test. Mean, coefficient of variance (C.V.), p-value for the effect of S rate (Pr>F), and least significant difference (LSD 0.10) are included.

		Grain	Grain		Earleaf			(	Grain	
Site	<b>Treatment</b> <sup>†</sup>	yield	moisture	N:S	Ν	S	N:	S I	N	S
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	ratio	g kg-	1	rat	io -	g kg <sup>-1</sup> -	
DPAC		0S	14.8	173 bc	15.3	28.3	1.9	12.5	12.4	1.0
		6S	14.7	172 bc	15.4	28.6	1.9	13.2	12.1	0.9
		11S	14.8	174 ab	15.3	28.7	1.9	12.0	12.2	1.0
		17S	14.8	175 a	15.	29.7	1.9	12.0	12.2	1.0
		22S	14.6	171 c	15.2	28.5	1.9	11.7	12.1	1.0
	22	2S 1B	14.8	175 a	15.4	29.6	1.9	12.0	12.1	1.0
		Mean	14.8	173	15.3	28.9	1.9	12.2	12.2	1.0
		C.V.	1.7	0.7	2.0	3.2	3.4	6.4	3.4	5.5
		Pr(>F)	0.74	0.01	0.90	0.20	0.33	0.15	0.89	0.11
		LSD 0.10	ns	0.2	ns	ns	ns	ns	ns	ns
				Pr>F for s	single D.F.	contrasts	of least-s	quares n	neans	
	OS vs	all S rates	0.68	0.61	0.81	0.28	0.37	0.54	0.29	0.97
	6S vs	s >S rates	0.75	0.14	0.44	0.51	0.29	0.01	0.71	0.006
	0B	vs 1B	0.25	0.002	0.34	0.11	0.37	0.59	0.74	0.66

					Earleaf			Grain	
Location	Treatment <sup>†</sup>	Yield	Moisture	N:S	Ν	S	N:S	Ν	S
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	ratio	g k	g <sup>-1</sup>	ratio	g ł	cg <sup>-1</sup>
NEPAC	0S	12.3 ab	213	16.3 a	27.9	1.7 c	13.8 a	12.2	0.9 d
	6S	12.0 b	206	16.0 ab	28.4	1.8 bc	13.6 a	12.5	0.9 cd
	11 <b>S</b>	12.6 a	212	15.3 c	28.6	1.8 ab	11.9 b	12.5	1.1 ab
	17S	12.5 a	211	15.8 ab	28.9	1.8 ab	12.2 b	12.5	1.0 ab
	22\$	12.7 a	205	15.7 bc	28.9	1.8 a	11.6 b	12.4	1.1 a
	22S 1B	12.7 a	211	15.5 bc	29.2	1.9 a	12.5 b	12.3	1.0 c
	Mean	12.5	210	15.8	28.6	1.8	12.6	12.4	1.0
	C.V.	3.5	5.6	3.2	3.1	3.5	8.9	4.5	8.7
	Pr(>F)	0.07	0.80	0.03	0.18	0.003	0.008	0.91	0.003
	LSD 0.10	0.4	ns	0.50	ns	0.06	1.1	ns	0.08
			Pr>F for si	ngle D.F.	contrasts	of least-	squares n	neans	
	0S vs all S rates	0.44	0.42	0.01	0.05	0.002	0.009	0.35	0.002
	6S vs >S rates	0.008	0.55	0.12	0.32	0.06	0.003	1.00	0.002
	0B vs 1B	0.92	0.42	0.57	0.55	0.34	0.16	0.64	0.07
Rice	0S	13.6	222	15.7 ab	30.2 b	1.9 c	15.0 a	11.8 bc	0.8 c
	6S	13.8	223	15.8 a	31.5 a	2.0 b	13.9 ab	11.7 c	0.8 c
	11 <b>S</b>	13.8	220	15.2 bc	31.4 a	2.1 a	13.8 ab	11.9 bc	0.9 c
	17S	13.9	220	15.5 ab	31.9 a	2.1 ab	12.4 cd	12.0 ab	1.0 b
	22S	13.9	223	15.3 ab	31.8 a	2.1 ab	11.6 d	12.3 a	1.1 a
	22S 1B	13.9	220	14.8 c	31.1 ab	2.1 a	12.8 bc	12.1 ab	1.0 b
	Mean	13.8	221	15.4	31.3	2.0	13.3	12.0	0.9
	C.V.	1.7	1.9	3.6	3.4	2.9	9.1	2.7	8.4
	Pr(>F)	0.65	0.82	0.06	0.10	0.001	< 0.001	0.06	< 0.001
	LSD 0.10	ns	ns	0.6	1.1	0.059	1.2	0.3	0.08
			Pr>F for si	ngle D.F.	contrasts	of least-	squares n	neans	
	0S vs all S rates	0.22	0.84	0.42	0.006	< 0.001	0.001	0.27	0.001
	6S vs >S rates	0.82	0.39	0.07	0.68	0.06	0.03	0.02	0.003
	0B vs 1B	0.75	0.42	0.13	0.28	0.19	0.08	0.23	0.02

Table 2.7, continued

† Sidedress S and B rates (kg ha-1).

				:	Earleaf			Grain	
Location	<b>Treatment</b> <sup>†</sup>	Yield	Moisture	N:S	Ν	S	N:S	Ν	S
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	ratio	g k	g <sup>-1</sup>	ratio	g kg <sup>-1</sup>	
SEPAC(1)	0S	13.9 a	275	14.8 ab	29.6	2.0 c	12.4	12.1 bc	1.0
	6S	13.7 b	276	15.0 a	30.1	2.0 bc	12.3	12.3 ab	1.0
	11 <b>S</b>	14.0 a	274	14.5 bc	30.4	2.1 ab	12.0	12.0 c	1.0
	17S	14.0 a	275	14.0 d	29.8	2.1 ab	13.9	12.2 ab	0.9
	228	13.9 a	275	14.3 cd	30.5	2.1 a	12.3	12.5 a	1.0
	22S 1B	13.9 a	274	14.2 cd	29.9	2.1 ab	11.4	12.4 a	1.1
	Mean	13.9	275	14.5	30.1	2.1	12.4	12.2	1.0
	C.V.	1.1	1.3	3.1	3.5	4.6	15.8	2.5	10.4
	Pr(>F)	0.009	0.89	0.005	0.67	0.06	0.42	0.05	0.17
	LSD 0.10	0.1	ns	0.4	ns	0.095	ns	0.3	ns
			Pr>F fo	r single D.l	F. contra	sts of lea	st-square	s means	
	OS vs all S rates	0.68	0.97	0.08	0.25	0.01	0.80	0.18	0.78
	6S vs >S rates	< 0.001	0.54	0.002	0.79	0.09	0.67	0.59	0.72
	0B vs 1B	0.85	0.56	0.21	0.28	0.85	0.17	0.16	0.17
SWPAC	0S	13.8	180	16.0	32.6	2.0	12.8	13.1	1.0
	12S	13.7	179	16.0	33.4	2.1	11.6	12.7	1.1
	228	13.9	178	15.8	33.2	2.1	11.2	13.1	1.2
	22S 1B	14.1	181	15.5	31.7	2.0	11.5	13.2	1.1
	Mean	13.8	179	15.8	32.7	2.1	11.8	13.0	1.1
	C.V.	4.0	3.9	2.5	3.5	3.7	8.4	4.1	8.2
	Pr(>F)	0.70	0.90	0.28	0.19	0.22	0.19	0.70	0.25
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns
			Pr>F fo	r single D.l	F. contra	sts of leas	st-square	s means	
	OS vs all S rates	0.70	0.70	0.80	0.37	0.11	0.05	0.59	0.13
	12S vs 22S	0.30	0.70	0.22	0.97	0.65	0.56	0.32	0.27
	0B vs 1B	0.90	0.40	0.67	0.07	0.09	0.65	0.97	0.63

Table 2.7, continued

					Earleaf			Grain	
Location	<b>Treatment</b> <sup>†</sup>	Yield	Moisture	N:S	Ν	S	N:S	Ν	S
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	ratio	g k	g <sup>-1</sup>	ratio	g kg <sup>-1</sup>	
TPAC	0S	13.9	192	15.0 a	31.4	2.1	12.7	11.7	0.9 c
	6S	13.9	192	14.5 bc	30.5	2.1	12.2	11.9	1.0 b
	11 <b>S</b>	13.8	192	14.8 ab	31.5	2.2	11.9	11.8	1.0 ab
	17S	13.9	192	14.2 c	30.8	2.2	11.9	11.9	1.0 ab
	22S	13.9	192	14.3 c	30.8	2.1	11.8	11.9	1.0 a
	22S 1B	14.0	192	14.2 c	30.3	2.1	12.1	11.9	1.0 ab
	Mean	13.9	192	14.5	30.9	2.1	12.1	11.9	1.0
	C.V.	1.2	0.5	3.2	2.8	3.6	4.4	2.6	3.8
	Pr(>F)	0.74	0.92	0.02	0.14	0.40	0.11	0.70	0.007
	LSD 0.10	ns	ns	0.46	ns	ns	ns	ns	0.04
			Pr>F fo	or single D	.F. contr	asts of 1	east-squa	tres means	
	0S vs all S r	ates 0.90	0.74	0.0	02 0.1	20 0	.09 0	.008 0.72	<0.00 1
	6S vs >S r	ates 0.25	0.47	0.8	30 0. <sup>2</sup>	21 0	.65 0	.14 0.20	0.13
	0B vs	1B 0.41	0.48	0.5	64 0.1	39 0	.46 0	.03 0.05	0.25

Table 2.7, continued

						Ear	eaf		Gı	rain
Location 7	[reatment <sup>†</sup>	Yield	I	Moisture	N:S	Ν	S	N:S	Ν	S
		Mg ha	- <sup>1</sup> §	g kg <sup>-1</sup>	ratio		g kg-1	ratio	g	kg-1
Blackford	0S	1	2.3 b	191 b		16.2 a	26.3 b	1.6 b		
whole field	17S	12	2.8 a	196 a		15.7	27.2 a	1.7 a		
		<b>r</b> 1.	~ ~	104		b	267			
	Ν	lean I	2.5	194		16.0	26.7	1.7		
	_	C.V. 3	.2	2.4		4.0	4.5	6.1		
	Pr	(>F) 0	.02	0.03		0.08	0.07	0.02		
	LSD	0.10 0	.3	3.5		0.43	0.80	0.07		
Bone	<b>o</b> 0S	1.	3.9	198		15.7	27.7	1.8		
	17S	14	4.0	196		15.8	28.6	1.8		
	Ν	Iean 1	3.9	197		16.2	27.9	1.7		
	(	C.V.  0	.9	4.0		5.00	5.3	7.5		
	Pr	(>F) 0	.34	0.77		0.59	0.68	0.54		
	LSD	0.10 n	8	ns		ns	ns	ns		
Whitake	r OS	1	1.7 b	196 b		16.5	25.3 b	1.5 b		
	17S	12	2.6 a	202 a		15.7	26.6 a	1.7 a		
	N	lean 1	2.1	199		16.1	25.9	1.6		
	(	C.V. 3	.9	3.2		4.99	2.19	5.4		
	Pr	(>F) 0	.004	0.06		0.17	0.002	0.04		
	LSD	0.10 0	.39	5.3		ns	0.77	0.12		
Martinsvill	e OS	11	2.4 b	178 b		16.6	24.9	1.5 b		
	17S	1	3.9 a	191 a		15.6	27.0	1.7 a		
	N	Iean 1	3.1	184		16.1	26.0	1.6		
		C.V. 3	.5	0.1		6.38	5.50	5.4		
	Pr	(>F) 0	.06	< 0.001		0.39	0.22	0.10		
	LSD	0.10 1	.1	0.32		ns	ns	0.2		
Sarana	c 0S	1	3.8 a	182		15.5	27.1	1.8		
~	178	1	3.1 b	184		15.2	26.3	1.7		
	N	lean 1	3.4	183		15.3	26.7	1.7		
	14	$\gamma V 2$	0	2 5		0.36	17	2.0		
	Dr	(>F) 0	.01	0.48		0.11	0.34	0.74		
	דד תא ד	010 0	.51 4	0. <del>1</del> 0		ns	ns	0.22		
	LSD	0.10 0	.4	ns		ns	ns	0.22		

Table 2.7, continued

			Moisture		Earlea	ſ		Gra	in
Location	<b>Treatment</b> <sup>†</sup>	Yield		N:S	Ν	S	N:S	Ν	S
		Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	ratio	g	kg <sup>-1</sup>	ratio	g k	g <sup>-1</sup>
Henry	0S	13.8 b	215						
	17S	14.1 a	218						
	Mean	13.9	217						
	C.V.	0.9	1.1						
	Pr(>F)	0.02	0.12						
	LSD 0.10	0.2	ns						

Table 2.7, continued

	Predicted crop response to fertilization											
	Is most likely	Will usually occur	Is likely depending on specific crop and site conditions	Actual corn grain yield response to S fertilization in this study								
Site		%	of the field									
DPAC17&19	0	0	100	Not responsive								
DPAC18&20	0	18	82	Not responsive								
NEPAC19	4	0	96	Not responsive								
NEPAC18&20	6	12	82	Not responsive								
Rice17&19	2	15	83	Responsive								
Rice18&20	0	52	48	Responsive 1 of 2 years								
SEPAC(1)19	99	0	1	Not responsive								
SEPAC(2)19	46	0	50	Not responsive								
SEPAC(1)18&20	90	0	10	Responsive 1 of 2 years								
SWPAC18	30	65	0	Responsive								
SWPAC19	94	3	0	Not responsive								
SWPAC20	0	65	0	Not responsive								
TPAC19	0	12	88	Not responsive								
TPAC20	0	0	100	Not responsive								
Blackford17	0	100	0	Not responsive								
Blackford18	0	100	0	Not responsive								
Blackford19	0	100	0	Not responsive								
Blackford20	17	83	0	Not responsive (Bono, Saranac)								
	5	95	0	Responsive (Whitaker, Martinsville)								
Shelby(1)18	0	100	0	High response								
	0	100	0	Low response								
Shelby(1)19	0	100	0	High response								
	0	100	0	Low response								
Shelby(2)19	0	100	0	Responsive								
Henry20	0	100	0	Responsive								

Table 2.8: Predicted crop response to sulfur fertilization for sites in Indiana using the sulfur response model developed by Ohio State University (<u>http://cura-gis-web.asc.ohio-state.edu/sam.htm</u>) and compared to findings from sulfur response field experiments on corn from 2017 through 2020. Values represent the percentage of the field responsive to sulfur fertilization classified in categories for low S requirement as indicated by the model.

Table 2.9: Regression models for earleaf S (g kg<sup>-1</sup>), earleaf N (g kg<sup>-1</sup>), earleaf N:S, grain yield (Mg ha<sup>-1</sup>), grain S (g kg<sup>-1</sup>), grain N (g kg<sup>-1</sup>), and grain N:S versus S rate, also grain yield (Mg ha<sup>-1</sup>) versus earleaf S (g kg<sup>-1</sup>), and grain yield (Mg ha<sup>-1</sup>) versus earleaf N:S. Adjusted R squared (R<sup>2</sup>) for linear regressions when  $p \le 0.10$ , and residual standard error (RSE) for linear-plateau regressions. Only site-years included where a significant response occurred in one or more of the independent variables.

Site	Earleaf S (y) vs. S rate (x)	R <sup>2</sup> or RSE	Earleaf N (y) & S rate (x)	R <sup>2</sup> or RSE	Earleaf N:S (y) & S rate (x)	R <sup>2</sup> or RSE
	g kg <sup>-1</sup>		g kg <sup>-1</sup>		N:S ratio	
DPAC19	y=2.00+0.0058x	0.73		ns	y=15.11-0.036x if x<26.8	0.18
NEPAC19	y=1.99+0.0065x	0.88		ns	y=15.52-0.034x	0.97
NEPAC20	y=1.74+0.0058x	0.77	y=28.00+0.047x	0.84		ns
Rice18	y=1.50+0.011x	0.99		ns	y=17.19-0.090x	0.83
Rice19	y=1.65+0.01x	0.54		ns	y=17.03-0.11x if x<18.2	0.11
Rice20		ns	y=30.17+0.26x if x<5.8	0.28		ns
<b>SEPAC(1)18</b>		ns	y=31.83-0.10x	0.99		ns
SEPAC(1)19	y=2.07+0.0031x	0.80		ns	y=14.89-0.024x	0.94
<b>SEPAC(1)20</b>	y=1.99+0.0076x	0.92		ns		ns
SWPAC19	y=1.79+0.0084x	0.72	y=31.13+0.054x	0.75	y=17.36-0.043x	0.66
Shelby(1)18	y=2.03+0.0056x	0.99		ns	y=18.40-0.23x if x<13.6	0.14
Site	Yield (y) & S rate (x)	<b>R<sup>2</sup> or RSE</b>	Yield (y) & earleaf S (x)	<b>R<sup>2</sup> or RSE</b>	Yield (y) & earleaf N:S (x)	<b>R<sup>2</sup> or RSE</b>
	Mg ha <sup>-1</sup>		Mg ha <sup>-1</sup>		Mg ha <sup>-1</sup>	
Rice18	y=11.36+0.083x	0.99	y=-0.14+7.58x if x < 1.7	0.26		ns
Rice19	y=11.2+0.11x if x<8.6	0.06	y=3.69+4.70x if x<1.8	0.05	y=18.32-0.41x	0.66
Rice20	y=13.69+0.0091x	0.69	y=8.06+2.86x if x<2.0	0.06		ns
Shelby(1)19		ns	Y=11.14+1.26x	0.99	y=17.05-0.21x	0.97

<sup>†</sup> Not enough data points for regression analysis or data not available.

	Table 2.9, <i>continued</i> :											
	Site	Grain S (y) & S rate (x)	R <sup>2</sup> or RSE	Grain N (y) & S rate (x)	<b>R<sup>2</sup> or RSE</b>	Grain N:S (y) & S rate (x)	<b>R<sup>2</sup> or RSE</b>					
		$g kg^{-1}$		g kg <sup>-1</sup>		N:S ratio						
	DPAC19	y=0.88+0.0031x	0.70	y=11.23+0.019x	0.57		ns					
	NEPAC19	y=0.95+0.0035x	0.87		ns	y=13.28-0.056x	0.71					
	NEPAC20	y=0.87+0.016x if x<11.6	0.04		ns	y=13.77-0.11x	0.78					
	Rice19	y=0.69+0.014x	0.96		ns	y=18.31-0.22x	0.88					
96	Rice20	y=0.79+0.012x	0.90	y=11.70+0.023x	0.75	y=14.96-0.15x	0.93					
•	<b>SEPAC(1)19</b>		ns	y=13.03+0.017x	0.46		ns					
	SWPAC19	y=0.87+0.0061x	0.56		ns	y=14.0-0.25x if x<13.2	0.10					
	SWPAC20	y=1.04+0.0059x	0.99		ns		ns					
	TPAC20	y=0.93+0.010x if x<7.0	0.01		ns		ns					
	Shelby(1)19	y=0.78+0.0091x if x<15.9	0.04		ns	y=14.50-0.12x	0.95					

<sup>†</sup> Not enough data points for regression analysis or data not available.

Table 2.10: Whole plant nutrient concentrations and nitrogen to sulfur ratio (N:S) measured at V3-V7 corn growth stages prior to S fertilization at sites in 2019 and 2020.

Site-year	N:S	Ν	Р	K	Ca	Mg	S	Zn	Mn	Fe	Cu	B
	ratio			g kg	-1					mg kg <sup>-1</sup>		
DPAC19	19	44.2	5.0	46.4	5.3	3.8	2.3	33	51	136	11	4
DPAC20	18	47.8	5.7	40.5	6.9	4.8	2.6	43	56	-	13	11
NEPAC19	17	44.5	2.9	43.5	5.7	3.3	2.6	29	55	208	10	3
NEPAC20	19	40.4	3.6	35.9	5.0	3.2	2.1	26	54	-	11	9
Rice19	24	43.5	4.9	38.2	4.5	3.4	1.8	22	46	110	8	4
Rice20	26	43.3	5.5	40.6	3.4	3.7	1.7	38	47	-	9	10
<b>SEPAC(1)19</b>	16	41.5	4.4	40.0	5.0	2.8	2.7	29	59	192	10	3
SEPAC(2)19	17	30.4	4.8	38.6	4.3	3.3	1.8	29	69	92	9	2
SEPAC(1)20	18	45.7	6.0	52.2	5.5	3.0	2.5	58	65	-	14	8
SWPAC19	17	45.6	3.9	50.2	5.5	3.5	2.7	23	92	175	8	8
SWPAC20	19	40.2	2.5	35.8	6.1	2.3	2.2	27	104	-	7	10
TPAC19	16	39.2	4.1	37.1	5.4	3.5	2.4	31	67	164	11	5
TPAC20	17	30.6	4.2	35.8	4.6	3.1	1.8	29	56	-	10	10
Shelby(1)19	14	29.4	2.9	39.9	7.9	3.1	2.2	20	61	253	8	4

- not determined

					2019							2020			
Site	Depth	$\mathbf{p}\mathbf{H}^{\dagger}$	OM <sup>‡</sup>	Clay↓	<b>Sand</b> <sup>‡</sup>	TC¶	S	$\mathbf{WPM}^{\mathrm{F}}$	pH	OM	Clay	Sand	ТС	S	WPM
	(cm)		g kg⁻	1 g	g 100g-1		mg	SO <sub>4</sub> -S kg <sup>-1</sup>		g kg-	1	g 100g-1		mg S	SO <sub>4</sub> -S kg <sup>-1</sup>
DPAC	0-20	7.1	27	33	24	CL	7	6.6	7.2	31	32	24	CL	8	7.3
all soils	20-40	7.7	22	37	26	CL	8		7.6	28	40	22	С	8	
	40-60	8.0	17	40	25	С	7		7.9	24	40	25	С	11	
Blount	0-20								7.3	28	33	22	CL	7	7.1
	20-40								7.7	26	40	20	SiC	9	
	40-60								7.9	23	38	27	CL	11	
Pewamo	0-20								7.1	35	32	26	CL	8	7.6
	20-40								7.5	30	40	24	С	8	
	40-60								7.8	25	42	22	С	11	
NEPAC	0-20	6.3	27	26	45	L	8	6.6	6.3	23	31	42	CL	8	6.9
	20-40	6.9	21	34	40	CL	4		7.0	20	30	39	CL	6	
	40-60	7.9	17	36	44	CL	4		7.8	19	40	36	С	8	
Rice	0-20	6.6	25	15	72	SL	9	7.4	6.4	23	8	78	SL	6	5.2
all soils	20-40	6.1	17	17	72	SL	8		5.9	17	12	80	SL	6	
	40-60	6.3	12	17	74	SL	5		6.1	13	12	77	SL	3	
Maumee	0-20								6.3	19	6	81	LS	9	6.9
	20-40								5.7	15	10	83	LS	7	
	40-60								5.9	12	10	81	LS	4	

Table 2.11: Selected soil chemical (pH, organic matter (OM), Mehlich-3 extractable  $SO_4$ -S, and weighted profile mean (WPM) of  $SO_4$ -S) and physical characteristics (clay, sand, and textural class (TC) for sites from 2017 to 2020. Textural class for DPAC and Rice in 2019 apply for those same sites in 2017 and DPAC, NEPAC, SEPAC(1), and Rice in 2020 apply for those same sites in 2018.

<sup>†</sup> solution 1:1 soil:water

 $^{\ddagger}$  organic matter determined by loss on ignition at 360 °C

<sup>‡</sup> hydrometer method

<sup>#</sup>Clay (C), clay loam (CL), silty clay (SiC), silty clay loam (SiCL), silt loam (SiL), loam (L); loamy sand (LS); sandy clay loam (SCL), sandy loam (SL), Loamy sand (LS) <sup>#</sup> (WPM) of SO<sub>4</sub>-S was calculated at 16 sites to create a single SO<sub>4</sub>-S concentration to represent the entire 0 to 60 cm soil depth using constant = 0.4

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					2019							2020			
Site	Depth	pН	OM <sup>‡</sup>	Clay↓	Sand↓	ТС	S	<b>WPM</b> <sup>¥</sup>	pH	OM	Clay	Sand	ТС	S	WPM
	(cm)		g kg <sup>-1</sup>	g 1(	00 g <sup>-1</sup>		mg	SO4 <sup>-</sup> S kg <sup>-1</sup>		g kg <sup>-1</sup>	g 10	00 g <sup>-1</sup>		mg S	SO <sub>4</sub> -S kg <sup>-1</sup>
Gilford	0-20								6.5	27	11	75	SL	4	3.5
	20-40								6.2	18	14	76	SL	5	
	40-60								6.4	15	15	73	SL	2	
SEPAC(1)	0-20	5.8	19	20	25	SiL	10	16.4	6.2	26	23	17	SiC	12	13.9
	20-40	5.3	14	26	20	SiL	24		5.8	20	28	13	SiCL	18	
	40-60	5.0	12	29	20	SiCL	43		5.6	18	33	14	SiCL	28	
SEPAC(2)	0-20	6.7	25	24	16	SiCL	9	7.8							
	20-40	7.0	16	30	14	SiCL	6								
	40-60	6.7	13	31	13	SiCL	11								
SWPAC	0-20	6.8	10	9	64	SL	7	5.3	7.0	11	12	75	SL	8	5.8
	20-40	6.6	7	14	64	SL	5		6.8	5	12	76	SL	5	
	40-60	6.6	8	16	65	SL	3		6.8	5	11	76	SL	4	
TPAC	0-20	6.3	26	26	19	SiL	10	8.5	6.6	21	24	25	SiL	6	5.9
	20-40	5.8	24	30	20	SiCL	9		5.9	20	30	24	CL	7	
	40-60	5.9	20	33	19	SiCL	10		5.8	20	29	24	CL	7	
Shelby(1)	0-20	6.3	25	16	43	L	8	6.3							
	20-40	6.7	21	22	28	SiL	6								
	40-60	7.0	18	41	27	С	4								

Table 2.11, continued

<sup>†</sup> solution 1:1 soil:water

<sup>‡</sup> organic matter determined by loss on ignition at 360 °C

<sup>‡</sup> hydrometer method

<sup>#</sup>Clay (C), clay loam (CL), silty clay (SiC), silty clay loam (SiCL), silt loam (SiL), loam (L); loamy sand (LS); sandy clay loam (SCL), sandy loam (SL), Loamy sand (LS)

 $\frac{1}{2}$  (WPM) of SO<sub>4</sub>-S was calculated at 16 sites to create a single SO<sub>4</sub>-S concentration to represent the entire 0 to 60 cm soil depth using constant = 0.4

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					2019							2020			
Site	Depth	pH <sup>†</sup>	OM <sup>‡</sup>	Clay↓	Sand <sup>‡</sup>	TC¶	S	<b>WPM</b> <sup>¥</sup>	pН	ОМ	Clay	Sand	ТС	S	WPM
	(cm)		g kg <sup>-1</sup>	g 10	00 g <sup>-1</sup>		mg S	SO4 <sup>-</sup> S kg <sup>-1</sup>		g kg <sup>-1</sup>	g 10	00 g <sup>-1</sup>		mg	SO4-S kg-1
Shelby(2)	0-20	6.1	20	23	51	SCL	6	5.7							
	20-40	5.9	18	24	50	SCL	7								
	40-60	6.1	17	27	52	SCL	5								
Blackford	0-20								6.2	34	31	35	CL	8	6.3
all soils	20-40								6.3	31	35	33	CL	7	
	40-60								6.7	27	37	33	CL	5	
Bono	0-20								6.3	39	32	40	CL	9	6.6
	20-40								6.6	30	35	34	CL	6	
	40-60								7.1	24	32	32	CL	5	
Whitaker	0-20								5.8	21	23	35	L	7	5.3
	20-40								5.7	22	31	34	CL	8	
	40-60								5.9	23	34	41	CL	5	
Martinsville	0-20								6.5	37	32	32	CL	6	6.7
	20-40								6.8	37	42	32	С	5	
	40-60								7.0	33	37	36	CL	7	
Saranac	0-20								6.6	49	44	24	С	8	6.3
	20-40								6.7	44	40	26	С	7	
	40-60								7.1	38	52	16	С	5	
Henry	0-20								6.6	16					

Table 2.11, continued

<sup>†</sup> solution 1:1 soil:water

<sup>‡</sup> organic matter determined by loss on ignition at 360 °C

<sup>‡</sup> hydrometer method

<sup>#</sup>Clay (C), clay loam (CL), silty clay (SiC), silty clay loam (SiCL), silt loam (SiL), loam (L); loamy sand (LS); sandy clay loam (SCL), sandy loam (SL), Loamy sand (LS)

 $\frac{1}{2}$  (WPM) of SO<sub>4</sub>-S was calculated at 16 sites to create a single SO<sub>4</sub>-S concentration to represent the entire 0 to 60 cm soil depth using constant = 0.4

## 2.5 Appendix

Site-year	Seeding rate	Seed brand	Hybrid	CRM	Plant stand
	Seeds m <sup>-2</sup>				plants m <sup>-2</sup>
DPAC17	7.4	Pioneer	P0895AMXT	108	
DPAC18	7.4	Pioneer	P1197AMXT	111	
DPAC19	7.4	Pioneer	P0574AMXT	105	
DPAC20	7.4	Pioneer	P1108Q	111	7.3
NEPAC18	7.4	Pioneer	P1197AMXT	111	
NEPAC19	7.4	Pioneer	P0574AMXT	105	7.1
NEPAC20	7.4	Pioneer	P1108Q	111	7.2
Rice17	6.9	Pioneer	P0825AMXT	108	
Rice18	7.4	Pioneer	P1197AMXT	111	
Rice19	7.4	Pioneer	P1197AMXT	111	7.6
Rice20	7.4	Pioneer	P1108Q	111	7.1
SEPAC(1)18	7.4	Pioneer	P1197 (non-transgenic)	111	
SEPAC(1)19	7.4	Pioneer	P1197	111	7.0
SEPAC(2)19	7.4	Pioneer	P1197	111	7.1
SEPAC20	7.4	Pioneer	P1197 (non-GMO)	111	
SWPAC18	6.7	Beck's	5829A4	109	
SWPAC19	6.7	Pioneer	P1197AMXT	111	6.3
SWPAC20	6.7	Pioneer	P1108Q	111	
TPAC19	7.4	Pioneer	P1197AMXT	111	6.9
TPAC20	7.4	Pioneer	P1108Q	111	7.2
Blackford17	7.9	Partners Brand	PB8320DTVT2Pro	108	
Blackford18	7.9	Partners Brand	PB8035 (non-transgenic)	108	
Blackford19	7.9	Stewart's	04CV288	104	
Blackford20	7.9	Stewart's	09DP409	108	
Shelby(1)18	8.0	Channel	210-26 (non-GMO)	110	
Shelby(1)19	8.0	Channel	209-15 VT2TRIB	109	7.7
Shelby(2)19	8.6	Pioneer	P1306W	113	
Henry20		Dekalb	DKC64-89RIB	114	

Table A-1: Seeding rate, hybrid, comparative relative maturity (CRM), and plant stand at sites from 2017 to 2019.

Site	Activity	2017	2018	2019	2020
DPAC	planting	17-May	7-May	7-Jun	14-May
	soil & plant sample			2-Jul	10-Jun
	sidedress	13-Jun	4-Jun	2-Jul	12-Jun
	earleaf sample			9-Aug	22-Jul
	grain harvest	$10\text{-Nov}^{\dagger}$	$25-Oct^{\dagger}$	21-Nov	11-Nov
NEPAC	planting		9-May	4-Jun	2-May
	soil & plant sample			28-Jun	4-Jun
	sidedress		25-Jun	1-Jul	8-Jun
	earleaf sample			9-Aug	23-Jul
	grain harvest		$16-Oct^{\dagger}$	6-Nov	22-Oct
Rice	planting	15-May	10-May	20-May	6-May
	soil & plant sample			20-May & 14-Jun	12-Jun
	sidedress	20-Jun	13-Jun	26-Jun	16-Jun
	earleaf sample			1-Aug	20-Jul
	grain harvest	$8\text{-Nov}^{\dagger}$	22-Oct <sup>†</sup>	5-Nov	28-Oct
SEPAC(1)	planting		11-May	4-Jun	1-May
	soil & plant sample			8-Jul	5-jun & 17-Jun
	sidedress		7-Jun	8-Jul	
	earleaf sample			8-Aug	28-Jul
	grain harvest		$2\text{-Oct}^{\dagger}$	16-Oct	22-Oct
SEPAC(2)	planting			18-May	
	soil & plant sample			13-Jun	
	sidedress			13-Jun	
	earleaf sample			22-Jul	
	grain harvest			27-Sep	
SWPAC	planting		1-May	28-May	12-May
	soil & plant sample			18-Jun	1-Jun
	sidedress		25-May	18-Jun	5-Jun
	earleaf sample			24-Jul	14-Jul
	grain harvest		19-Sep <sup>†</sup>	8-Oct	1-Oct
TPAC	planting			3-Jun	1-Jun
	soil & plant sample			3-Jul	19-Jun
	sidedress			3-Jul	9-Jul
	earleaf sample			5-Aug	29-Jul
	grain harvest			5-Dec	9-Nov

Table A-2: Dates of planting, sidedress, and in-season measurements (soil nutrients, plant tissue nutrients, earleaf nutrients, and ear nutrients and grain yield) at sites from 2017 to 2020.

† ear nutrients not measured

Table A-3, continued												
Site	Activity	2017	2018	2019	2020							
Blackford	planting	17-May	7-May	6-Jun	4-May							
	sidedress	14-Jun	29-May	27-Jun	10-Jun							
	earleaf sample			2-Aug	18-Jul							
	grain harvest	$30\text{-}Oct^{\dagger}$	16-Oct <sup>†</sup>	28-Oct	20-Oct							
Shelby(1)	planting		5-May	29-May								
	soil & plant sample			26-Jun								
	sidedress			28-Jun								
	earleaf sample			31-Jul								
	grain harvest		20-Sep <sup>†</sup>	23-Oct								
Shelby(2)	planting			15-Apr								
	sidedress			7-Jun								
	earleaf sample			22-Jul								
	grain harvest			17-Oct								
Henry	planting				7-May							
	sidedress				9-Jun							
	grain harvest				13-Oct							

† ear nutrients not measured

Site-year	Ν	Р	K	S	Zn	Time of	Source
		I	kg ha <sup>-1</sup>			appreation	
DPAC17			75			pre-plant	0-0-49.8
	32	13		0.5		pre-plant	19-7.4-0
NEPAC18			112			fall 2017	0-0-49.8
	12	26		2		fall 2017	11-22.8-0
NEPAC20			28			fall 2019	0-0-49.8
	9	19		1		fall 2019	11-22.8-0
Rice17			69			pre-plant	0-0-49.8
		34				pre-plant	0-20.2-0
Rice18			56			pre-plant	0-0-49.8
		23				pre-plant	0-20.2-0
				3	6	pre-plant	Zinc Sulfate 35.5% Zn
Rice19			56			pre-plant	0-0-49.8
		23				pre-plant	0-20.2-0
				3	6	pre-plant	Zinc Sulfate 35.5% Zn
Rice20			84			pre-plant	0-0-49.8
SEPAC(1)18			212			pre-plant	0-0-49.8
	51	58		5		pre-plant	18-20-0
SEPAC(1)19	32	36		3		pre-plant	18-20-0
	45	10		0.7		planting	22-4.8-0
SEPAC(2)19			56			pre-plant	0-0-49.8
	29	33				pre-plant	18-20-0
SEPAC(1)20			84			pre-plant	0-0-49.8
SWPAC19			70			pre-plant	0-0-49.8
TPAC19	45	18		0.7		planting	19-7.4-0
TPAC20	45	18		0.7		planting	19-7.4-0
Blackford17	4	7	3			in-furrow	6-10.5-4.9
Blackford18	4	7	3			in-furrow	6-10.5-4.9
Blackford19	4	7	3			in-furrow	6-10.5-4.9
Blackford20	11	23	56			fall 2019	5-10.0-23.2
	4	7	3			in-furrow	6-10.5-4.9
Shelby(2)19	34					pre-plant	28-0-0

Table A-3: Pre-plant and at planting fertilizer and lime applications at sites from 2017 to 2020. Absence of sites indicates no fertilizer was applied.

Site	Treatment	Rows	Seeds	Seed	Р	K	Mg	Zn	Mn	Fe	Cu	В	Al
		per e	ar	g		- g kg-1				mg	kg <sup>-1</sup>		
DPAC	0S	15.0	464	360	2.7	3.5	1.2	17	4	15	1	1	1
	7S	14.8	473	361	2.6	3.4	1.1	17	4	14	1	1	2
	13S	15.0	463	353	2.5	3.3	1.1	16	4	14	1	1	1
	208	14.8	464	358	2.4	3.2	1.0	16	4	14	1	1	2
	278	14.8	461	351	2.5	3.4	1.1	16	4	15	1	1	2
	34S	14.8	459	365	2.7	3.3	1.1	17	4	14	1	1	1
	Mean	14.9	464	358	2.6	3.4	1.1	16	4	14	1	1	2
	C.V.	2.5	5.6	3.3	9.2	6.6	9.2	8	14	15	28	39	79
	Pr>F	0.9	1.0	0.5	0.4	0.6	0.5	0.6	0.8	1.0	0.2	0.8	0.6
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NEPAC	0S	14.7	470	375	2.7	3.4	1.2	18	4	15	2	2	1
	7S	15.2	492	376	2.6	3.2	1.2	20	4	13	1	2	1
	13S	14.8	479	373	2.9	3.6	1.3	18	4	14	1	2	1
	208	15.0	479	371	2.4	3.0	1.1	17	4	14	1	2	1
	27S	15.0	479	367	2.4	3.1	1.1	16	4	14	1	2	1
	34S	14.7	463	372	2.5	3.3	1.1	17	4	14	2	2	2
	Mean	14.9	477	372	2.6	3.2	1.2	18	4	14	1	2	1
	C.V.	3	4	3	21.5	16.6	20.3	23	15	14	35	26	64
	Pr>F	0.5	0.3	0.6	0.6	0.5	0.5	0.6	0.3	0.7	0.2	1.0	0.4
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Rice	0S+0.4B	16.8	516 b	295	2.8	3.9	1.2 b	16	4	13	1 b	1	1
	8S+0.4B	17.0	539 ab	296	2.7	3.8	1.1 b	15	4	14	1 b	1	1
	17S+0.4B	16.8	538 ab	298	2.8	3.9	1.2 ab	16	5	15	2 a	1	2
	25S+0.4B	17.0	539 ab	303	2.8	3.8	1.2 ab	16	4	15	1 b	1	1
	30S+0.4B	17.0	528 b	298	2.9	4.0	1.3 a	15	5	15	1 b	1	1
	258	17.4	562 a	304	3.0	4.0	1.3 a	16	4	15	1 b	1	2
	Mean	17.0	537	299	2.8	3.9	1.2	16	4	15	1	1	1
	C.V.	3	4	3	7.5	5.7	7.8	9	14	13	28	31	95
	Pr>F	0.3	0.09	0.6	0.4	0.5	0.08	0.4	0.6	0.3	0.009	0.8	0.7
	LSD 0.10	0.5	25	ns	ns	ns	0.10	ns	ns	ns	0.4	ns	ns

Table A-4: Average rows and average seeds per ear, average weight for 1,000 seeds, and primary and secondary nutrients concentration measured in corn grain at physiological maturity in 2019. Means with different letters within a site indicate differences between sulfur rates at p < 0.1 using Tukey LSD test. Mean, coefficient of variance (C.V.), p-value for the effect of sulfur rate (Pr>F), and least significant difference (LSD 0.10) per site is included.

Site	Treatment	Rows	Seeds	Seed	Р	K	Mg	Zn	Mn	Fe	Cu	В	Al
		per e	ear	g	g kg <sup>-1</sup>					mg kg-	1		
SEPAC(1)	0S	17.7 a	538	295	2.1 c	3.3 d	1.1 d	14 c	4	9	1	1	1
	7S	16.7 c	525	308	2.7 a	3.9 a	1.4 a	18 a	5	12	2	1	1
	13S	17.3 ab	530	302	2.5 ab	3.6 bc	1.2 bc	16 bc	5	11	2	1	1
	20S	17.3 ab	558	299	2.4 bc	3.4 cd	1.1 cd	16 bc	5	10	1	1	1
	27S	17.3 ab	554	303	2.6 a	3.7 ab	1.3 ab	16 ab	5	12	1	1	1
	34S	17.0 bc	555	302	2.5 ab	3.5 bc	1.2 bc	16 bc	5	10	1	1	1
	Mean	17.2	543	302	2.5	3.6	1.2	16	5	11	1	1	1
	C.V.	2	4	3	6.9	5.2	7.0	7	11	20	30	22	31
	Pr>F	0.07	0.4	0.7	0.02	0.02	0.02	0.06	0.3	0.3	0.2	0.5	0.6
	LSD 0.10	0.52	ns	ns	0.25	0.27	0.13	2	ns	ns	ns	ns	ns
SEPAC(2)	0S	17.8 a	559 c	276	2.3	3.6	1.1	17	4	13	2	1	1
	13S	18.0 a	588 bc	275	2.2	3.4	1.1	16	4	13	2	1	2
	0S+1B	17.8 a	636 a	285	2.3	3.6	1.1	17	4	13	2	1	2
	13S+1B	17.8 a	611 ab	288	2.3	3.6	1.1	17	4	13	2	1	1
	2B	18.0 a	593 bc	279	2.2	3.6	1.1	17	4	13	2	1	1
	13S+2B	17.2 b	581 bc	286	2.4	3.6	1.1	17	4	13	2	1	1
	Mean	17.8	595	281	2.3	3.6	1.1	17	4	13	2	1	1
	C.V.	2	6	3	8.2	6.1	9.0	7	11	11	30	0	43
	Pr>F	0.04	0.08	0.2	0.7	0.6	0.9	0.8	0.9	1.0	1.0	0.4	0.2
	LSD 0.10	0.42	41.92	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
SWPAC	0S+2B	17.0	561 b	310	3.1	4.4	1.1	15	4	12	1	2	1
	8S+2B	17.7	602 a	311	3.1	4.4	1.1	17	4	13	1	2	1
	17S+2B	17.3	553 b	309	3.3	4.6	1.2	17	4	13	1	1	2
	25S+2B	17.0	566 b	310	3.1	4.4	1.1	16	4	14	1	1	1
	34S+2B	17.0	579 ab	314	3.2	4.6	1.1	17	4	13	1	2	2
	25S	17.0	569 b	310	3.1	4.4	1.1	15	4	13	1	2	1
	Mean	17.2	572	310	3.2	4.5	1.1	16	4	13	1	2	1
	C.V.	3	3	3	7.4	6.5	8.7	8	10	19	41	39	60
	Pr>F	0.6	0.09	1.0	0.8	0.9	0.8	0.6	0.7	0.9	0.9	0.7	0.7
	LSD 0.10	ns	27.3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
TPAC	0S+2B	16.7	528	394	3.0	4.1	1.3	20 a	5 a	17 a	2	2	1
	8S+2B	17.0	534	396	3.0	4.0	1.2	18 c	5 bc	15 ab	2	2	2
	17S+2B	17.0	531	396	3.0	4.0	1.2	18 c	4 c	13 c	2	2	2
	25S+2B	16.5	513	389	3.0	4.0	1.2	18 c	5 ab	13 bc	2	2	2
	34S+2B	16.5	517	397	3.1	4.0	1.3	20 ab	5 a	14 bc	2	2	2
	25S	16.8	537	391	2.9	3.8	1.2	18 bc	5 bc	13 bc	2	2	1

Table A-4, continued

Site	Treatment	Rows	Seeds	Seed	Р	K	Mg	Zn	Mn	Fe	Cu	В	
		per ear		g	g kg <sup>-1</sup>			mg kg <sup>-1</sup>					
	Mean	16.8	527	394	3.0	4.0	1.2	18	5	14	2	2	
	C.V.	3	5	2	8.0	6.7	9.2	8	9	15	29	22	
	Pr>F	0.5	0.5	0.4	0.7	0.4	0.6	0.08	0.02	0.03	1	0.14	
	LSD 0.10	ns	ns	ns	ns	ns	ns	1	0.4	2	ns	ns	
Shelby(2)	0S	15.0	547	278	3.0	3.7	1.1	20	4	12	1	1	
	11 <b>S</b>	14.6	536	276	2.8	3.5	1.1	18	4	12	2	1	
	22S	14.8	536	284	2.7	3.4	1.1	17	4	11	1	1	
	Mean	14.8	539	280	2.8	3.5	1.1	18	4	12	2	1	
	C.V.	3	4	2	10.8	9.2	11.8	12	12	13	39	23	
	Pr>F	0.4	0.7	0.2	0.4	0.3	0.8	0.3	0.8	0.4	0.5	0.4	
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
Shelby(1)	0S	16.8	588	316	2.5	3.3	1.0	18	4	12	2	1	
	11 <b>S</b>	16.5	590	320	2.4	3.3	1.1	18	4	14	2	1	
	17S	16.5	585	311	2.2	3.2	1.1	17	5	16	2	1	
	22S	16.5	583	319	2.2	3.1	1.0	17	4	13	2	1	
	22S+0.4B	17.0	598	312	2.5	3.4	1.1	17	5	15	2	1	
	Mean	16.7	589	316	2.3	3.2	1.0	17	4	14	2	1	
	C.V.	3	3	3	9.1	7.1	10.1	8	15	19	18	21	
	Pr>F	0.4	0.8	0.6	0.1	0.4	0.8	0.9	0.3	0.4	0.7	0.4	
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	

Table A-4, continued
Site	Treatment	Rows	Seeds	Seed	Р	K	Mg	Zn	Mn	Cu	В
		per ea	ar	g		g kg <sup>-1</sup>			• mg kg	-1	-
DPAC	0S	16.3 b	573	332 ab	2.9	3.6	1.1	21	6	4	4
	6S	17.3 a	595	328 bc	2.7	3.5	1.0	19	5	3	3
	11S	17.0 a	587	337 a	2.8	3.5	1.1	20	6	4	4
	17S	17.3 a	594	329 bc	2.8	3.7	1.1	20	6	4	3
	228	17.0 a	596	317 d	2.8	3.6	1.1	20	6	4	3
	22S+B	16.8 ab	585	323 cd	2.9	3.7	1.1	20	6	3	3
	Mean	16.9	588	328	2.8	3.6	1.1	20	6	3	3
	C.V.	3	4	2	8	6	6	6	14	15	27
	Pr>F	0.05	0.7	0.01	0.7	0.7	1.0	0.5	0.6	0.5	0.6
	LSD 0.10	0.5	ns	8	ns	ns	ns	ns	ns	ns	ns
NEPAC	0S	17.0	590	284	2.3	3.5	1.1	23 a	5	5	2
	6S	17.0	578	274	2.2	3.4	1.1	20 b	5	5	2
	11 <b>S</b>	16.7	588	281	2.6	3.6	1.1	20 b	5	4	3
	17S	16.7	588	290	2.3	3.4	1.1	20 b	5	3	3
	22S	17.2	588	283	2.4	3.6	1.1	20 b	5	4	2
	22S+B	16.8	588	287	2.1	3.3	1.0	19 b	4	3	3
	Mean	16.9	587	283	2.3	3.4	1.1	20	5	4	3
	C.V.	2	5	6	18	9	8	10	22	53	19
	Pr>F	0.3	1.0	0.6	0.5	0.5	0.6	0.08	0.7	0.5	0.2
	LSD 0.10	ns	ns	ns	ns	ns	ns	2.0	ns	ns	ns
Rice	0S	16.8 a	653	300	2.6	3.4	1.0	23	4	3	3
	6S	16.7 ab	639	305	2.8	3.5	1.0	20	4	3	3
	11 <b>S</b>	16.2 c	658	304	2.5	3.3	1.0	20	4	3	3
	17S	16.2 c	638	297	2.5	3.4	1.0	20	4	3	2
	228	16.7 ab	648	301	2.6	3.5	1.0	19	4	3	3
	22S+B	16.3 bc	638	297	2.5	3.4	1.0	18	4	3	3
	Mean	16.5	646	301	2.6	3.4	1.0	20	4	3	3
	C.V.	3	4	3	10	5	6	16	10	15	23
	Pr>F	0.05	0.6	0.4	0.2	0.4	0.6	0.2	0.5	0.4	0.7
	LSD 0.10	0.4	ns	ns	ns	ns	ns	ns	ns	ns	ns
SEPAC(1)	0S	18.2	679	305	2.1	3.6	0.9	19	6	3	3
	6S	18.3	663	303	2.2	3.5	0.9	21	7	3	3
	11 <b>S</b>	18.3	686	299	2.2	3.7	1.0	21	6	3	4
	17S	18.3	674	304	2.0	3.5	0.9	20	6	3	3
	228	18.0	682	306	2.2	3.5	1.0	20	6	3	3
	22S+B	18.0	658	303	2.4	3.8	1.0	21	7	3	3

Table A-5: Average rows and average seeds per ear, average weight for 1,000 seeds, and primary and secondary nutrients concentration measured in corn grain at physiological maturity in 2020. Means with different letters within a site indicate differences between sulfur rates at  $p \le 0.10$  using Tukey LSD test. Mean, coefficient of variance (C.V.), p-value for the effect of sulfur rate (Pr>F), and least significant difference (LSD 0.10) per site is included.

Site	Treatment	Rows	Seeds	Seed	Р	K	Mg	Zn	Mn	Cu	В
		per e	ar	g		g kg <sup>-1</sup> -			- mg kg	-1	
	Mean	18.2	674	303	2.2	3.6	1.0	20	6	3	3
	C.V.	3	4	2	12	6	7	8	13	20	13
	Pr>F	0.8	0.6	0.7	0.1	0.2	0.2	0.5	0.4	0.5	0.5
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
SWPAC	0S	17.8	569	324	3.5	4.1	1.3	19	7	3	3
	11 <b>S</b>	18.0	579	319	3.3	4.0	1.2	18	6	3	3
	228	17.8	577	325	3.4	4.1	1.2	18	6	3	3
	22S+B	17.8	601	331	3.2	4.0	1.2	18	6	3	3
	Mean	17.8	581	325	3.4	4.0	1.2	18	6	3	3
	C.V.	3	4	3	9	7	10	9	16	31	14
	Pr>F	0.8	0.4	0.4	0.7	0.8	0.7	0.9	0.7	0.7	0.8
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
TPAC	0S	17.7	645	290	2.8	3.8	1.0	19	7	4	3
	6S	17.7	652	286	2.9	3.8	1.0	19	7	3	3
	11 <b>S</b>	17.7	656	289	2.9	3.9	1.0	20	7	4	3
	17S	17.8	659	286	3.0	3.9	1.0	19	7	4	2
	22S	17.3	649	289	2.9	3.8	1.0	19	7	3	3
	22S+B	17.2	652	290	2.7	3.7	0.9	18	6	3	3
	Mean	17.6	652	288	2.8	3.8	1.0	19	7	3	3
	C.V.	3	4	2	6	4	5	8	11	19	29
	Pr>F	0.3	0.9	0.8	0.1	0.2	0.1	0.3	0.6	0.8	0.4
	LSD 0.10	ns	ns	7	ns	ns	ns	ns	ns	ns	ns

Table A-5, continued

Site	Treatment	Р	K	Ca	Mg	Zn	Mn	Fe	Cu	В	Al
			g kį	g <sup>-1</sup>				mg kg	-1		
DPAC	0S	4.4	23.4	4.3	3.2	21 a	44	85	10	5	4
	0S-17S	4.2	24.8	3.5	2.7	18 c	36	78	9	5	2
	0S-34S	4.3	24.2	4.1	3.0	20 ab	44	86	10	5	7
	6S-0S	4.1	22.7	3.8	3.2	18 c	42	75	9	4	8
	6S-11S	4.1	24.3	4.1	3.0	18 bc	47	81	10	4	6
	6S-28S	4.4	23.9	4.1	3.0	20 ab	42	85	10	5	10
-	Mean	4.3	23.9	4.0	3.0	19.0	42.6	81.7	9.8	4.7	6.2
	C.V.	6.9	5.3	8.5	9.1	5.8	10.2	6.2	8.1	23.4	95.3
	Pr>F	0.60	0.48	0.13	0.36	0.05	0.18	0.11	0.47	0.92	0.59
	LSD 0.10	ns	ns	ns	ns	1.6	ns	ns	ns	ns	ns
NEPAC	0S	2.9 b	24.9	4.4 ab	2.8	17	45	78	7	2 c	27
	0S-17S	2.6 c	25.4	4.1 b	2.5	17	44	80	7	4 a	29
	6S-11S	3.1 a	24.7	4.6 a	2.8	18	47	82	8	2 bc	19
	6S-22S	2.8 b	23.9	4.8 a	2.9	18	45	85	7	3 ab	18
-	Mean	2.8	24.7	4.5	2.8	17.3	45.3	81.2	7.3	2.5	23.1
	C.V.	3.9	2.8	5.4	9.9	3.8	18.8	5.5	7.6	28.4	31.1
	Pr>F	0.01	0.19	0.06	0.37	0.46	0.97	0.33	0.46	0.06	0.29
	LSD 0.10	0.2	ns	0.4	ns	ns	ns	ns	ns	1.1	ns
Rice	0S	3.0	26.0	3.3	2.9	16	36	83	8	2	10
	0S - 17S	3.1	25.6	3.3	2.9	15	33	86	8	3	14
	0S - 28S	3.1	26.7	3.3	3.0	15	32	89	9	3	24
	6S - 0S	2.9	26.1	3.4	2.8	16	34	86	8	3	11
	6S - 11S	2.9	25.4	3.4	3.2	16	33	85	8	3	19
	6S - 22S	3.1	26.3	3.3	3.0	15	31	83	8	2	20
-	Mean	3.0	26.0	3.3	3.0	15.5	33.2	85.4	8.1	2.6	16.5
	C.V.	8.1	4.3	9.5	9.7	5.1	16.2	8.6	9.2	40.6	52.4
	Pr>F	0.81	0.76	1.00	0.74	0.19	0.88	0.92	0.68	0.75	0.35
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table A-6: Nutrient concentrations in corn earleaf at silking stage in 2018. Means with different letters within a site indicate differences between sulfur rates at  $p \le 0.10$  using Tukey LSD test. Mean, coefficient of variance (C.V.), p-value for the effect of sulfur rate (Pr>F), and least significant difference (LSD 0.10) per site is included.

Site	Treatment	Р	K	Ca	Mg	Zn	Mn	Fe	Cu	В	Al
			g kg	g <sup>-1</sup>				mg kg	g <sup>-1</sup>		
SEPAC(1)	05	4.0	25.4	4.7	2.1	21 a	79	89	11	3	8
	0S - 17S	3.7	26.5	4.3	2.0	19 b	74	80	10	3	1
	0S - 28S	3.7	25.9	4.4	2.1	18 c	74	78	10	3	9
	6S - 0S	3.9	25.7	4.3	1.9	19 b	69	86	10	2	14
	6S - 11S	3.6	26.3	3.9	2.0	17 c	70	73	9	3	6
	6S - 22S	3.8	26.0	4.4	2.0	19 bc	68	79	10	3	6
	Mean	3.8	26.0	4.3	2.0	18.9	72.3	81.1	9.9	2.8	7.3
	C.V.	4.3	5.0	7.7	5.6	5.9	8.1	9.2	7.9	23.2	102.8
	Pr>F	0.17	0.90	0.24	0.51	0.03	0.31	0.20	0.16	0.55	0.51
	LSD 0.10	ns	ns	ns	ns	1.7	ns	ns	ns	ns	ns
SWPAC	05	3.7	26.8	4.1	1.10	18	83	82	9	7	14
	0S - 34S	3.6	26.8	4.0	1.17	16	69	84	9	8	17
	6S - 0S	3.7	27.9	4.0	1.10	17	85	80	9	6	16
	6S - 28S	3.9	28.1	4.4	1.10	18	91	90	10	7	5
	Mean	3.7	27.4	4.2	1.1	17.4	81.9	84.1	9.3	7.3	12.9
	C.V.	5.0	3.3	9.6	4.5	11.3	15.3	11.2	11.4	25.5	81.5
	Pr>F	0.51	0.23	0.59	0.35	0.61	0.30	0.66	0.55	0.75	0.54
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Shelby(1)	V <sub>3</sub> N‡	3.7 a	23.5	5.5	2.1	27 a	67 a	109	15 a	1	3
	Split N	3.1 b	23.2	4.8	2.1	23 b	48 d	85	11 b	1	11
	V <sub>3</sub> N & 22S	3.1 b	22.1	5.3	2.2	24 b	58 bc	104	14 a	1	11
	Split N & 22S	3.2 b	20.5	5.6	2.6	22 b	58 bc	103	14 a	1	20
	V <sub>3</sub> N & 34S	3.4 ab	22.0	5.3	2.2	24 b	63 ab	105	14 a	1	7
	Split N & 34S	3.3 b	22.6	5.2	2.3	23 b	53 cd	101	13 a	2	6
	Mean	3.3	22.3	5.3	2.2	23.8	57.6	101.1	13.6	1.3	9.7
	C.V.	7.3	7.0	10.1	14.2	6.6	9.1	11.2	9.7	50.9	100.3
	Pr>F	0.08	0.32	0.52	0.51	0.02	0.01	0.24	0.09	0.81	0.41
	LSD 0.10	0.4	ns	ns	ns	2.3	7.7	ns	1.9	ns	ns

Table A-6, continued

significant difference (LSD 0.10) per site is included. Site Treatment DM Р K Ca Mg Zn Mn Fe Cu B Al ----- g kg<sup>-1</sup>---------- g kg<sup>-1</sup> -------------g DPAC **0**S 22.6 31 95 bc 11 5 4.8 3.3 5.0 3.3 62 5 6S 4.9 21.7 5.0 33 4 3.4 3.4 60 100 a 11 6 13S 93 d 5 4.7 3.3 21.4 5.0 3.7 26 58 11 4 29 20S 5.0 3.5 21.6 4.9 57 95 cd 4 3.5 11 4 27S 4.8 3.4 22.2 4.9 52 99 ab 12 4 3.6 28 5 34S 4.9 3.3 21.4 4.8 3.5 28 56 97 ab 11 5 4 4.9 3.4 21.8 4.9 3.5 29 58 97 11 5 4 Mean C.V. 2.9 4.4 6.0 3.1 10.2 5.0 27.0 17.8 12.8 3.6 18.3 Pr>F 0.2 0.5 0.7 0.2 0.5 0.5 0.4 0.03 0.1 0.3 0.9 4 LSD 0.10 ns NEPAC **0**S 3 5.1 3.1 20.2 30 55 93 11 4.8 3.5 6 6S 20.6 4.9 3.3 4.9 3.5 32 56 91 11 6 4 13S 5.2 3.1 20.5 4.8 3.4 33 56 101 11 6 4 20S 5.0 3.1 20.9 59 95 12 4.7 3.4 32 6 4 3 27S 5.0 20.4 93 3.1 4.7 3.4 31 58 11 6 34S 5.2 3.1 19.6 32 59 93 12 4.8 3.5 6 4 3.1 20.4 94 11 4 Mean 5.1 4.8 3.4 32 57 6 C.V. 5.8 5.9 6.7 5.6 11.6 18.3 11.0 6.7 12.8 20.4 6.6 Pr>F 0.6 0.5 0.7 0.7 1.0 0.5 1.0 0.6 0.2 0.3 0.3 LSD 0.10 ns Rice 0S 0.4B 5.7 3.4 22.5 4.0 a 2.9 19 a 32 69 c 7 c 4 a 4 8S 0.4B 5.8 3.5 22.6 3.7 b 2.8 19 a 30 74 ab 8 ab 4 4 ab 17S 0.4B 5.8 23.0 3.7 b 19 a 29 76 ab 3.6 2.9 8 ab 4 bc 4 25S 0.4B 5.8 3.4 22.9 3.4 c 2.8 17 b 28 72 bc 8 ab 4 c 4 30S 0.4B 5.8 3.6 23.5 3.6 b 2.8 19 ab 79 a 9 a 4 26 4 bc 25S 5.6 3.5 22.8 3.6 bc 2.9 74 ab 8 bc 4 17 b 28 4 c 74 8 4 5.7 3.5 22.9 3.7 2.9 18 29 4 Mean C.V. 3.0 5.6 7.5 7.0 6.8 8.7 27.2 3.6 5.1 6.7 14.6 Pr>F 0.3 0.4 0.4 0.002 0.9 0.08 0.3 0.09 0.02 0.09 1.0 2 LSD 0.10 0.20 6 1 0.4 ns ns ns ns ns ns

Table A-7: Earleaf dry matter (DM) and primary and secondary nutrients concentrations measured in corn earleaf at silking stage in 2019. Means with different letters within a site indicate differences between sulfur rates at  $p \le 0.10$  using Tukey LSD test. Mean, coefficient of variance (C.V.), p-value for the effect of sulfur rate (Pr>F), and least significant difference (LSD 0.10) per site is included.

Site	Treatment	DM	Р	K	Ca	Mg	Zn	Mn	Fe	Cu	В	Al
		g		g kg	-1				mg	g kg <sup>-1</sup>		
SEPAC(1)	0S	4.0	3.3	22.2	5.3	2.1 ab	26	69	94	10	2	8
	6S	4.0	3.1	20.5	5.1	2.2 a	29	74	93	10	3	8
	13S	3.9	3.2	20.5	4.4	2.0 bc	26	65	98	9	2	7
	208	3.9	3.1	19.3	4.3	1.8 c	22	65	89	9	3	8
	275	3.9	3.2	18.8	4.6	2.1 ab	22	66	89	9	2	6
	34S	3.8	3.2	19.2	4.4	2.0 bc	21	67	90	10	2	7
	Mean	3.9	3.2	20.1	4.7	2.0	24	67	92	10	2	7
	C.V.	7.6	4.4	10.4	9.7	5.6	21.5	11.1	6.7	9.3	28.3	21.1
	Pr>F	1.0	0.5	0.4	0.1	0.03	0.5	0.7	0.5	0.7	0.8	0.5
	LSD 0.10	ns	ns	ns	ns	0.17	ns	ns	ns	ns	ns	ns
SEPAC(2)	0S	5.4	3.1 c	21.2 c	5.9 a	3.2 a	24	70 c	91	9 c	4 ab	8 bc
	13S	5.5	3.1 c	24.0 a	5.1 c	2.6 b	23	77 bc	90	9 bc	4 ab	7 c
	1B	5.1	3.4 b	24.3 a	5.0 c	2.6 b	28	82 ab	95	10 a	4 b	8 bc
	13S 1B	5.4	3.7 a	23.5 ab	5.3 bc	: 3.0 a	27	73 c	93	10 a	5 a	10 ab
	2B	5.4	3.4 b	22.5 bc	5.8 a	3.0 a	24	74 bc	100	10 ab	4 ab	13 a
	13S 2B	5.5	3.3 bc	21.4 c	5.6 ab	o 3.0 a	22	87 a	99	10 ab	3 c	11 ab
	Mean	5.4	3.3	22.8	5.4	2.9	25	77	95	10	4	10
	C.V.	6.1	6.6	5.6	6.7	9.1	15.5	10.8	8.4	6.1	14.2	34.7
	Pr>F	0.4	0.002	0.003	0.004	0.02	0.1	0.04	0.2	0.01	0.001	0.06
	LSD 0.10	ns	0.24	1.4	0.40	0.29	ns	9	ns	1	1	4
SWPAC	0S 2B	5.0	4.7	29.8	3.9	2.3	17	73	89	10	6	5
	8S 2B	4.7	4.9	30.3	3.7	2.4	17	66	86	10	6	5
	17S 2B	5.2	4.9	30.1	3.9	2.6	18	71	97	12	6	6
	25S 2B	4.9	4.9	29.9	3.8	2.5	19	76	92	12	6	5
	34S 2B	4.9	4.7	30.0	3.9	2.4	18	72	92	12	7	5
	25S	5.0	4.5	27.6	4.0	2.6	17	70	95	11	6	6
	Mean	5.0	4.8	29.6	3.9	2.5	18	71	92	11	6	5
	C.V.	6.3	5.8	4.0	9.6	10.6	4.8	16.9	8.9	9.6	8.1	21.6
	Pr>F	0.7	0.4	0.1	1.0	0.8	0.1	0.9	0.7	0.4	0.3	0.4
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table A-7, continued

Site	Treatment	DM	Р	K	Ca	Mg	Zn	Mn	Fe	Cu	В	Al
		g		g k	g <sup>-1</sup>				mg k	g <sup>-1</sup>		
TPAC	0S 2B	4.4 b	3.1	19.8	5.4	3.1	30	75	85	10 c	5	5
	8S 2B	4.4 b	3.2	20.0	5.9	3.2	31	77	89	11 a	6	4
	17S 2B	4.6 a	3.1	19.3	5.8	3.4	29	73	86	10 bc	6	4
	25S 2B	4.6 a	3.2	19.4	5.6	3.1	29	78	84	11 b	6	4
	34S 2B	4.5 ab	3.2	19.9	5.7	3.2	31	78	89	11 a	6	5
	25S	4.6 a	3.2	20.1	5.6	3.1	29	74	89	11 b	7	6
-	Mean	4.5	3.2	19.7	5.6	3.2	30	76	87	11	6	5
	C.V.	3.4	4.0	4.3	5.6	7.5	7.5	7.8	7.3	3.5	12.0	42.5
	Pr>F	0.06	0.3	0.5	0.2	0.5	0.3	0.5	0.6	< 0.001	0.1	0.6
	LSD 0.10	0.1	ns	ns	ns	ns	ns	ns	ns	0.4	ns	ns
Shelby(2)	0S	4.5	3.5	30.9	4.0	1.4 b	24	60 b	81 b	9 b	2	7
	11 <b>S</b>	4.3	3.6	32.3	4.2	1.5 b	26	83 a	93 a	11 a	3	8
	22S	4.0	3.6	32.6	4.4	1.6 a	25	77 a	95 a	11 a	3	8
-	Mean	4.2	3.6	31.9	4.2	1.5	25	73	89	11	2	8
	C.V.	7.6	4.1	4.8	6.3	6.7	14	14	3	4	35	20
	Pr>F	0.1	0.4	0.2	0.2	0.04	0.7	0.02	< 0.001	< 0.001	0.3	0.6
	LSD 0.10	ns	ns	ns	ns	0.12	ns	12	4	1	ns	ns
Shelby(1)	0S	5.1	3.0	19.3 b	5.6	2.5	22	70 c	71 c	10 b	5 d	4 a
	11 <b>S</b>	5.3	3.0	20.8 a	6.1	2.5	25	82 ab	88 a	13 a	5 cd	3 a
	17S	5.1	2.9	20.7 a	5.8	2.6	23	89 a	84 ab	13 a	5 bc	2 b
	22S	5.4	3.0	21.5 a	5.8	2.2	24	77 bc	82 b	12 a	5 b	3 a
	22S 0.4B	5.3	3.0	20.7 a	5.9	2.5	23	76 bc	87 ab	12 a	7 a	3 a
-	Mean	5.2	3.0	20.6	5.8	2.5	23	79	82	12	5	3
	C.V.	4.2	6.4	4.7	4.9	9.1	7.4	11.8	6.3	8.5	6.7	15.2
	Pr>F	0.2	0.9	0.09	0.2	0.3	0.3	0.10	0.004	0.01	< 0.00	10.005
	LSD 0.10	ns	ns	1.2	ns	ns	ns	ns	7	1	0.4	1

Table A-7, continued

Site DM Р B Treatment K Ca Mg Zn Mn Cu ----- g kg<sup>-1</sup>---------- mg kg<sup>-1</sup> -----g 7 DPAC **0**S 4.8 3.1 18.8 5.9 22 38 10 b 4.0 6S 4.9 3.0 5.9 22 40 10 b 18.7 4.1 6 11S 22 7 4.8 3.0 18.6 6.0 4.1 39 11 ab 17S 3.1 3.9 22 39 4.8 18.9 6.1 11 ab 6 7 22S 4.7 3.0 21 18.0 6.0 4.3 48 11 a 22S+B22 8 4.8 3.1 19.0 5.8 3.8 37 10 b 4.8 3.0 18.7 5.9 4.0 22 40 10 7 Mean C.V. 3.0 3.7 5.3 2.2 6 5 11 5.7 16 Pr>F 0.5 0.6 0.8 0.1 0.1 0.9 0.4 0.06 0.1 LSD 0.10 ns ns ns ns ns ns ns 0.6 ns NEPAC 0S9 4.3 20 2.8 17.5 5.8 3.4 54 5 b 6S 4.2 2.9 9 17.5 5.8 3.5 22 54 5 b 11S 4.3 2.9 18.4 5.6 3.4 20 55 9 5 b 17S 4.3 2.9 20 10 5 b 18.5 5.6 3.3 53 10 22S 4.3 22 55 3.0 18.6 5.5 3.4 6 b 9 22S+B4.3 2.9 3.2 20 18.1 5.5 57 7 a 2.9 21 55 10 6 Mean 4.3 18.1 5.6 3.4 C.V. 3.8 3.5 4.9 5.1 6.2 9 13 7 9 Pr>F 0.7 0.4 0.2 0.2 0.3 0.2 1.0 0.4 < 0.001 LSD 0.10 ns ns ns ns ns ns ns ns 0.5 Rice 0S4.2 3.4 24.3 3.8 2.3 18 35 10 10 6S 4.3 3.4 24.2 3.7 2.2 18 39 10 10 10 11S 4.3 3.5 24.4 2.3 19 39 10 3.8 17S 10 4.3 3.4 24.3 3.8 2.3 17 37 10 22S 4.3 3.5 24.6 3.7 2.3 18 37 10 10 22S+B4.3 24.9 3.9 2.4 20 38 10 10 3.4 4.2 3.4 24.5 3.8 2.3 18 38 10 10 Mean C.V. 4.7 11 11 4.1 3.6 4.8 8.7 18 11 Pr>F 0.9 0.6 0.8 0.3 0.6 0.6 0.5 1.0 1.0 LSD 0.10 ns ns ns ns ns ns ns ns ns

Table A-8: Earleaf dry matter (DM) and primary and secondary nutrients concentration measured in corn earleaf at silking stage in 2020. Means with different letters within a site indicate differences between sulfur rates at  $p \le 0.10$  using Tukey LSD test. Mean, coefficient of variance (C.V.), p-value for the effect of sulfur rate (Pr>F), and least significant difference (LSD 0.10) per site is included.

Site	Treatment	DM	Р	K	Ca	Mg	Zn	Mn	Cu	В
		g		g	kg <sup>-1</sup>			mg k	g <sup>-1</sup>	
SEPAC(1)	0S	5.1 a	3.2	22.6	4.0	1.9	26	66	11	6b
	6S	4.7 b	3.2	22.9	4.1	2.0	28	71	11	6b
	11 <b>S</b>	5.0 a	3.2	22.1	4.1	2.0	26	76	11	6b
	17S	5.0 a	3.2	22.9	4.0	2.0	28	70	11	6b
	228	4.9 a	3.3	22.7	4.2	2.1	26	73	11	6b
	22S+B	4.9 a	3.2	22.0	4.1	2.1	30	72	12	8a
	Mean	4.9	3.2	22.5	4.1	2.0	27	72	11	6
	C.V.	3.8	3.5	5.9	4.6	5.5	18	10	13	13
	Pr>F	0.05	0.7	0.8	0.7	0.2	0.7	0.3	0.5	0.002
	LSD 0.10	0.2	ns	ns	ns	ns	ns	ns	ns	0.8
SWPAC	Control	4.0	4.1	25.1	4.7	2.0	19	83	13	8 b
	12S	3.9	4.2	24.9	4.8	2.1	20	83	13	7 b
	228	4.0	4.2	24.8	4.9	2.2	25	84	13	8 b
	22S+B	4.0	4.0	26.5	4.5	2.0	20	81	12	10 a
	Mean	3.9	4.1	25.3	4.7	2.1	21	83	13	8
	C.V.	3.5	3.8	8.1	10.8	7.7	21	12	7	13
	Pr>F	0.4	0.4	0.6	0.6	0.3	0.3	1.0	0.6	0.02
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns	1.4
ТРАС	0S	5.5	3.8	22.4	6.2	2.8 ab	18	58	11	7
	6S	5.6	3.8	22.6	6.2	2.9 a	18	58	11	7
	11 <b>S</b>	5.1	3.9	22.4	6.2	2.9 a	18	57	12	7
	17S	5.5	3.9	22.3	6.3	3.0 a	17	57	11	7
	22S	5.5	3.8	23.4	5.9	2.6 bc	17	60	11	8
	22S+B	5.3	3.9	23.3	5.9	2.5 c	17	60	11	9
	Mean	5.4	3.9	22.7	6.1	2.8	17	58	11	8
	C.V.	4.4	3.3	5.3	4.6	9.4	8	8	7	16
	Pr>F	0.1	0.7	0.4	0.2	0.02	0.7	0.7	0.7	0.1
	LSD 0.10	ns	ns	ns	ns	0.26	ns	ns	ns	ns
Blackford	Control	3.7	3.0	20.3	5.0	1.6	23	48	10	10
All soils	17S	3.8	3.0	20.6	5.2	1.7	23	44	11	10
	Mean	3.8	3.0	20.4	5.1	1.7	23	46	10	10
	C.V.	6.4	2.9	4.3	9.9	6.1	7	44	16	10
	Pr>F	0.4	0.8	0.4	0.4	0.02	0.8	0.7	0.1	0.7
	LSD 0.10	ns	ns	ns	ns	0.07	ns	ns	ns	ns

Table A-8, continued

Site	Treatment	DM	Р	K	Ca	Mg	Zn	Mn	Cu	В
		g		g k	g <sup>-1</sup>			mg	kg <sup>-1</sup>	
Bono	Control	3.9	3.2	19.5 b	5.7	1.8	28	29	10	10
	17S	3.9	3.2	20.2 a	5.7	1.8	27	31	11	11
	Mean	3.9	3.1	19.9	5.4	1.7	26	34	11	10
	C.V.	6.6	3.9	1.5	9.3	7.5	8	5	10	11
	Pr>F	0.7	1.0	0.02	0.9	0.5	0.4	0.1	0.5	0.4
	LSD 0.10	ns	ns	0.41	ns	ns	ns	ns	ns	ns
Whitaker	Control	3.5	3.0	21.1 b	4.5	1.5 b	21	75	9	10
	17S	3.5	3.1	21.8 a	5.0	1.7 a	22	57	11	10
	Mean	3.5	3.1	21.4	4.8	1.6	22	66	10	10
	C.V.	8.1	3.3	1.7	12.4	5.4	5	45	25	5
	Pr>F	0.8	0.71	0.05	0.2	0.04	0.3	0.4	0.2	0.11
	LSD 0.10	ns	ns	0.50	ns	0.12	ns	ns	ns	ns
Martinsville	Control	3.8	2.9	21.2	4.6	1.5 b	21	49	9	8
	17S	4.0	2.9	19.9	5.0	1.7 a	22	53	10	9
	Mean	3.9	2.9	20.5	4.8	1.6	22	51	9	8
	C.V.	7.2	5.4	5.9	6.8	5.4	7	22	10	9
	Pr>F	0.5	0.9	0.3	0.3	0.10	0.7	0.7	0.5	0.7
	LSD 0.10	ns	ns	ns	ns	0.21	ns	ns	ns	ns
Saranac	Control	3.8	3.0	18.9	5.5	2.3	21	18	10	10
	17S	4.0	3.0	19.6	5.0	1.9	22	23	10	10
	Mean	3.9	3.0	19.3	5.3	2.1	22	20	10	10
	C.V.	7.2	0.3	1.8	9.9	10.2	8	22	2	1
	Pr>F	0.5	0.3	0.3	0.5	0.33	0.7	0.5	0.2	0.7
	LSD 0.10	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table A-8, continued

Table A-9: Recommended Mehlich-3 soil test critical levels for phosphorus, potassium, calcium, and magnesium for corn in Indiana (Culman et al., 2020) and reference sufficiency ranges for corn at vegetative stage and at silking (R1) (Campbell, 2013)

Nutrient		Vegetative stage	Earleaf at silking
	Soil critical levels	(>10 cm in height to tasseling)	(R1)
	mg kg <sup>-1</sup>	g kg <sup>-1</sup>	
Ν		30.0-40.0	28.0-40.0
Р	20–40	3.0-5.0	2.5-5
K	100–170	20.0-30.0	18.0-30.0
Ca	200	2.5-8.0	2.5-8.0
Mg	35-50	1.5-6.0	1.5-6.0
S		1.5-4.0	1.5-6.0
		mg kg <sup>-1</sup>	
Fe		30-250	30-250
Mn		20-150	15-150
Zn		20-70	20-70
Cu		5-25	5-25
В		5-25	5-25

Site-year	Depth	$\mathbf{p}\mathbf{H}^{\dagger}$	OM <sup>‡</sup>	SO₄-S <sup>↓</sup>	Texture
	cm		g kg <sup>-1</sup>	mg kg <sup>-1</sup>	
ACRE15	0-20	6.4	42	6	
	20-40	6.7	31	4	
	40-60	6.9	23	3	
DPAC15	0-20	6.6	34	6	
	20-40	7.0	29	4	
	40-60	7.5	25	4	
NEPAC15	0-20	6.1	30	10	
	20-40	6.8	23	6	
	40-60	7.2	19	5	
PPAC15	0-20	6.3	40	5	
	20-40	6.5	24	3	
	40-60	6.7	20	3	
SEPAC15	0-20	6.1	28	7	
	20-40	5.9	21	12	
	40-60	5.7	18	19	
TPAC15	0-20	5.8	27	6	
	20-40	5.4	25	6	
	40-60	5.6	22	7	
Blackford17 <sup>¶</sup>	0-20	6.8	29	7	
Rice17 <sup>¶</sup>	0-20	6.7	19	6	
SEPAC(1)17	0-20	6.1	21	0	
NEPAC18¶	0-20	6.2	26	5	
Rice18 <sup>¶</sup>	0-20	6.8	22	6	
SEPAC(1)18 <sup>¶</sup>	0-20	6.4	21	-	
SWPAC18¶	0-20	6.7	13	7	LS
	20-40	6.6	13	7	
Shelby(1)18 (more responsive) <sup>†</sup>	0-20	6.3	26	-	
Shelby(1)18 (less responsive) <sup>†</sup>	0-20	6.5	23	-	
Shelby(2)19	0-20	6.0	21	5	SCL
More response to S	20-40	6.0	19	6	SCL
*	40-60	6.0	18	5	SCL
Shelby(2)19	0-20	6.2	20	7	L
Less response to S	20-40	6.0	18	8	L
*	40-60	6.2	17	5	SCL

Table A-10: Soil pH, organic matter (OM), sulfate-S ( $SO_4$ -S), and soil texture at sites in Indiana on fields that had sulfur response trials in 2017 – 2020, and from fields sampled in 2015 (Moser, 2016).

<sup>†</sup> solution 1:1 soil:water

<sup>‡</sup> determined by loss on ignition at 360 °C

<sup>1</sup> SO<sub>4</sub>-S by Mehlich-3 extractions quantified by inductively coupled plasma spectroscopy

<sup>¶</sup> sulfur experiment at this site-year

- not measured

Site-year	Depth	pH <sup>†</sup>	OM <sup>‡</sup>	SO₄-S↓	Texture
	cm		g kg <sup>-1</sup>	mg kg <sup>-1</sup>	
Shelby(2)19	0-20	5.9	19	6	SCL
no response to S	20-40	5.6	16	7	SCL
	40-60	5.9	15	5	SCL
Blackford20	0-20	6.2	34	9	CL
Bono	20-40	6.3	31	6	CL
	40-60	6.7	27	5	CL
Blackford20	0-20	6.3	39	7	L
Whitaker	20-40	6.6	30	8	CL
	40-60	7.1	24	5	CL
Blackford20	0-20	5.8	21	6	CL
Martinsville	20-40	5.7	22	5	С
	40-60	5.9	23	7	CL
Blackford20	0-20	6.5	37	8	С
Saranac	20-40	6.8	37	7	С
	40-60	7.0	33	5	С

Table A-10, continued

<sup>†</sup> solution 1:1 soil:water
 <sup>‡</sup> determined by loss on ignition at 360 °C
 <sup>‡</sup> SO<sub>4</sub>-S by Mehlich-3 extractions quantified by inductively coupled plasma spectroscopy
 <sup>¶</sup> sulfur experiment at this site-year

- not measured

						201	19									2020	)				
Site	Depth	Ν	Р	K	Ca	Mg	Zn	Mn	Fe	Cu	В	Ν	Р	K	Ca	Mg	Zn	Mn	Fe	Cu	В
											- mg kg <sup>-</sup>	1									
DPAC	0-20	3.4	27	131	2537	566	2.3	95	142	2.0	0.4	7.5	30	123	2511	534	1.6	63	166	2.5	0.4
	20-40	9.7	1	115	5185	920	1.0	101	71	1.5	0.2	8.8	7	110	3624	762	1.0	46	113	2.4	0.3
	40-60	2.7	3	123	3873	978	1.3	95	91	1.8	0.2	6.1	3	105	4617	798	0.9	63	92	2.3	0.2
NEPAC	0-20	10.4	33	118	2055	317	1.9	58	142	2.0	0.3	9.4	28	100	1427	276	1.4	61	125	1.4	0.2
	20-40	7.6	5	98	2514	442	1.1	94	107	1.9	0.3	10.4	7	61	2054	376	0.9	47	102	1.3	0.1
	40-60	13.6	2	100	5026	453	1.2	57	86	1.6	0.2	7.6	2	59	4986	439	0.9	75	86	1.2	0.2
Rice	0-20	4.3	86	142	1249	334	1.7	10	154	1.5	0.2	4.9	58	133	1056	263	2.0	9	171	1.3	0.3
	20-40	2.1	29	55	1033	294	0.9	9	113	1.5	0.1	3.2	25	62	893	265	1.3	12	112	1.3	0.2
	40-60	1.5	9	41	1034	269	0.6	9	77	1.4	0.1	2.3	10	43	998	208	0.9	10	67	1.2	0.4
SEPAC(1)	0-20	9.9	22	94	963	159	0.9	158	129	0.9	0.1	7.5	23	129	1105	176	1.8	139	108	1.1	0.1
	20-40	14.7	7	85	1043	153	0.7	91	103	0.7	0.1	8.8	11	61	1059	161	1.2	91	98	0.6	0.1
	40-60	7.5	2	94	987	191	0.7	48	101	0.8	0.2	6.1	4	63	1191	179	0.6	45	79	0.5	0.1
SEPAC(2)	0-20	8.7	20	92	1728	239	3.1	215	101	1.8	0.5										
	20-40	7.4	5	70	2018	268	1.2	107	79	0.9	0.2										
	40-60	4.5	3	70	2138	275	0.6	45	63	0.6	0.2										
SWPAC	0-20	11.2	55	141	752	140	1.7	161	160	0.9	0.1	5.4	77	126	716	105	2.2	142	223	1.5	0.2
	20-40	2.9	34	115	694	133	0.9	91	140	1.1	0.1	2.6	49	79	610	69	1.1	95	207	1.2	0.2
	40-60	3.0	44	116	1057	117	0.9	48	139	1.2	0.1	1.4	57	63	616	52	0.7	59	188	0.9	0.1
TPAC	0-20	7.7	39	171	2058	301	1.9	68	143	1.6	0.2	13.9	31	146	1691	208	1.4	83	112	1.4	0.4
	20-40	16.4	12	114	2023	323	1.0	29	139	1.6	0.1	7.1	12	98	1676	263	0.8	36	110	1.3	0.2
	40-60	10.2	6	124	2513	422	0.7	21	115	1.3	0.1	12.0	10	106	1895	329	0.7	25	114	1.4	0.2
Shelby(1)	0-20	20.7	32	134	2101	800	1.2	47	153	2.6	0.3										
	20-40	6.7	9	113	2817	618	1.0	47	113	2.4	0.4										
	40-60	4.7	3	122	3138	406	1.0	77	92	2.0	0.4										

Table A-11: Selected chemical soil properties of sites in 2019 and 2020. Inorganic nitrogen (N) extracted with 1N KCl and measured with a SEAL AQ2 discrete analyzer (method 353.2, revision 2.0). For the other nutrients, Mehlich-3 extractions with nutrients quantified by inductively coupled plasma spectroscopy.

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