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Optimizing Soybean (*Glycine max L.*) Yield with Nitrogen and Sulfur Applications

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I am submitting herewith a thesis written by Kacey A. Cannon entitled "Optimizing Soybean (*Glycine max L.*) Yield with Nitrogen and Sulfur Applications." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Angela McClure, Major Professor

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Optimizing Soybean (*Glycine max L.*) Yield with Nitrogen and Sulfur Applications

A Thesis Presented for the
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Abstract

Although soybean (*Glycine max* L.) yields have increased over the past decade, even greater improvement is in demand. Nontraditional practices such as applying nitrogen (N) to soybean for yield optimization, might be one way to meet this demand. Also, sulfur (S) is becoming a more important limiting nutrient in production due to higher yielding crops, lower S containing production inputs, and reduced supply from the environment. The N study involved two environments, irrigated and dryland, in Milan (35.9198° N, 88.7589° W) and Jackson (35.6145° N, 88.8139° W), TN in 2015 and 2016. Urea fertilizer treatments were 34, 67, and 101 kg N ha^{-1} (per hectare). Soybean height, nodes per plant, total biomass weight, biomass N concentration, total nodules per plant, total active nodules per plant, total adolescent nodules per plant, total nodule weight per plant, 100 seed weight, and yield were collected to evaluate treatment effects. Data analysis concluded that N applications significantly increased plant height, plant nodes, and plant biomass of soybean. However, N treatments significantly reduced active and adolescent nodule production. Soybean yield was not significantly increased by the N applications. The irrigated sites yielded at or below the dryland comparison, probably due to lodging, which may have compromised yield potential. The S study included soybean and corn (*Zea mays* L.) experiments, which were conducted in an S deficient soil in Milan, TN in 2015 and 2016. Ammonium sulfate treatments were 11, 23, and 34 kg S ha^{-1} (per hectare). Plant height, leaf S concentrations, seed S and N concentrations, 100 seed weight, and yield were collected to evaluate treatment effects. Data analysis concluded that S application significantly increased leaf S concentrations in corn but not soybean. Soybean and corn seed S was significantly increased but not seed N. Soybean yield was not increased, but corn yield was significantly increased 16% across all S rates, with no significant differences detected among S rates. Overall, results indicate N affected soybean growth and nodule development while the impact on yield was not demonstrated. In deficient soils, S fertilizer may improve corn yields, but may not be economical for soybean.

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Introduction

Crop History and Production

Soybean

Soybean (*Glycine max*) is an annual legume belonging to the family Fabaceae. The origin of this crop is not clear, but botanists believe it is derived from *Glycine ussuriensis*, which is a legume native to central China (Editors, 2014). Cultivation of this crop in China began more than 5,000 years ago for food and medicinal purposes (U.S. Soybean, 2006). It was not until after the Chinese-Japanese war of the mid-1890s that this crop became localized in China (Benson and Gibson, 2005). In 1908, soybean finally attracted the world's attention after shipments were made to Europe (Benson and Gibson, 2005).

In 1804, a Yankee clipper ship coming from China first brought soybeans to the U.S, and U.S. farmers first grew soybeans in 1829 (U.S. Soybean, 2006). However, soybean may have been introduced to the American Colonies as early as 1765 as "Chinese vetches" (Benson and Gibson, 2005). Therefore, early soybean cultivation in the U.S. was probably for forage rather than seed production (Benson and Gibson, 2005).

In 1904, George Washington Carver at the Tuskegee Institute in Tuskegee, Alabama, began to investigate the crop. His research led to the discovery of the valuable protein and oil that the bean provided, which changed the way people viewed the crop as a forage (U.S. Soybean, 2006). It was not until the 1920's that soybean acreage greatly expanded into the U.S. Corn Belt (Benson and Gibson, 2005).

Most edible fats and oils were imported into the U.S. before World War II. Due to trade disruption at the start of the war, this oil supply was cut, resulting in the need for a new source for oil. Therefore, U.S. processors turned to soybean oil to fill the demand. The U.S. soybean crop expanded to 212 million kilograms produced on 2 million hectares by 1940 (Benson and

Gibson, 2005 and U.S. Soybean, 2006). In the 1980's, the U.S. became the world's dominant soybean producer, with China and Brazil following. Soybeans have become the United States' second largest cash crop and number one export crop. More than half of the absolute value of the crop comes from exports of whole soybeans, soybean meal, and soybean oil; resulting in 40% of the world's soybean trade originating in the United States (Benson and Gibson, 2005). Good and Irwin (2014) reported that the United States' average soybean yield nearly doubled from the early 1960's to early 2010's, from 1,549 kg ha^{-1} to 2,897 kg ha^{-1} as producers increased production by an average 25 kilograms per hectare per year since 1960. In 2013, Tennessee ranked 15th nationally for soy production, producing more than 1.9 billion kilograms of seed (Cook, 2015). In recent years, soybeans have replaced some cotton acreage in the west Tennessee region because of declining cotton prices and higher input costs. Of the 28,340,080 U.S. hectares planted in soybean, 404,858 of those hectares are in west Tennessee counties, with Dyer, Obion, Gibson, Lauderdale, and Weakley counties leading in production (Fuqua, 2011; McBryde, 2016). With the development of higher yielding cultivars containing herbicide tolerant traits, soybean production has increased dramatically in the last few decades. Tennessee soybean yields in the 1960's were 1,345 to 2,017 kg ha^{-1} while growers can now produce a yield of 4,034 kg ha^{-1} on the same acreage (Fuqua, 2011).

Corn

Corn (*Zea mays L.*) is an annual grass crop belonging to the Poaceae family, which is known throughout most of the world as maize. Corn is thought to have originated from its wild grass ancestor, teosinte (Beadle, 1932). However, it is unclear if early selective breeding by farmers or natural selection resulted in the off-type that is produced today (Galinat, 1988; Mabberly, 1997). Cultivation of this crop first began in Mesoamerica more than 8,000 years ago

(Galinat, 1988). Native Americans taught European colonists to grow the indigenous grain, and, since its introduction into Europe by Christopher Columbus and other explorers, corn has spread to all areas of the world suitable for its cultivation (Editors, 2016).

Corn is the primary United States feed grain, accounting for approximately 95 percent of total grain production and use (USDA, 2015). More than 36 million hectares are planted annually (USDA, 2015). During the 2014-2015 crop marketing year, the United States grew 360 million tons, and 13 percent of the production was exported to more than 100 different countries (U.S. Grains Council, 2016). In recent years, corn has become the second most important grain crop in Tennessee, following soybean. Tennessee ranks 17th nationally for corn production, and produced 720,000 tons in 2015 (USDA, 2015). Of the 36 million U.S. hectares planted in corn, 315,655 of those hectares are in the state of Tennessee, with the western counties of Obion, Weakley, Gibson, Henry, and Dyer leading in production (UTCrops, 2016).

Crop Growth and Development

Soybean

Soybean cultivars can display either a determinant or indeterminate growth habit. A determinant growth habit means that vegetative growth and reproductive growth happen at separate times during the season, while indeterminate means that vegetative growth overlaps with reproduction (ISU, 1985). Vegetative growth is described in “V” stages, and reproduction in “R” stages. Vegetative growth begins with germination and emergence of the cotyledon followed by the production of first the unifoliate then trifoliate leaves at main stem nodes. The number of leaves and nodes produced will vary due to the specific variety, planting date, and environment (ISU, 1985). According to Fehr et al. (1971), once the soybean starts to flower, it is considered being in a reproductive (R) growth stage. Reproduction begins with flowering and ends at maturity and takes place over 8 stages: beginning bloom (R1), full bloom (R2), beginning pod (R3), full pod (R4), beginning seed (R5), full seed (R6), beginning maturity (R7), and full maturity (R8). The length of time for reproductive development varies depending on several factors including temperature, maturity group, and photoperiod or day length. The maturity group classification for soybean cultivars in the U.S. is based on development response to photoperiod (Heatherly and Elmore, 2004). Day length is the main driving factor for flower initiation and soybean reproductive development, because short days (long dark periods) initiate the flowering process. The extent of the required dark period varies among different maturity groups (Holshouser, 2010).

Corn

Corn (*Zea mays*) displays a determinant growth habit, meaning that the vegetative and reproductive stages happen at separate times (Ritchie et al., 1993). The vegetative growth stages are referred to as “V” stages, and the reproductive stages are referred to as “R” stages. The vegetative stages begin with germination and emergence of the coleoptile and continues through each “V (n)” stage, where (n) represents the last leaf stage before the emergence of the tassel (Ritchie et al, 1993). V (T), or tassel emergence, is the final vegetative stage when the whole tassel is visible and in modern hybrids pollination begins shortly before silking. Corn reproductive development from silking to maturity takes place over 6 stages; silking (R1), kernel blister (R2), kernel milk (R3), kernel dough (R4), kernel dent (R5), and physiological maturity (R6) (Ritchie et al., 1993). Photoperiod is not a main component in corn development as it is in soybean; rather, corn is dependent on growing degree units or heat accumulation (Shaw, 1988). The amount of heat required for development varies among different hybrids.

Nitrogen Demand and Fixation Ability in Soybean

According to Shober and Taylor (2014), the nitrogen (N) demand for soybeans is relatively high due to the large protein content in the seed. Soybean N removal in the seed is estimated at 157 kg ha^{-1} for yields of 2,689 kg ha^{-1}, while an irrigated crop yielding 4,706 kg ha^{-1} would remove about 272 kg N ha^{-1} in the seed. Soybeans are a leguminous crop, meaning the plant provides itself with N through a symbiotic relationship with nitrogen-fixing bacteria of the species *Bradyrhizobium japonicum* (Franzen, 1999). Symbiotic dinitrogen (N_2) fixation is the biological process by which the atmospheric dinitrogen gas (N_2) is converted to ammonia with the aid of a key enzyme called nitrogenase (Sulieman and Phan Tran, 2014). Through this symbiotic relationship, photosynthetically-derived carbohydrates and minerals are supplied to the bacteria; and, in exchange, the bacteria supply the fixed nitrogen to the legume host. This process is accomplished through bacteria living inside the cells of *de novo* formed organs, the nodules, which usually develop on the roots of leguminous plants (Sulieman and Phan Tran, 2014). On average, N_2 fixation provides 50-60% of N needed in soybean, with the rest coming from nitrate and ammonium N in the soil (Salvagotti et al., 2008).

Legumes are a family of dicotyledoneous plants, most of which form a symbiosis in their root systems with N fixing microbes in specialized nodules (Bruning and Rozema, 2013). N_2 fixation is extremely energy intensive for the plant, because of the energy required to break the triple bond of dinitrogen gas. Therefore, if high levels of N are already present in the soil, the plant will reduce the fixation process. Legume nodules are complex organs, containing several interacting processes that operate at specific levels, including; nodule formation, carbon metabolism, oxygen supply, cellular redox, and transmembrane transport (Sulieman and Phan Tran, 2014).

Nodule formation requires several steps. Scheaffer and Moncada (2012) state that hairs first protrude from the roots and release root exudates, which are chemical signals to the rhizobia. The exudates attract certain rhizobia to the root. Next, the hairs entrap the rhizobia by curling around the bacteria. Then, the rhizobia break down and digest the cell wall of the hairs resulting in the infection of the root by the bacteria. The rhizobia infect by forming an infection thread in the center of the root, where it divides and multiplies. Then, the bacteria cause the root cells to divide, resulting in the formation of a nodule from the swelling of the root cells to the surface of the root. Once the rhizobia are dwelling inside the nodules, they lose their cell wall; therefore, becoming bacteroids, which develop a nitrogenase enzyme to fix atmospheric N. Nitrogenase is irreversibly damaged when exposed to oxygen (Bruning and Rozema, 2013). The host plant produces leghemoglobin, which is a protein related to the human hemoglobin, to regulate the oxygen levels in the nodule. If the nodules are active, the inside of the nodule will possess a pink pigment, which is the leghemoglobin (Sheaffer and Moncada, 2012). Nodules on annuals, such as soybean, are short lived and will be constantly replaced throughout the growing season. At the time of pod filling (R5), the legume host focuses on the development of the seed, providing less energy to nodules, which leads to the loss of the N fixing ability of the nodules (Flynn and Idowu, 2015).

Legumes are important both ecologically and agriculturally because they are responsible for a considerable part of the global flux of N, from atmospheric N_2 to fixed forms such as ammonia, nitrate, and organic N (Zahran, 1999). The maximum N_2 fixation potential by soybean is estimated to be 337 kg ha^{-1} under ideal environmental conditions (Shober and Taylor, 2014). The majority of the fixed N is available for the crop, but the rest is incorporated into the soil microbial cycle and eventually lost by denitrification and leaching as nitrate (Bruning and

Rozema, 2013).

Role of Nitrogen and Nitrogen Fertilizers

Dinitrogen fixation is one way to provide a crop or plant with adequate N or add to the soluble soil N pool. Additionally, soil organic matter is continuously decomposed by soil microbes which, in return, release plant available N into the soil solution. West Tennessee's silt loam soils have approximately 1.3% soil organic matter (NRCS, 2017).

Commercial fertilizers are important sources of N in the production of some crops. Crop removal, nutrient leaching, volatilization, and erosion are some of the main reasons for nutrient loss in soil (Simplot, 2016). Nitrogen, phosphorus, and potassium are the three macronutrients for plant viability. Plants cannot use the elemental form of any nutrient; it must be in a reduced state. Plants can only take up N when it is the form of nitrate (NO_3^-) or ammonium (NH_4^+). Of these two forms, nitrate is absorbed in the largest quantity by the plant root system (Mugaas, 2011).

Nitrogen has many key roles within the plant. Adequate N promotes vegetative growth, which aids in the recovery from injury and environmental stresses. Nitrogen is also a key factor in the production of chlorophyll, which gives the plant its green pigment (Mugaas, 2011). It is also necessary for the synthesis of amino acids into proteins and for regulation of the uptake of other nutrients, and it is the basic ingredient of nucleic acids and enzymes (Simplot, 2016). High demand for N may result in the soil being left deficient of adequate N for plant productivity during the growing season. Inadequate levels of N in the soil solution will slow plant growth, decrease photosynthetic capacity and food production, slow injury recovery time, and decrease tolerance to stresses (Mugaas, 2011).

Urea and Urease

Fertilizer urea has surpassed ammonium nitrate as the most popular worldwide N fertilizer because of its lower production and transportation costs. According to D.W. James (2010), like other commercial N fertilizers, urea is manufactured from anhydrous ammonia (NH_3). The high N content of urea (46% N) is the main reason for the lower cost of this N fertilizer form. Freight, storage, and handling costs are all less than lower N containing fertilizers such as ammonium nitrate (34% N) and ammonium sulfate (21%). The chemical formulation for urea is $(NH_2)2CO$, therefore, this synthetic fertilizer is an organic form of N. Urea is the first organic compound to be artificially synthesized through chemical processes using inorganic compounds (Agro-Products, 2008). Freidrich Wohler carried out the chemical research in 1828, at which time he concluded that potassium cyanate, when treated with ammonium sulfate, produces urea. Wohler's discovery helped bring about the organic revolution. According to Lorenc (2008), Wohler's landmark achievement was that until then only living organisms were believed to be able to produce organic compounds, and these compounds were thought to be special, requiring a crucial force to create them. Therefore, Wohler bridged the gap between the living and non-living worlds.

Urease is an enzyme that is mainly found in seeds, micro-organisms, and is in nearly all soils (Lorenc, 2008). Lorenc (2008) states that when the substrate urea is present in the soil, via urine or fertilizer, soil microbes feed on the urea, producing the enzyme urease that transforms urea to ammonium bicarbonate. Urease is known to hydrolyze urea to ammonium bicarbonate at rates that are approximately 10^{14} times the rate of uncatalysed reactions (Upadhyay, 2012). Once dissolved in water, urea is converted to ammonium bicarbonate within a few days following the application of a naturally occurring enzyme called urease (IPNI, 2016). When the hydrolization of urea by the urease enzyme occurs, much of the ammonium that is created is held on soil cation exchange sites, but may be lost through volatilization if it converts to ammonia, sometimes resulting in a loss of up to 50% (IPNI, 2016).

Urease Inhibitors

According to Sutton (2005), urease inhibitors block the conversion of urea to ammonium for a period of one to two weeks, allowing time for incorporation of the fertilizer into the soil by rainfall or other means. Urease inhibitors are available as a granule or prill treatment or as a slow release fertilizer formulation. According to the IPNI (2016), the most popular fertilizer treatment type of urease inhibitor is N-(n-Butyl) thiophosphoric triamide (NBTPT or NBPT), which is the active ingredient of the commercial product, Agrotain™. According to Robertson and Vitousek (2009), slow release fertilizers commonly are pelletized formulations coated with a substance or membrane that slows solubility. Sulfur coated urea is the oldest of these technologies, produced by coating urea pellets with a layer of molten sulfur that is additionally coated with a sealant. The polymer coating creates a semi-permeable membrane that slows the nitrification process. Sutton (2005) states that certain urease inhibitors contain additional ingredients that function as nitrification inhibitors, whereby the conversion of ammonium (NH_4^+) to nitrate (NO_3^-) is slowed by reducing or interfering with the metabolism of nitrosomonas and nitrococcus bacteria. Therefore by deterring thses bacteria populations, the conversion of ammonium to nitrate is significantly reducedSutton (2005) further states that the different inhibitor technologies are used to make efficient use of urea fertilizers, reduce nitrate run off and leaching, and reduce ammonia and greenhouse gas emissions.

Applying Nitrogen to Nitrogen Fixers

Some researchers suggest that N fertilization is not necessary for inoculated soybean (Freeborn et al., 2001; Schmitt et al., 2001; Barker and Sawyer, 2005; Sogut, 2006); whereas others indicate that biological N fixation is not sufficient to meet the N demand of the high yield crop, and N fertilization is necessary to improve yield and quality of soybean at certain application times or rates (Purcell and King, 1996; Gan et al., 2002, 2003; Osborne and Riedell, 2006; Ray et al., 2006).

According to Salvagiotti et al. (2008), in order to achieve high yield potential, soybean must maintain high photosynthesis rates and store large amounts of N in seeds. Thus, an ideal crop canopy must enable full light interception and adequate storage of N in leaves to maintain photosynthesis for converting incoming radiation into new biomass and, eventually, grain yield. Dinitrogen fixation and mineral soil or fertilizer N are the main sources of meeting the N requirement of high yielding soybeans. Many factors will affect the response of the soybean to N fertilization, such as temperature, soil type, soil water and organic matter content, and genotype (Caliskan et al., 2008). Maximum N_2 fixation occurs between the R3 and R5 stages of soybean development, and any deficiencies between crop N demand and N supply by N fixation must be satisfied by N uptake from other sources (Salvagiotti et al., 2008).

When adequate soil N is available then N fixation is inhibited, but there are some situations when N fertilization is helpful to the plant development. Hardason et al. (1984), suggests that during the early period of plant growth, when nodules have not fully developed, the young plant depends on soil N and N stored within the cotyledons for normal growth. If the soil N is inadequate to meet the needs for the seedling, growth can be stunted. Therefore, a low rate of N might be beneficial to encourage both growth and N_2 Fixation. Furthermore, as soybean roots and nodules age, their ability to fix N can be significantly reduced compared to that of early season N_2 fixation levels. Thus, foliar applications of N could possibly supplement an N deficit, particularly in a high yield situation. Hodgins et al. (2015) stated that increasing soybean yield goes hand in hand with a larger N demand. The ability to maintain N fixation by the rhizobia during the late season can be difficult and can impede the crop's ability to supply all of the N required for maximum grain filling and seed N content. Although Hodgins et al., (2015) did not significantly increase soybean yield with late season foliar N applications, there is justification for further research to investigate the effects of N applications on a soybean crop.

Potential Effect of Nitrogen Fertilization on Nitrogen Fixation

Salvagiotti et al. (2008) reviewed 108 studies and a total of 637 data sets, which showed a negative exponential relationship between N fertilizer rate and N fixation when N was applied to the soil surface. The results indicated that when zero N was applied, the maximum rate of N_2 fixation was reached (337 kg N ha^{-1}) but where 100 or 300 kg ha^{-1} of N fertilizer was applied, fixation rates only reached a maximum of 129 and 17 kg N ha^{-1}, respectively. Some studies reviewed by Salvagiotti showed that N placed at 20 cm and deeper had a decreased inhibitory effect on fixation. A potential problem with deep placement of N is it could be lost by leaching before the plants are able to reach the N for use (Salvagiotti et al., 2008). Considering deep placement of N is only practical before planting or during early growth stages, a more practical way to minimize the inhibition of N fixation could be to use a slow release N source, such as a polymer coated urea.

Caliskan et al. (2008) conducted an experiment on effects of N and iron fertilization on growth, yield, and fertilizer efficiency of soybean in a Mediterranean type soil with a high pH. Half of the N treatments were broadcast applied by hand immediately before planting, and the second half was applied at full flowering (R2). Urea was used as the N sources in both applications. Soybeans responded to N fertilization, although a decrease in seed yield was found with the combination of a higher N (>80 kg ha^{-1}) and iron (400 g ha^{-1}) rate. Their study also showed that both leaf area and biomass growth or photosynthetic ability (µmol m^2 s^1) can be increased with N fertilization. When photosynthetic rates are increased with the rate of N fertilization, the N can be credited with increasing the amount of chlorophyll pigments, because N is one of the main ingredients of chlorophyll. With N fixation beginning around the V2 growth stage or about three weeks after planting, an application of a small starter dose of N may promote early growth and yield in many circumstances (Caliskan et al., 2008). Caliskan et al., (2008) found that application of a starter N fertilizer improved early growth of soybean plants at N rates from 20 to 40 kg ha^{-1}, but biomass differences due to rates were not observed, probably due to the short time between emergence and sampling. Yield increased as N dose increased under zero Fe conditions, but decreased with increased N dose over 80 kg ha^{-1} at high (400 g ha^{-1}) Fe rates. The digression was due to the reduction of N fixation ability in a high N environment because of the iron. In all, their research pointed out that N fertilization before planting and during early reproductive stages can promote early growth and, ultimately, yield of inoculated soybean in a Mediterranean environment when N is under the influence of high iron fertilizer applications (Caliskan et al., 2008).

Other studies revealed higher amounts of N in a starter dose can be detrimental to N fixation, i.e. infection, nodulation, or the N fixing capability of the *Bradyrhizobium* bacteria, but do not significantly affect yield (Beard and Hoover, 1971; Koutroubas et al., 1998). However, Afza et al., (1987) reported that lower N rates applied at pod filling (less than 40 kg/ha) did not inhibit N fixation and increase yield by approximately 37%. A Mississippi concluded that applying a high rate of N (>291 kg N ha^{-1}) to supplement fixed N, increased soy yields over the zero N check by 327 and 442 kg ha^{-1} under irrigated and non-irrigated growing conditions, respectively (Ray et al., 2006). This study, therefore, revealed that N fixation may not be satisfactory for maximizing soybean yield, however Heatherly (2004) concluded that supplementing fixed N with fertilizer N is not profitable.

According to Schmidt, as soybean yields continue to increase and yields in the 4,034-5,379 kg ha^{-1} range and higher become more common, N fixation and soil N mineralization will reach capacity in many growing environments. Thus, an increasing number of N shortfalls are almost certain to occur (Schmidt, 2016).

Economics is the main driving factor in row crop agriculture. If producers are not able to make a larger profit by adding another input into their system, the additional input will be discarded. Additional N fertilizer in a soybean production system has been viewed as not economical for many years because of the legume crop's ability to conduct N_2 fixation.

However, as stated before, modern soybean cultivars are requiring more N for a variety of reasons. Wesley et al. (1998) significantly increased irrigated soybean yields when four different N sources, including urea, were applied at 23 or 45 kg N ha^{-1} on a silt loam soil at eight different sites in Kansas. In this study it was found that producers would benefit from a mid-season application (R3) of N at 23 kg N ha^{-1}. The economic analysis indicated a producer would increase profit by \$104.50 ha^{-1} at 1998 N prices and a soybean price of \$17.30 ha^{-1}, which is significantly lower than the current years' price of \$24.70 ha^{-1}. N cost was assumed to be \$0.66 kg^{-1}, whereas, in recent years N cost has slightly increased to \$0.79 kg^{-1} (Smith et al., 2017), and with the addition of Agrotain Ultra™ the N cost per hectare increases to approximately \$1.00 ha^{-1}. Although input costs have increased in recent years, if optimal timing and N rate to increase yield is identified, N fertilizer additions to a soybean crop may become profitable for Tennessee producers in the near future.

Role of Sulfur in Soybean and Corn Production

Among the 17 essential nutrients for plant growth, sulfur (S) is a requirement for all crops. It is a secondary macronutrient, behind N, phosphorus, and potassium. A balance of these nutrients is essential in accomplishing optimum plant health and yield goals. Soybean requires 0.0058 kilograms S per hectoliter and corn requires 0.0015 kilograms S per hectoliter (Mosaic, 2014; Davidson, 2015). As stated by Place et al. (2007), in the soil, S can exist as organic S compounds, sulfides (S^-), elemental sulfur (S), and sulfate (SO_4^{2-}). Most of the S in the soil is found in soil organic matter (SOM), which is not readily available to plants; therefore, it must undergo a mineralization process to be converted to sulfate for plant uptake (Place et al., 2007). In the sulfate form, it plays an important role in protein synthesis and is essential for many different plant processes since it is a main component of amino acids, proteins, and peptides. Also, S is important for the formation of chlorophyll as well as the success of nodulation and N fixation in soybeans. According to Davidson (2015), S is a component of ferredoxin, an iron-S protein found in the chloroplasts. Ferredoxin also plays a metabolic role in both N fixation and sulfate reduction and in the absorption of N by rhizobacteria living in the nodules. Root nodules are high in protein, and nitrogenase has iron and S cofactors. Therefore, S, being a component of two amino acids, can limit N fixation and, ultimately, yield in legumes (Davidson, 2014).

Sulfur and Nitrogen

Like N, S can leave the soil solution by plant uptake, leaching, and volatilization, which soil disturbance increases (Place et al., 2007). Deficiency symptoms of N and S are often mistaken for each other. Both deficiencies exhibit interveinal chlorosis and stunted plant growth. However, S is considered an immobile nutrient inside the plant, meaning that the plant cannot easily move it to where it is needed the most, i.e. younger tissue. Thus, S deficiency symptoms will be seen in the younger tissue before the older tissue. Conversely, N is very mobile in the plant, and symptoms will be seen in the older tissue before the younger. According to Agrisolutions (2011), an adequate balance between N and S is vital to maintain maximum N use efficiency, plant vigor, water use efficiency, phosphate use, carbohydrate production and utilization, rate of grain fill, and maturity. The ratio is a result of the close relationship between S and N in the production of key plant proteins.

Why Apply Sulfur?

Nutrient uptake patterns indicate S is accumulated more during the grain filling period than the vegetative growth stages. Therefore, S deficiency in the late season could result in the loss of yield. While a considerable portion of most nutrients that are taken up by grain is assembled from plant tissues, most S in grain is taken up from the soil (Bender et al., 2013; Hest, 2014). Roberson (2012) writes, prolonged rainfall events cause accelerated leaching of sulfate from the soil profile. In result of the S being water soluble and mobile, the S in the upper soil profile will leach into the lower rooting zone. Typically S will accumulate in subsoil horizons containing more clay, which could be up to 46 or more centimeters below the soil surface. Roberson (2012) continues, another factor that affects S plant availability is the size of the plant root system. Once germination has occurred, the corn seedling relies on the radicle as the primary root system. Two weeks after germination, the radicle degrades and seminal roots form from the first node of the corn plant. Seminal roots are 1.3 cm or less deep in the soil surface and will grow deeper over time. Thus, during early season, shallow roots may not effectively locate soil S in the upper soil profile.

Soybean have largely been proven the opposite. Lawson (2012) investigated soybean response to S fertilization in Iowa on an irrigated coarse sand soil with low organic matter. Calcium sulfate was used as the S source and applied at 0, 11, 23, 46 kg S ha^{-1}. It was found that overall foliage S concentration was increased with increased S rate, but yields were not significantly affected. Therefore, there was no economic return for the S applications to the soybean crop. However, Boem et al. (2007) found that fertilizing soybean with gypsum at 15 kg S ha $^{-1}$ and ammonium sulfate at 15 kg S ha $^{-1}$ or 13 kg N ha $^{-1}$ on loam, sandy loam, and silt loam soils in Buenos Aires, Argentina resulted in a yield increase. Soybean seed yields were increased with the addition of S at all sites, indicating that S deficiency can negatively impact soybean yield.

Sawyer et al. (2011) found that corn yield in Iowa was increased by S applications. Gypsum was surface broadcast-applied shortly after planting at 0, 11, 23, and 46 kg S ha $^{-1}$. Several locations throughout Iowa were evaluated on both fine-textured and coarse-textured soils. The yield increase for the fine-textured soils averaged 1021 kg ha $^{-1}$, at the maximum S rate of 11 kg S ha $^{-1}$ as gypsum. However, Wortmann et al., (2009) reported, over a three year period, no yield increase with S of 20 kg ha^{-1} on either loamy sand, sandy loam, silt loam, or silty clay loam soils. Therefore concluding that S fertilization was likely to not increase corn yields on medium or fine textured soils or on sandy soils with more than 10 mg kg^{-1} soil organic matter (Wortmann et al., 2009).

Justification for Fertilizer N or S

In the last 20 years or so, soybean hectares have dramatically increased, and yield has also increased with the years. Although yields have shown great improvement, even greater improvement is needed. These improvements must be accomplished quickly and efficiently, in order to meet the dietary needs of 1 x 10^{10} people expected to occupy the earth by 2050 (Specht, 1999). Cropland is becoming more limited; therefore, an increase in production per acre seems to be one way to meet demand. Nontraditional practices, such as applying N fertilizers to soybean for yield optimization, might be one of many solutions to fill this demand. Higher yielding varieties on the market, stress conditions hindering the N fixation process and N solubility in the soil solution, the requirement for a greater quality grain, and the ambition to improve the livelihoods of the American farmers are the main reasons for pursuing this nontraditional management practice.

Davidson (2014) contends that S has become a more important limiting nutrient in production for several reasons; including, higher crop yields that require more S, fewer S impurities in modern fertilizers, less use of S-containing pesticides, reduced S emissions to the atmosphere, and soil organic matter levels that are too low to provide enough S. Tennessee's switch to conservation tillage (no-till) in the 1970s, might have a hindering effect on S availability in the spring, due to cooler soil temperatures and no-till reducing the rate of decomposition of organic matter, which is the main source of soil sulfate (Morrison, 2009). Thus, these S shortfalls indicate a need for further research to provide Tennessee producers with information to maximize their soy and corn crop.

The University of Tennessee has currently done minimal research on the effects of N on soybean and the impact of an S application on soybean or corn yield. Therefore, research which focused primarily on soybean, generated data that will be used to develop appropriate N and S recommendations for Tennessee producers.

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Part II.

Effect of Nitrogen Rate and Timing on Soybean (*Glycine max L.*) Growth, Development, and Yield

Abstract

Although soybean (*Glycine max*) yields have increased over the past decade, even greater improvement is in demand. Nontraditional practices such as applying nitrogen (N) to soybean for yield optimization, might be one of many solutions to fill this demand. Dryland double crop and irrigated field environments were used to evaluate various N application rates and timings for soybean (*Glycine max L.*) yield optimization in 2015 and 2016 at Milan and Jackson, TN. Urea was broadcast-applied by hand at-planting, V2 or R2 stage soybeans. Application rates were 34, 67 or 101 kg N ha and a zero N control was included for comparison. Early and mid-season plant measurements of biomass, height, node number, whole plant and leaf N concentration, as well as seed weight, and yield were significantly affected by both year and environment. N treatment significantly increased early and mid-season plant height, early season biomass, mid-season plant node numbers, and early season whole plant N. At planting and V2 applied N only numerically increased whole plant early season N levels, and did not affect mid-season plant levels or leaf N levels. Early and mid-season nodule numbers were not significantly decreased by N fertilizer treatments within dryland or irrigated environments, suggesting that N rates did not have a strong impact on nodulation at these application timings. However, applications of N at-planting or V2 may have delayed nodulation, significantly reducing early season average nodule weights which decreased as N rate increased. Early season active and adolescent nodule numbers per plant were significantly reduced with increasing N rate. Irrigated soybeans were taller in both 2015 and 2016, and with addition of N treatments, height increased as N rate increased. Dryland environment produced greater early season biomass with some N treatments compared to the zero N control than the irrigated growing conditions. In 2015 and 2016 total nodule numbers and early season nodule weights were lower under irrigation compared to

dryland growing conditions Seed weights in the dryland tended to be larger than the irrigated environment in both years. Only year and environment affected yield. The high yield environment (irrigated) yielded at or below the dryland, probably due to lodging, which may have compromised yield potential and may explain why N treatments did not affect soybean yield. Results indicate N affected soybean growth and some aspects of nodule development while N impact on yield was not demonstrated. A clear yield benefit is necessary to offset N fertilizer cost and make N fertilizer supplements a profitable practice.

Introduction

Soybean has become a top cash crop in the United States (U.S.) and Tennessee's number one row crop in recent years (UTCrops, 2015). Even though soybean yields have increased substantially since first being cultivated in the U.S., there is some interest in incorporating nontraditional practices such as N (N) applications into a soybean cropping system in order to support high yields. As available cropland decreases, producing more on a limited amount of land remains a priority as long as inputs are economical and justified.

Being a leguminous species, soybean provides itself with N through a symbiotic relationship in root nodules with rhizobium bacteria, *Bradyrhizobium japonicum;* therefore, mineral N additions are not normally needed. The majority of the plant's total N content is derived from the dinitrogen fixing rhizobia (Bruning and Rozema, 2013) with the remainder taken up from available soil N. Through the symbiotic bacterial relationship, dinitrogen gas (N_2) is reformed into ammonia with the assistance of a crucial enzyme, nitrogenase (Sulieman and Phan Tran, 2014). Through the plant-bacterial affiliation, photosynthetically-derived carbohydrates and minerals are furnished to the rhizobia in exchange for the fixed N from the bacteria. This process is achieved through rhizobia living inside the cells of *de novo* formed organs or nodules, which form on the roots of leguminous plant species (Sulieman and Phan Tran, 2014).

Two different N sources available to soybeans are the N fixed by the plant and the N contributed by soil organic matter. A typical soybean plant provides itself with 50-60% of N needed, with the rest coming from nitrate and ammonium N in the soil (Salvagiotti et al., 2008). Soil organic matter is constantly decomposed by soil microbes, resulting in the release of plant available N into the soil solution.

Nitrogen is necessary for a variety of plant growth and development processes. The most important of these processes is stimulating vegetative growth, being a main factor in chlorophyll production, and assisting in environmental stress, injury, and disease recovery (Mugaas, 2011). Also, N is a component of amino acids for proteins, balances the uptake of other nutrients, and is a key element in critical compounds, such as nucleic acids and enzymes (Simplot, 2016). If the N level is deficient in the soil solution and not supplied through N fixation, the result will be decreased plant productivity of legumes, decreased photosynthetic capacity and food production, slow injury recovery time, and reduced tolerance to stresses (Mugaas, 2011).

With the higher yield potential of modern legume cultivars, more N is required from the bacterial relationship and soil solution; thus, supplementing the difference between crop demand and N supply with mineral fertilizers may be necessary to reach modern yield goals. Some researchers have proposed that N fertilization is not needed for a normal inoculated soybean crop (Salvagiotti et al., 2008), but others have suggested that biological N fixation and soil uptake of N is not enough to meet crop demand (Caliskan et al., 2008). For soybean to reach higher yield goals, the crop must sustain high photosynthetic rates and accumulate large amounts of N in the seeds (Salvagiotti et al., 2008). Maximizing canopy development permits full light interception and sufficient storage of N in leaves to manage photosynthesis that is not limited by N to transform solar radiation into new biomass and ultimately grain yield (Salvagiotti et al., 2008). Maximum biological N fixation occurs between the R3 and R5 growth stages of soybean; therefore, any shortages between crop demand and N fixation supply must be supplemented by N uptake from other sources (Salvagiotti et al., 2008).

However, when the soil solution is immersed with N, N fixation rates may be reduced because uptake of soil N requires less energy than dinitrogen fixation by the plant and high N rate supplements may be detrimental to nodulation and N fixation by plant. Dinitrogen fixation is highly energy intensive for the plant, because of the necessary energy required to break the triple bond of dinitrogen gas. Therefore, if high levels of N are already present in the soil, the plant will reduce the fixation process (Sulieman and Phan Tran, 2014).

Currently, contemporary research is focused on how soybean yields can be bettered through N supplements. A 2008 review of N rate and timing studies (Salvagiotti et al.,) indicated there may be a negative relationship between N fertilizer rate and N fixation when N was applied to the soil surface. The results indicated that where zero N was applied, maximum N_2 fixation reached 337 kg N ha^{-1}; but where 100 or 300 kg ha $^{-1}$ of N fertilizer was applied, fixation rates only reached a maximum of 129 and 17 kg N ha $^{-1}$ respectively. Caliskan et al. (2008) conducted a study on effects of N and iron fertilization on growth, yield, and fertilizer efficiency of soybean in a Mediterranean type soil with a high pH. Soybeans responded to N fertilization, although seed yield decreased a combination of a higher N (>80 kg ha^{-1}) and iron (400 g ha^{-1}) rates. Soybean yield increased as N dose increased under zero Fe conditions, but decreased with increased N dose over 80 kg ha^{-1} under 400 g ha^{-1} Fe application. Beard and Hoover (1971) concluded that higher amounts of N in a starter dose can be detrimental to the N fixation system but at the same time does not significantly affect yield. By contrast, Afza et al. (1987) reported that lower N rates (less than 40 kg ha^{-1}) did not inhibit N fixation and increased yield of soybean by approximately 37%. A Mississippi study, conducted by Ray et al. (2005), concluded that applying a high rate of N (>291 kg N ha^{-1}) to replace fixed N increased soybean yields above the zero N check by 327 and 442 kg ha^{-1} in irrigated and non-irrigated environments, respectively. Although results revealed N fixation by itself may not maximize soybean yields, Heatherly (2004) concluded that replacing fixed N with fertilizer N is not profitable.

As soybean yields steadily increase and 4,034-5,379 kg ha^{-1} and greater yields become more common, biological N fixation and soil N mineralization will reach capacity in numerous growing environments. N deficiencies, particularly in high yield environments, may become more common. Therefore, the objective of our research was to determine N rate and timing that may increase seed yield of soybean as a profitable production practice.

Materials and Methods

Irrigated and non-irrigated field experiments evaluating N application rates and timings were conducted in 2015 and 2016 at the Milan Research and Education Center in Milan, TN (35.9198° N, 88.7589° W) and in 2016 at the West Tennessee Research and Education Center in Jackson, TN (35.6145° N, 88.8139° W) These experiments employed a randomized complete block design with separate plots consisting of four rows measuring 3 m wide and 9 m long, with a row spacing of 76 cm in 2015, and six rows measuring 4.6 m wide and 9 m long, with a row spacing of 76 cm in 2016. The irrigated sites were located in Milan on a Loring silt loam (2015) and a Falaya silt loam (2016), and the non-irrigated sites were located in Milan (2015) and Jackson (2016) on a Lexington silt loam. Weeds and pests were controlled using standard University of Tennessee recommendations both years (Steckel, et. al., 2016; Stewart and McClure, 2016).

The N optimizing experiment included two environments, irrigated or high yield and non-irrigated. The irrigated test was planted on May 7, 2015 and May 8, 2016; the non-irrigated test was planted on June 18, 2015 and June 13, 2016, behind a wheat crop. Asgrow 4632 (Monsanto Company; St. Louis, MO) was the soybean variety tested both years. Seed were planted at a depth of 2.5 cm and at a population of 345,800 seeds ha^{-1}.

Before planting in 2016, a composite 15-cm soil sample from each irrigated plot was collected for soil N analysis to estimate soil N contribution. These samples were analyzed by Brookside Laboratories, Inc. in New Bremen, Ohio using the weight loss on ignition method for organic matter N and Mehlich III extraction method for available N expressed as kilograms per hectare (Table 3). Soil samples from the dryland environment were not collected.

Agrotain Ultra™ treated urea (46-0-0) was applied to both soybean environments in four treatments at different growth stages both years. The four treatments were 0, 34, 67, and 101 kg actual N ha $^{-1}$ evenly broadcast applied by hand at-planting, at the second vegetative growth stage (V2), and at the second reproductive growth stage (R2) (Tables 1, 2). Also, in 2015, 45 kg ha^{-1} of phosphorus (P) and potassium (K) were applied to the irrigated field due to low P and K levels detected in soil sample analysis. However, the dryland environment had high P and K levels so none was added. In 2016, the irrigated field was high in P and K so none was added, but the dryland had 54 kg N ha^{-1}, 101 kg P ha^{-1}, 112 kg K ha^{-1}, and 18 kg sulfur (S) ha^{-1} applied the winter of 2015 due to low levels of P, K, and S. The N was applied for the reason that winter wheat was grown prior to the soybean crop.

In 2015 and 2016, soybean node number, plant heights, dry plant biomass weights, and leaf and whole plant tissue N (2016 only) were determined early and mid/late season to measure the effects of the N applications on plant growth and development. To identify N treatment effects on yield, 100 seed weights and yield were recorded at harvest. To determine potential N treatment effect on root nodulation, visible nodules were counted mid-season at R1 to R2 growth stages, and mid/late season counts were made when soybeans were at R4 to

R5 growth stages. In 2016, nodule internal color and weights were recorded as part of the early season nodule data collection.

For in-season measurements, 10 plants were randomly collected, using shovels to dig up the root ball/plant, from two non-harvest rows of each plot (border rows). Once root balls/plants were collected, roots were gently washed and separated from the plant. Nodules on the roots were counted by hand, and nodes of the plant were also counted by hand. Plant height was recorded using a standard wood meter stick. Height measurements were made from where the roots were detached to the youngest developed trifoliate. Nodule color analysis was conducted during the early season collection date by taking a five plant subsample from the 10 random plants collected from each plot. Nodules were then counted and weighed on a high precision lab scale, and each nodule was cut open with a scalpel to determine internal color: pink (mature or active) green (adolescent), or white (immature) (Kandel and Endres, 2012).

At random, ten newest fully developed trifoliate leaves were collected from each plot for N content analysis at both early and mid-season collection dates. Trifoliate leaves and each aboveground plant biomass sample were labeled and dried for 48 hours at 60°C in small lab ovens. Dry biomass weights were then recorded on a high precision lab scale. Once dried, the whole plants and trifoliate samples were ground separately using a lab grade tissue grinder. Then, 0.15 grams of the ground tissue from each sample was separately weighed on a high precision lab grade scale and put into small individual aluminum foil packets. The aluminum packets, containing plant tissue, were then put into a LECO Corporation TruSpec CN Nitrogen Determinator, and the N content was determined using a dry combustion method.

The two center rows of each plot were harvested with a Kincaid plot combine

(Kincaid Equipment Manufacturing; Haven, KS), and plot weights were converted to 13% moisture to estimate yield. At harvest, 100 seeds were collected from each plot harvest subsample. These seeds were then weighed and expressed in grams per hundred seed. A 0.45 kg seed sample was shipped to Brookside Laboratories for N content analysis which involved being digested with nitric acid and hydrogen peroxide in a CEM MARS Express microwave system. The digested samples were then analyzed in an Elementar Vario EL Cube combustion analyzer (Greg Meyer, Agricultural Laboratory Manager, Brookside Laboratories, Inc., greg@blinc.com).

Data were analyzed with SAS (ver.9.4; SAS Institute; Cary, NC) using the GLIMMIX procedure. Type III statistics were used to test all fixed effects and the interactions of the fixed effects. All data were considered fixed effects except for replications, which was categorized as a random effect. When interactions were not detected among fixed effects, the data were averaged across year and/or environment. The least square means were based on an alpha of 0.05 and utilized for mean separations. The DANDA.sas developed by Dr. Arnold Saxton in 2013, is a design and analysis macro that was used to build the GLIMMIX procedures and convert the mean separations to letter groupings. Data were analyzed across years for mean separations and regressions among treatments.

Results and Discussion

Based on analysis of pre plant soil N samples, soil used in the irrigated 2016 site did supply some N for plant use (Table 2). Weather conditions in 2015 were slightly cooler than 2016 with more timely rainfall at the REC Milan (Figure 1). In 2016, the West TN REC received more timely rains than the REC at Milan during the growing season. There was more observed lodging at harvest both years in the irrigated sites with the variety used in our studies.

The main effects for treatment, environment, and year and interaction of main effects on plant measurements, seed weight, and yield are summarized in Table 3. Both year and environment significantly affected all plant data measurements, seed weight, and yield. Nitrogen treatment significantly affected early season and mid-season plant height, early season biomass and mid-season node numbers but did not have a significant impact on other plant measurements, seed weight, or yield across both years.

Soybean Development: Biomass, Height, and Nodes

At the early season biomass sampling time of R1 to R2, soybean plants weighed more in 2016 when compared to 2015 by approximately 20 grams per 10 plants (Table 4). The increase is probably due to more timely rainfall events that occurred in 2016 at the dryland location. Nitrogen treatment effects across both environments and years were highly significant on early season biomass (Table 5). All N rates numerically increased early season biomass compared to the zero N check (Table 5). N rates of 67 to 101 kg ha $^{-1}$ at-planting or 101 kg ha $^{-1}$ at V-2 produced biomass greater than the zero N check. Nitrogen may have been available to the plant longer when applied at-planting than at V2, therefore producing a greater effect on early season vegetation. Similarly, Deibert et al. (1979) found that biomass was significantly increased from 2,400 kg to 3,222 kg by N fertilizer treatments at-planting with maximum production accomplished by the highest N rate, 134 kg applied N ha^{-1}.

A full linear regression model explained 98% of the early season biomass differences (Figure 3). Nitrogen affected biomass, and slopes differed among both N timings and environments. Individual regressions had R-squares ranging from 0.04 to 98, with irrigated at-planting N applications having the lowest R-square value. At-planting (AP) N increased biomass by 0.20 g kg^{-1} ha $^{-1}$ in an irrigated environment (R-square = 0.98) but did not increase early season biomass in a dryland environment. Irrigated and dryland environments received adequate rainfall within the first week to incorporate fertilizer, but cooler temperatures after the irrigated at-planting applications may have enabled the higher biomass increase compared to dryland. When N was applied at V2, early season dryland biomass increased more with an increased in N rate than irrigated at 0.39 grams compared to 0.14 g kg^{-1} N ha $^{-1}$, respectively. Since irrigation was not applied this early to either of the irrigated sites, this difference may be due to more timely rains received in the dryland sites. Even though these N timings and rates increased biomass compared to the zero control, P values were not significant.

Since all figure lines were insignificant, further regressions were conducted to detect the location of the significant data. Figure 4 explains 18% of the differences between the zero control, at-planting, and V2 N timings. Timing of application did affect early season biomass, slopes differed among the timings, and P values were significant. Biomass was increased the most by 0.27 grams when N applications were applied at V2, but also had the lowest intercept compared to the zero control and at-planting N applications. Further investigation of regressions revealed that early season biomass was higher by both at-planting and V2 applications in the dryland environment than the irrigated. Figure 5 explains 92% of the differences between the two environments across the at-planting N applications. Environments did affect at-planting N biomass with the dryland increasing by 0.10 grams, and the irrigated not having any increase. Figure 6 explains 96% of the differences between the two environments across the V2 N applications. Environments did affect V2 N biomass with the dryland increasing by 0.27 grams, and the irrigated not having any increase. These results for irrigated having lower early season biomass is probably due to the irrigated environment in both 2015 and 2016 receiving more rainfall and having cooler temperatures compared to the dryland.

Mid-season biomass was measured at R4 to R5 stage soybeans, or about one month after

R2 N treatments were applied. Overall in 2016, soybeans produced greater mid-season growth compared to plants of similar stage sampled in 2015; and dryland plants weighed more than irrigated plants in both years (Table 6), probably due to more timely rainfall in 2016.

In contrast to the early season biomass results, there was no significant mid-season biomass increase with N treatment averaged over year and environment (Table 3). However, a year by treatment interaction was detected (Table 3); therefore, N rate and mid-season biomass are listed in Table 6. In 2015, N application did not significantly increase biomass compared to the untreated check at any N rate, but there were differences observed in 2016 (Table 6). Adversely, Bhangoo and Albritton (1976) concluded that in all three years of the experiment, all N rates of 56, 112, 224, and 446 kg ha^{-1} increased mature vegetative growth over the zero control. Nitrogen at 67 kg ha $^{-1}$ at-planting significantly increased mid-season biomass over the zero rate check; and numerically, the same mid N rate at V2 and R2 increased biomass compared to other rates (Table 6). In 2016, N application at R2 resulted in the lowest biomass weights across environments, which were significantly lower than N at 67 or 34 kg ha $^{-1}$ at-planting or at 67 kg ha $^{-1}$ at V2 stage soybean (Table 6). No trend or pattern could be explained. Early season applications of N might be utilized more for the production of vegetation, but once the reproductive stages begin, the plant might focus more on producing pods and seed rather than producing more vegetation; which could explain a lack of accumulated biomass but does not explain the decreased biomass that was observed at mid-season. In addition, Deibert et al. (1979) resolved that there was a limited response to all N fertilizer treatments applied at-planting or full bloom (R2).

Irrigated soybeans were taller in 2015, both early and mid-season (Table 7). The addition of N numerically increased early season plant height for all treatments, but the effect of N treatment on height was variable. Only at-planting N at 34 or 101 kg ha $^{-1}$ and V2 N at 101 kg ha $^{-1}$ significantly increased early season height across environments and years (Table 8), while only N at 101 kg ha $^{-1}$ at-planting continued to affect mid-season height (Table 9).

Irrigated soybeans did not consistently produce more nodes than non-irrigated soybeans (Table 10). Numerically, the addition of N increased mid-season plant node numbers for most treatments. One N rate of 67 kg ha $^{-1}$ applied either at-planting or V2 timing consistently increased main stem nodes over the zero N check (Table 11).

Leaf and Plant Tissue Analysis

The application of N increased early season whole plant N but did not alter mid-season plant N levels (Table 12). In contrast, there was no increase in leaf N level due to N treatment either early or mid-season (Table 12). All N rates numerically increased whole plant early season N levels, however, only at-planting N at 329 kg ha $^{-1}$ or 329 to 494 kg ha $^{-1}$ applied at V2 soybean significantly increased early season whole plant N levels (Table 13). Irrigated sites had higher early season leaf N levels, but lower mid-season whole plant N levels compared to non-irrigated locations (Table 14). Deibert et al., (1979) found that average N concentration of plant dry matter at the R2 growth stage was significantly higher than the zero control and N concentrations increased with increased N rates. In addition, Bhanghoo and Albritton (1976) concluded that total N concentration of mature biomass was increased with N additions.

A full linear regression model explained only 63% of the differences in early season whole plant N (Figure 7), indicating plant N accumulation was affected by other variables. Individual regressions had R-squares ranging from 0.009 to 1.0, with at-planting N in a dryland environment having the lowest, and V2 N in a dryland environment producing the strongest correlation between early season biomass with N rate (R-square = 1.0). Dryland V2 N applications significantly increased early season whole plant N by 0.005% per kg N ha $^{-1}$.

Soybean Nodulation

Early and mid-season total soybean nodule numbers were affected by year and environment but not N treatment when averaged over year and environment (Table 3), suggesting that N fertilizer does not have a strong impact on overall nodulation at these application timings. Year by environment was significant for early and mid-season nodule number (Table 3). Early season total nodules per plant in 2015 and 2016 and 2015 mid-season counts were significantly lower under irrigation (Table 15). These results may be due to greater early season moisture in the irrigated environment making the fertilizer treatments quickly available to the plants than the dryland environment; therefore early season nodulation may have been restrained by increased N availability under irrigation.

In 2016, additional measurements of early season average nodule weight, number of active, adolescent and immature nodules, and average mid-season nodule weight showed some interesting results. Environment impacted all nodule measurements (Table 16). The irrigated site had lower average nodule weights early season, but higher nodule weights mid-season (Table 17). Smaller nodules in an irrigated environment is probably due to more plant soluble N in the irrigated soil solution with the early fertilizer applications. N treatment significantly affected early season nodule weight as well as the number of early season active and adolescent nodules but not immature nodule number or mid-season nodule weight (Table 18). The application of N at-planting or V2 significantly reduced average nodule weight early season and with V2 N applications weight generally decreased as N rate increased (Table 18).

At-planting N applications of 67 to 101 kg ha $^{-1}$ significantly reduced active nodule numbers, while N treatments at V2 did not significantly affect active nodule numbers (Table 18).

Early season adolescent nodule production was reduced by all N treatments and timings with the exception of the lowest N rate applied at-planting (Table 18). These results directly correlate with the 2008 review by Salvagiotti et al. which showed that there is a negative relationship between N fertilizer rate and N fixation when N is applied to the soil surface. Their results indicated N fixation rates where zero N was applied were significantly higher than those where N rates were increased. Further, Sutharsan et al. (2016) found that 70 kg N ha^{-1} along with other nutrients applied reduces total root nodulation by 37% compared to the zero control. Additionally it has been reported that high levels of N inhibit nodule formation, number of infection sites on the root, nodule development, N fixation in pre-existing nodules, and nitrogenase activity (Zahran, 1999).

A full linear regression model explained 86% of differences in adolescent nodule numbers (Figure 8). Individual regressions had R-squares ranging from 0.02 to 99, with dryland at-planting N applications having the lowest R-square and irrigated V2 applications having the strongest correlation between adolescent nodule numbers and nitrogen rate. Dryland at-planting and V2 N treatments resulted in the reduction of adolescent nodules by 0.04 and 0.05 nodules, respectively; but in irrigated environment, adolescent nodule numbers were increased by 0.02 nodules with at-planting treatments and decreased by 0.003 nodules with V2 applications. Dryland V2 N additions was the only treatment to significantly reduce adolescent nodule numbers ($P = 0.023$).

Seed Weight and Yield

Seed weight is a measure of seed size or density and is one of the yield components of soybean. Our data indicated no difference in seed size among N treatments in dryland and irrigated environments. Seed weights in the dryland environment were larger than in an irrigated environment both years (Table 19). Likewise, work by Brevedan et al., (1978) showed no differences in seed size across all N treatments applied over two years in the field or one year in the greenhouse.

There was no N rate or timing effect on yield; however, a year by environment interaction was observed (Table 4). A mean separation analysis of data showed differences among environments, with dryland out producing the irrigated environment in 2016 (Table 19). The high yield irrigated environment yielded at or below the dryland environment, indicating yield potential was compromised. Lodging of the irrigated plants near harvest was a severe problem that is believed to have affected yields in both years because of the extra moisture consistently entering the system; whereas, the dryland experiment had better standing plants at harvest time.

Deibert et al., (1979) reported that yield of mature nodulating soybean yield was not affected by N treatments, although, seed yields of non-nodulating soybeans were significantly higher when N applications were delayed until full bloom (R2). Data supporting positive yield affects with N application may be found from Bhangoo and Albritton (1976) who reported a significant yield increase from all rates of N applied compared to the zero control over a three year period using rates of 0, 56, 112, 224, and 448 kg NH_4NO_3 ha^{-1} applied at-planting on a Calloway silt loam soil. An additional comparison of 448 kg ha^{-1} rate split applied at-planting and at full bloom also resulted in a soybean yield increase. The same trend was seen by Deibert et al. (1979), where yield was increased substantially when N treatments were applied at full bloom, suggesting that soybean plants use soil N, applied or residual, at later growth stages when shifting from vegetative growth to seed production.

Conclusion

Year, environment, and N treatments affected soybean development according to the analysis of N rate and timing. Dryland plants had more biomass in both years than irrigated compared to the zero control, and all N treatments across environments increased early season biomass. More timely rainfall events in 2016 increased plant development compared to 2015. Earlier at-planting applications were more beneficial to vegetative development than later applications (R2) when the plant's main focus was on seed production. Increased vegetation is beneficial to high yield soybean production, because of the need for high photosynthetic rates to maximize plant health and reproduction. More timely rainfall events in 2016 increased plant development compared to 2015. Overall, total nodulation was not affected by N treatments when averaged across both years, but was affected by environment with dryland having more nodules than irrigated. Although, overall nodulation was not affected, most N treatments did significantly decrease nodule size and maturity especially in an irrigated environment. The data indicated that the applications of N caused an inhibition of active and adolescent nodulation compared to the zero control when applied at-planting and the V2 growth stage. The effect of the inhibition was lessened when the applications were delayed to V2. However, 34 kg N ha^{-1} applied at-planting had the least effect on active, adolescent, and early season total nodule weight per plant compared to the other treatments. One hundred seed weights results indicated that there were no differences observed in seed size, although dryland tended to produce larger seeds. Yield data exhibited no differences among N treatments. A year by environment interaction was detected, showing that the dryland out produced the irrigated in 2016 but not in 2015. When averaged across both years, numerically the dryland slightly out produced the irrigated environment. Lodging in the irrigated environment was a severe problem in both 2015 and 2016 that was assumed to have jeopardized yield data; whereas, the dryland had better stand ability at harvest both years. Therefore, further research is needed to try to correct the lodging issue to accomplish better yield data and overall plant health issues observed in the irrigated environment. As the study stands now, it is not economical for a Tennessee producer to include N applications into their soybean production systems, because the N treatments did not significantly increase yield numbers. Thus, if yield is not increased, profit is not increased resulting in the inability to offset the N fertilizer cost in a Tennessee soybean production system.

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Appendix

Tables & Figures

Table 1. Agrotain Ultra™ treated urea application rates applied at planting, V2 and R2 growth stages application dates to Asgrow 4632 soybean variety experiments in Milan, TN in 2015 and 2016 and in Jackson, TN in 2016

Irrigated	**Dryland**	**Growth Stage**[a]	**Rate (kg N ha^{-1})**			
5/8/2015	6/18/2015	Planting	0	34	67	101
5/6/2016	6/13/2016		0	34	67	101
6/2/2015	7/6/2015	V2	0	34	67	101
6/2/2016	7/1/2016		0	34	67	101
7/2/2015	8/3/2015	R2	0	34	67	101
6/23/2016	7/26/2016		0	34	67	101

[a] Growth stage refers to soybean growth stage of V2 and R2.

Table 2. 2015 and 2016 irrigated and dryland sites' pre-plant estimated soil organic matter, soil available nitrogen, phosphorus, potassium, magnesium, and sulfur at the UT Milan Research and Education Center in Milan, TN and the UT West Tennessee Research and Education Center in Jackson, TN

Year	Location	Environment	Soil Type	Soil SOM[a]	Soil Available N	Soil Available P	Soil Available K	Soil Available Mg	Soil Available S
				g kg^{-1}	*kg ha^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*
2015	Milan, TN	Irrigated	Loring Silt Loam	24	.	36	145	135	7
2015	Milan, TN	Dryland	Lexington Silt Loam	23	.	44	152	66	.
2016	Milan, TN	Irrigated	Loring Silt Loam	15.5	114.16	33.65	116.275	120.65	9.4
2016	Jackson, TN	Dryland	Lexington Silt Loam	33	.	29	154	94	8

[a] SOM = Soil organic matter

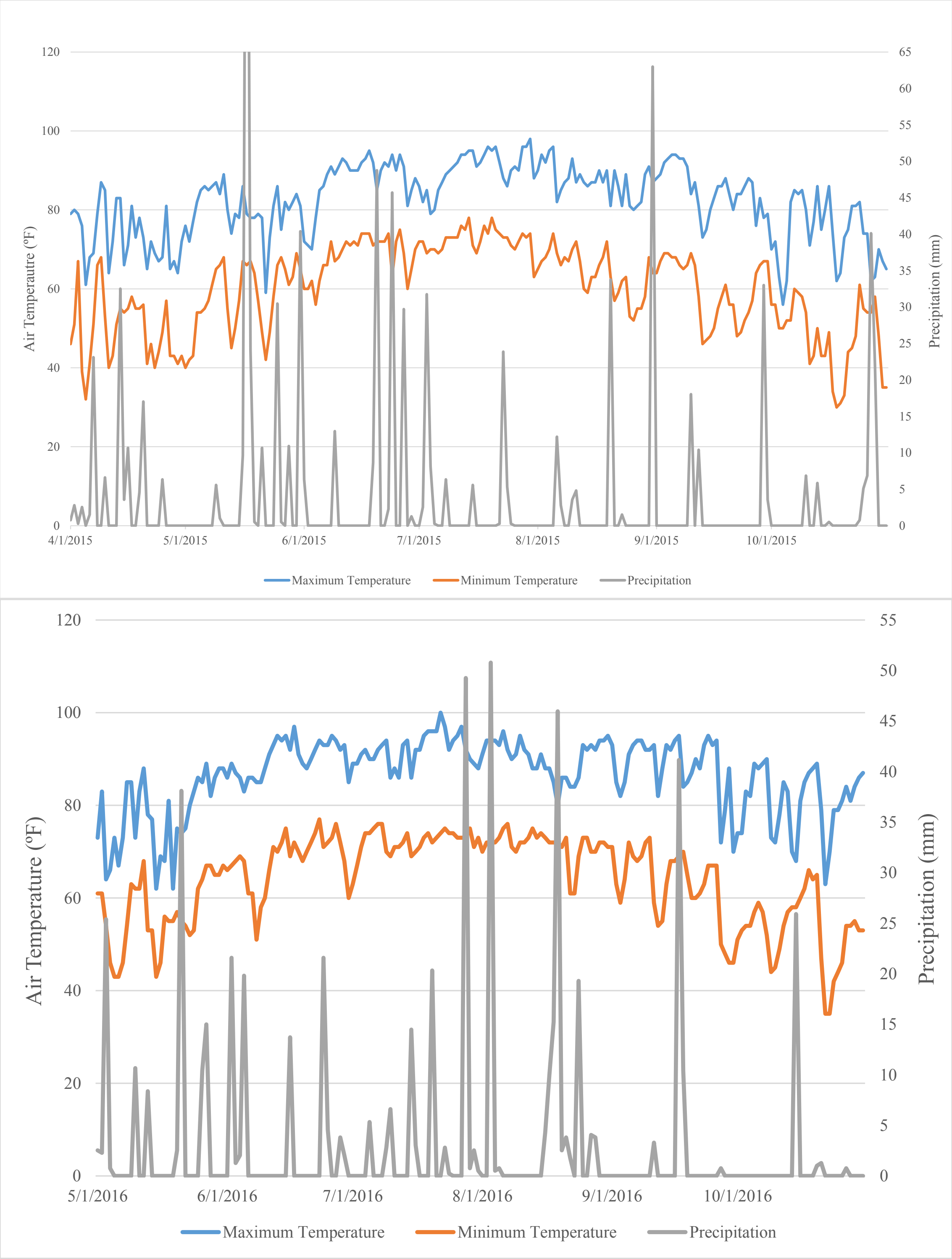


Figure 1. Weather conditions for 2015 (Milan, TN) & 2016 (Jackson, TN) (maximum, minimum air temperature, and precipitation)

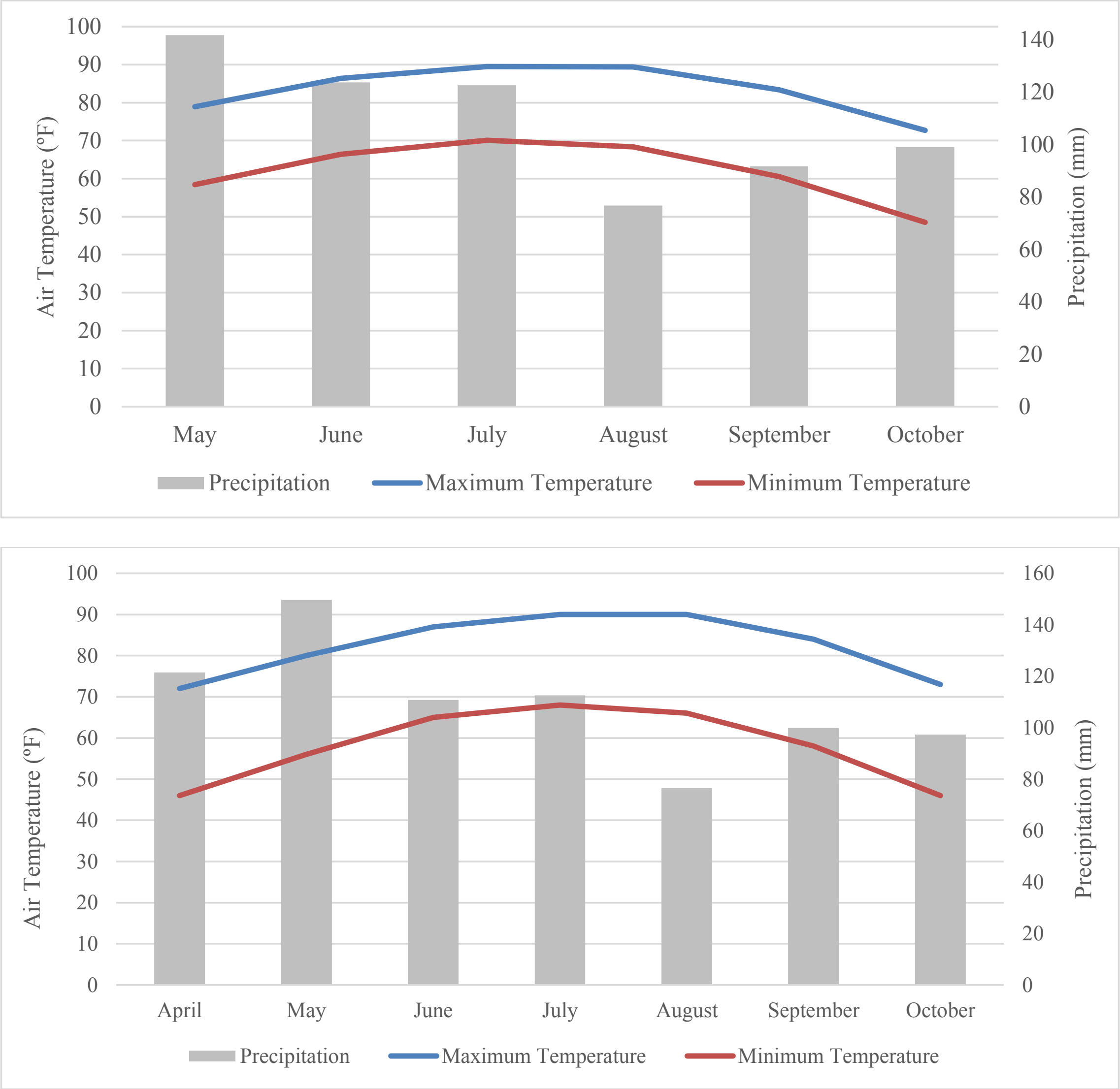


Figure 2. 30 year observed climatic normals for Jackson, TN (top) and Milan, TN (bottom)

Table 3. Significance of the main effects of N treatments on soybean plant height, early season plant nodes, early season root nodules, early season plant biomass, mid-season plant height, mid-season plant nodes, mid-season root nodules, mid-season plant biomass, harvest seed weight, and yield in Milan, TN in 2015 and 2016 and in Jackson, TN in 2016

Effect[a]	**df**	**Early Height[b]**	**Early Node[c]**	**Early Nodule[d]**	**Early Biomass[e]**	**Mid Height[b]**	**Mid Node[c]**	**Mid Nodule[d]**	**Mid Biomass[e]**	**Seed Weight[f]**	**Yield[g]**
Year	1	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.011**	**<0.0001**	***0.0006***	**<0.0001**	***0.0141***
Treatment	9	***0.012***	0.1632	0.0625	***0.0083***	***0.0491***	***0.0268***	0.4804	0.233	0.4688	0.6948
Environment	1	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***0.0007***	***0.001***	***<0.0001***	***<0.0001***	***0.0039***
Year*Treatment	9	0.6777	0.3058	0.5732	0.8687	0.2555	0.3191	0.7072	***0.0415***	0.7083	0.7300
Year*Environment	1	***<0.0001***	***0.2637***	***0.0073***	0.7526	***<0.0001***	***<0.0001***	***<0.0001***	0.6664	***<0.0001***	***<0.0001***
Treatment*Environment	9	0.9648	0.5846	0.3163	0.3319	0.8737	0.3477	0.8057	0.9727	0.8947	0.8304
Yr*Treatment*Environment	9	0.7674	0.6521	0.4782	0.2745	0.5363	0.4411	0.4611	0.2947	0.8918	0.8131

[a] Treatment consisted of Agrotain Ultra™ treated urea applied at-planting, V2 and R2 growth stages at 0, 34, 67, and 101 kg N ha^{-1}

[b] Plant height was measured in centimeters at R1 and R6 growth stages

[c] Nodes per plant were counted by hand at R1 and R6 growth stages

[d] Root nodules per plant were counted by hand at R1 and R6 growth stages

[e] Total plant biomass was measured in grams (g 10 plants^{-1}) on a high precision lab grade scale at R1 and R6 growth stages

[f] Seed weight was measured in grams per hundred seeds on a high precision lab grade scale at harvest

[g] Yield consisted of soybean yield (kg ha^{-1}) adjusted to 13% moisture

Table 4. Early and mid-season biomass (g 10 plants^{-1}) year*environment means in Milan, TN in 2015 and 2016 and in Jackson, TN in 2016

Timing of Sampling[a]	**Year**	**Environment**[b]	**Biomass**[c]
Early	2015	IRR	51.46 b
		DRY	79.07 a
	2016	IRR	71.99 b
		DRY	97.50a
Mid	2015	IRR	310.40 b
		DRY	437.70 a
	2016	IRR	352.20 b
		DRY	491.18 a

[a] Samples were collected at the R1 growth stage in the early season and at the R6 growth stage in the mid-season

[b] IRR = Irrigated Environment, DRY = Dryland Environment

[c] Plant biomass was measured in grams (g 10 plants^{-1}) on a high precision lab grade scale at the R1 and R6 growth stages

Table 5. Early season biomass (g 10 plants^{-1}) treatment means in Milan, TN and in Jackson, TN across 2015 and 2016

Trt[a]	**N Rate**[b]	**N Timing**[c]	**Biomass**[d]
1	0	.	64.44 c
2	34	Planting	74.50 abc
3	67	Planting	80.88 ab
4	101	Planting	80.75 ab
5	34	V2	68.00 c
6	67	V2	72.19 bc
7	101	V2	84.05 a

[a] Treatment consisted of Agrotain Ultra™ treated urea applied at-planting, V2 and R2 growth stages at 0, 34, 67, and 101 kg N ha^{-1}

[b] Nitrogen rates are in kilograms of N per hectare.

[c] N timing refers to nitrogen application at-planting or soybean growth stage of V2.

[d] Plant biomass was measured in grams (g 10 plants^{-1}) on a high precision lab grade scale at the R1 growth stage

Table 6. Mid-season biomass (g 10 plants^{-1}) year*treatment means in Milan, TN in 2015 and 2016 and in Jackson, TN in 2016 across the irrigated and dryland environments

Year	Trt[a]	N Rate[b]	N Timing[c]	Biomass[d]
2015	1	0	.	344.63 a
	2	34	Planting	408.50 a
	3	67	Planting	347.00 a
	4	101	Planting	366.00 a
	5	34	V2	416.00 a
	6	67	V2	375.88 a
	7	101	V2	395.75 a
	8	34	R2	384.00 a
	9	67	R2	339.88 a
	10	101	R2	362.88 a
2016	1	0	.	426.38 bcd
	2	34	Planting	423.75 bcd
	3	67	Planting	508.38 a
	4	101	Planting	456.50 abc
	5	34	V2	390.00 cd
	6	67	V2	474.50 ab
	7	101	V2	424.75 bcd
	8	34	R2	350.63 d
	9	67	R2	389.63 cd
	10	101	R2	372.37 d

[a] Treatment consisted of Agrotain Ultra™ treated urea applied at-planting, V2 and R2 growth stages at 0, 34, 67, and 101 kg N ha^{-1}

[b] Nitrogen rates are in kilograms of N per hectare.

[c] N timing refers to nitrogen application at-planting or soybean growth stage of V2 and R2.

[d] Plant biomass was measured in grams (g 10 plants^{-1}) on a high precision lab grade scale at the R6 growth stage

Table 7. Early and mid-season plant height (cm plant^{-1}) year*environment means in Milan, TN in 2015 and 2016 and in Jackson, TN in 2016

Timing of Sampling[a]	**Year**	**Environment**[b]	**Height**[c]
Early	2015	IRR	88.56 a
		DRY	44.50 b
	2016	IRR	35.65 b
		DRY	54.40 a
Mid	2015	IRR	122.61 a
		DRY	106.60 b
	2016	IRR	118.74 a
		DRY	121.60 a

[a] Samples were collected at the R1 growth stage in the early season and at the R6 growth stage in the mid-season
[b] IRR = irrigated environment, DRY = dryland environment
[c] Plant height was measured in centimeters at the R1 growth stage in the early season and at the R6 growth stage in the mid-season

Table 8. Early season plant height (cm plant^{-1}) treatment means in Milan, TN and Jackson, TN across 2015 and 2016

Trt[a]	**N Rate**[b]	**N Timing**[c]	**Height**[d]
1	0	.	52.99 c
2	34	Planting	57.25 ab
3	67	Planting	55.97 abc
4	101	Planting	58.52 a
5	34	V2	54.41 bc
6	67	V2	54.68 bc
7	101	V2	56.61 ab

[a] Treatment consisted of Agrotain Ultra™ treated urea applied at-planting, V2 and R2 growth stages at 0, 34, 67, and 101 kg N ha^{-1}
[b] Nitrogen rate is kilograms of N per hectare
[c] N timing refers to nitrogen application at-planting or soybean growth stage of V2.
[d] Plant height was measured in centimeters at the R1 growth stage in the early season

Table 9. Mid-season plant height (cm plant^{-1}) treatment means in Milan, TN and Jackson, TN across 2015 and 2016

Trt[a]	N Rate[b]	Growth Stage[c]	Height[d]
1	0	.	115.74 bc
2	34	Planting	118.60 ab
3	67	Planting	119.06 ab
4	101	Planting	120.94 a
5	34	V2	116.33 bc
6	67	V2	116.92 abc
7	101	V2	119.72 ab
8	34	R2	117.15 abc
9	67	R2	113.26 c
10	101	R2	116.17 bc

[a] Treatment consisted of Agrotain Ultra™ treated urea applied at-planting, V2 and R2 growth stages at 0, 34, 67, and 101 kg N ha^{-1}

[b] Nitrogen rate is kilograms of N per hectare

[c] N timing refers to nitrogen application at-planting or soybean growth stage of V2.

[d] Plant height was measured in centimeters at the R6 growth stage in the mid-season

Table 10. Early and mid-season average node per plant year*environment means in Milan, TN in 2015 and 2016 and in Jackson, TN in 2016

Timing of Sampling[a]	Year	Environment[b]	Nodes per plant[c]
Early	2015	IRR	10.30 b
		DRY	11.37 a
	2016	IRR	9.99 a
		DRY	8.48 b
Mid	2015	IRR	22.21 a
		DRY	19.39 b
	2016	IRR	19.61 b
		DRY	20.68 a

[a] Samples were collected at the R1 growth stage in the early season and at the R6 growth stage in the mid-season

[b] IRR = irrigated, DRY = dryland

[c] Nodes per plant were counted by hand at the R1 growth stage in the early season and at the R6 growth stage in the mid-season

Table 11. Mid-season average node per plant treatment means in Milan, TN and Jackson, TN across 2015 and 2016

Trt[a]	**N Rate[b]**	**N Timing[c]**	**Nodes per plant[d]**
1	0	.	19.86 bc
2	34	Planting	20.10 bc
3	67	Planting	21.34 a
4	101	Planting	20.97 ab
5	34	V2	20.10 bc
6	67	V2	21.43 a
7	101	V2	20.56 abc
8	34	R2	19.75 c
9	67	R2	20.21 bc
10	101	R2	20.40 abc

[a] Treatment consisted of Agrotain Ultra™ treated urea applied at-planting, V2 and R2 growth stages at 0, 34, 67, and 101 kg N ha^{-1} [b] Nitrogen rate is kilograms of N per hectare

[c] N timing refers to nitrogen application at-planting or soybean growth stage of V2.

[d] Nodes per plant were counted by hand at the R6 growth stage

Table 12. Significance of the main effects of the N treatments on early season whole plant percent nitrogen, early season leaf percent nitrogen, mid-season whole plant percent nitrogen, mid-season leaf percent nitrogen, and harvested seed percent nitrogen in Milan, TN and Jackson, TN in 2016

Effect[a]	**df**	**Early Season Whole Plant N[b]**	**Early Season Leaf N[c]**	**Mid-Season Whole Plant N[b]**	**Mid-Season Leaf N[c]**	**Harvested Seed N[d]**
Treatment	9	***0.0043***	0.3523	0.1000	0.456	0.6900
Environment	1	0.0767	***0.0062***	***<0.0001***	0.244	0.1243
Treatment*Environment	9	0.5644	0.2695	0.2458	0.662	0.8827

[a] Treatment consisted of Agrotain Ultra™ treated urea applied at-planting, V2 and R2 growth stages at 0, 34, 67, and 101 kg N ha^{-1}

[b] Whole plant N was measured in g kg^{-1} at the R1 growth stage in the early season and at the R6 growth stage in the mid-season

[c] Leaf N was measured in g kg^{-1} at the R1 growth stage in the early season and at the R6 growth stage in the mid-season

[d] Seed N was measured in g kg^{-1} from a 0.45 kg seed sample after harvest

Table 13. Early season whole plant and leaf N, mid-season whole plant and leaf N, and harvest seed N (g kg$_{-1}$) treatment means in Milan, TN and Jackson, TN across 2015 and 2016

Trt[a]	**N Rate**[b]	**N Timing**[c]	**Early Season Whole Plant N**[d]	**Early Season Leaf N**[e]	**Mid-Season Whole Plant N**[f]	**Mid-Season Leaf N**[g]	**Harvest Seed N**[h]
1	0	.	25.30 b	35.73 a	23.86 a	33.67 a	59.65 a
2	34	Planting	27.00 ab	36.10 a	25.99 a	32.46 a	60.00 a
3	67	Planting	29.10 a	37.72 a	24.77 a	32.80 a	60.02 a
4	101	Planting	27.50 ab	36.97 a	23.20 a	33.68 a	58.73 a
5	34	V2	27.50 ab	35.61 a	24.92 a	33.09 a	59.40 a
6	67	V2	28.90 a	37.04 a	24.03 a	32.59 a	58.84 a
7	101	V2	29.10 a	37.95 a	23.62 a	31.90 a	59.51 a
8	34	R2	.	.	24.81 a	30.37 a	59.04 a
9	67	R2	.	.	24.25 a	32.27 a	58.84 a
10	101	R2	.	.	23.64 a	30.55 a	58.62 a

[a] Treatment consisted of Agrotain Ultra™ treated urea applied at-planting, V2 and R2 growth stages at 0, 34, 67, and 101 kg N ha^{-1}

[b] Nitrogen rate is kilograms of N per hectare

[c] N timing refers to nitrogen application at-planting or soybean growth stage of V2.

[d] Whole plant N was measured in percentage at the R1 growth stage

[e] Leaf N was measured in percentage at the R1 growth stage

[f] Whole plant N was measured in percentage at the R5 growth stage

[g] Leaf N was measured in percentage at the R5 growth stage

[h] Seed N was measured in percentage after harvest

Table 14. Early season leaf and mid-season whole plant N (g kg^{-1}) environment means across treatments in Milan, TN and Jackson, TN across 2015 and 2016

Environment[a]	**Early season leaf N**[b]	**Mid-season whole plant N**[c]
IRR	37.70 a	22.00 b
DRY	35.70 b	26.60 a

[a] IRR = irrigated environment, DRY = dryland environment

[b] Early season leaf N was measured in percentage at the R1 growth stage

[c] Whole plant N was measured in percentage at the R6 growth stage

Table 15. Early season total nodules per plant year*environment means in Milan, TN in 2015 and 2016 and in Jackson, TN in 2016

Timing of Sampling[a]	Year	Environment[b]	Nodules per plant[c]
Early	2015	IRR	40.22 b
		DRY	49.65 a
	2016	IRR	22.77 b
		DRY	42.79 a
Mid	2015	IRR	59.02 b
		DRY	80.75 a
	2016	IRR	50.27 a
		DRY	43.54 b

[a] Samples were collected at the R1 growth stage in the early season and at the R6 growth stage in the mid-season
[b] IRR = irrigated environment, DRY = dryland environment
[c] Nodules per plant were counted by hand at the R1 growth stage in the early season and at the R6 growth stage in the mid-season

Table 16. Significance of the main effects of the N treatments on total early season nodule weight per plant, total early season active nodule production per plant, total early season adolescent nodule production per plant, total early season immature nodule production per plant, and total mid-season nodule weight per plant in Milan, TN and Jackson, TN in 2016

Effects[a]	df	Early Nodule Wt. [b]	Early Active[c]	Early Adolescent[d]	Early Immature[e]	Mid Nodule Wt.[b]
Treatment	6	**0.0349**	***0.0158***	***0.0078***	0.3628	0.698
Environment	1	***0.0288***	***0.0185***	***0.005***	***<0.0001***	***0.0033***
Treatment*Environment	6	0.2036	0.0655	0.2262	0.1829	0.5155

[a] Treatment consisted of Agrotain Ultra™ treated urea applied at-planting, V2 and R2 growth stages at 0, 34, 67, and 101 kg N ha^{-1}
[b] Nodule weight was measured in grams on a high precision lab grade scale at the R1 growth stage in the early season and at the R6 growth stage in the mid-season
[c] Early active nodules per plant were counted by hand at the R1 growth stage
[d] Early adolescent nodules per plant were counted by hand at the R1 growth stage
[e] Early immature nodules per plant were counted by hand at the R1 growth stage

Table 17. Early and mid-season average nodule weight per plant (g) environment means across treatments in Milan, TN and Jackson, TN in 2016

Timing of Sampling[a]	**Environment**[b]	**Nodule weight per plant**[c]
Early	IRR	0.38 b
	DRY	0.58 a
Mid	IRR	0.86 a
	DRY	0.67 b

[a] Samples were collected at the R1 growth stage in the early season and at the R6 growth stage in the mid-season
[b] IRR = irrigated environment, DRY = dryland environment
[c] Nodule weight per plant was measured in grams on a high precision lab grade scale at the R1 growth stage in the early season and at the R6 growth stage in the mid-season

Table 18. Early season average nodule weight per plant (g), early season active nodule per plant, and early season adolescent nodules per plant treatment means across environments in Milan, TN and Jackson, TN in 2016

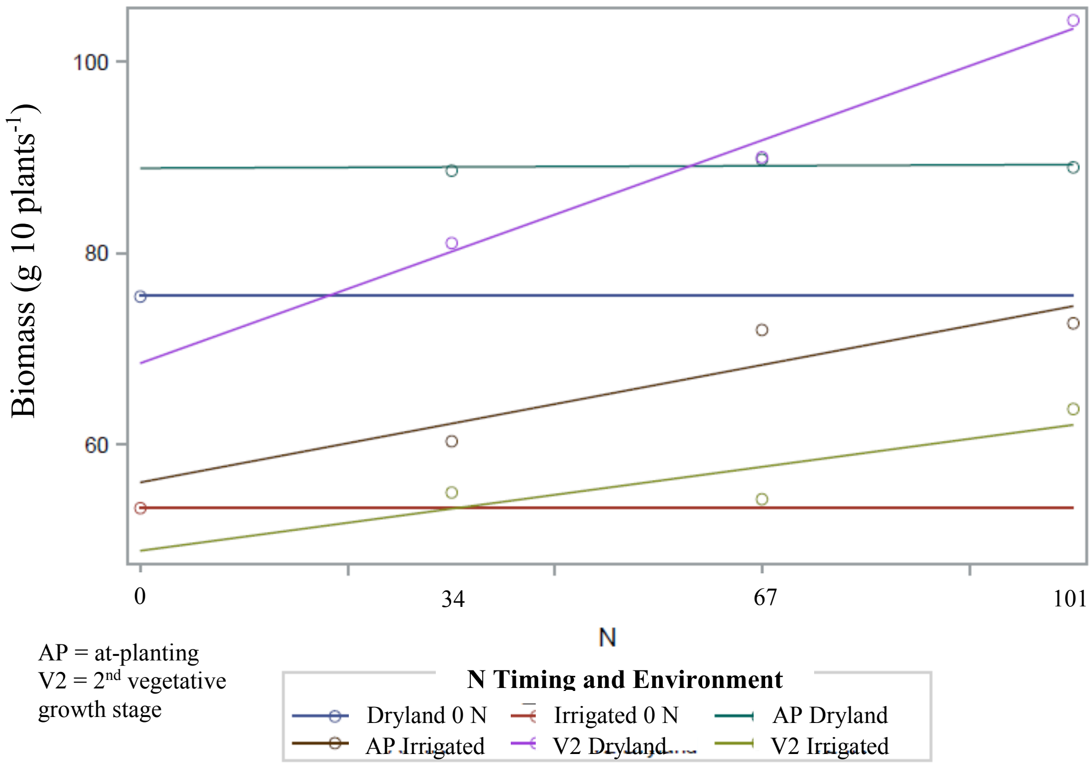
Trt[a]	**N Rate**[b]	**N Timing**[c]	**Nodule weight**[d]	**Active**[e]	**Adolescent**[f]
1	0	.	0.86 a	14.33 ab	4.08 a
2	34	Planting	0.53 b	14.95 a	3.23ab
3	67	Planting	0.31 b	7.48 c	1.60 c
4	101	Planting	0.41 b	9.38 c	1.68 c
5	34	V2	0.47 b	10.43 bc	2.65 bc
6	67	V2	0.39 b	10.23 bc	2.56 bc
7	101	V2	0.38 b	10.98abc	2.04 bc

[a] Treatment consisted of Agrotain Ultra™ treated urea applied at-planting, V2 and R2 growth stages at 0, 34, 67, and 101 kg N a^{-1}
[b] Nitrogen rate is kilograms of N per hectare
[c] N timing refers to nitrogen application at-planting or soybean growth stage V2
[d] Nodule weight per plant was measured in grams on a high precision lab grade scale at the R1 growth stage
[e] Active nodules per plant were counted by hand at the R1 growth stage
[f] Adolescent nodules per plant were counted by hand at the R1 growth stage

Table 19. Seed weight (g) and yield (kg ha^{-1}) year*environment means in Milan, TN in 2015 and 2016 and in Jackson, TN in 2016

Year	**Environment**[a]	**100 seed wt.**[b]	**Yield**[c]
2015	IRR	14.06 b	4,324 a
	DRY	14.80 a	4,205 a
2016	IRR	12.33 b	4,176 b
	DRY	14.43 a	4,675 a

[a] IRR = irrigated environment, DRY = dryland environment
[b] Seed weight was measured in grams per hundred seed on a high precision lab grade scale at harvest
[c] Yield consisted of soybean yield (kg ha^{-1}) adjusted to 13% moisture

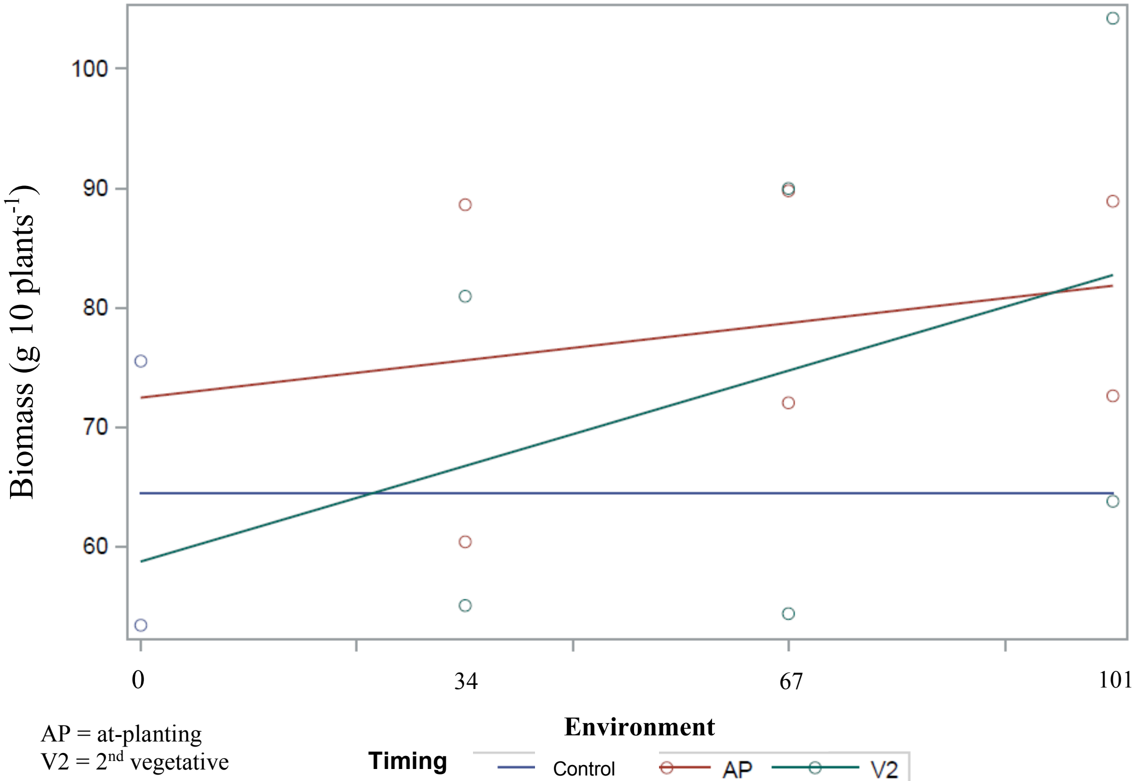


AP = at-planting
V2 = 2nd vegetative
growth stage

Figure 3. The effect of Agrotain Ultra™ treated urea rates of 0, 34, 67, and 101 kg N ha^{-1} applied at planting, and the V2 growth stage on early season biomass.

AP Dryland	AP IR	V2 Dryland	V2 IR
Y = 88.83 + 0.004x	Y = 56.08 + 0.204x	Y = 68.50 + 0.388x	Y = 48.96 + 0.146x
$R^2 = 0.0448$	$R^2 = 0.7882$	$R^2 = 0.9833$	$R^2 = 0.6967$
p = 0.8643	p = 0.3045	p = 0.0825	p = 0.3713

Dryland 0 N	Irrigated 0 N
Y = 75.50 + 0.00x	Y = 53.38 + 0.00x
$R^2 = 0.9875$	$R^2 = 0.9875$
p = <0.0001	p = <0.0001



AP = at-planting
V2 = 2nd vegetative
growth stage

Figure 4. The effect of Agrotain Ultra™ treated urea rates of 0, 34, 67, and 101 kg N ha^{-1} applied at planting, and the V2 growth stage across environments on early season biomass.

Control	AP	V2
Y = 64.44 + 0.00x	Y = 72.46 + 0.10x	Y = 58.73 + 0.27x
R^2 = 0.178	R^2 = 0.178	R^2 = 0.178
p = 0.0006	p = 0.004	p = 0.013

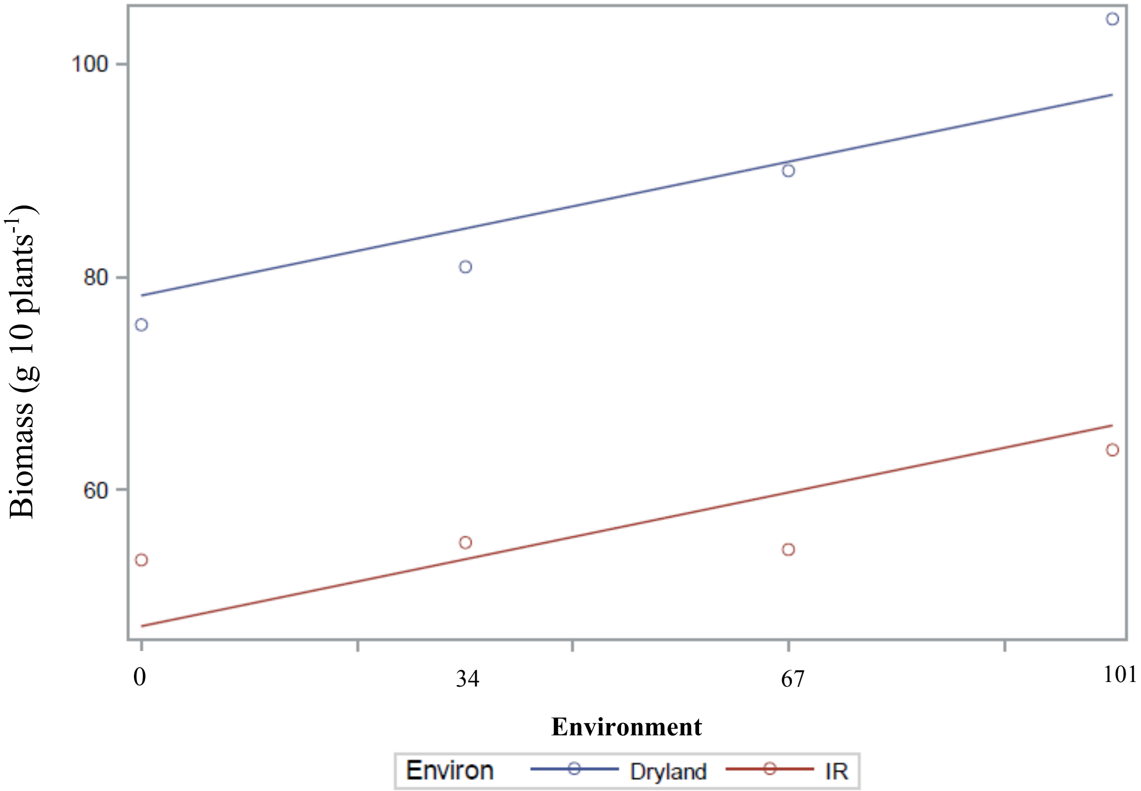


Figure 5. The effect of Agrotain Ultra™ treated urea rates of 0, 34, 67, and 101 kg N ha^{-1} applied within dryland and irrigated environments across at planting treatments on early season biomass.

Dryland	Irrigated
Y = 62.08 + 0.10x	Y = 20.75 + 0.00x
R^2 = 0.923	R^2 = 0.923
p = 0.0011	p = 0.2465

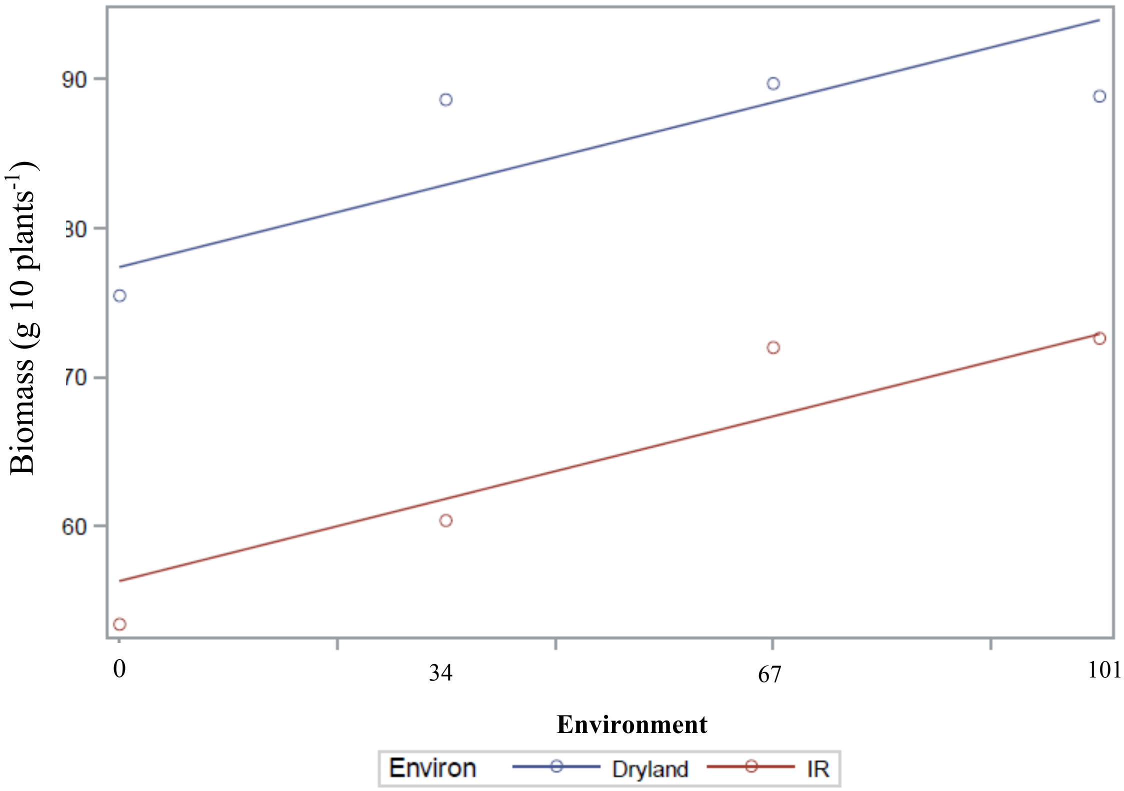


Figure 6. The effect of Agrotain Ultra™ treated urea rates of 0, 34, 67, and 101 kg N ha^{-1} applied within dryland and irrigated environments across V2 treatments on early season biomass.

Dryland	Irrigated
Y = 41.71 + 0.27x	Y = 34.04 + 0.00x
R^2 = 0.964	R^2 = 0.964
p = 0.0053	p = 0.0484

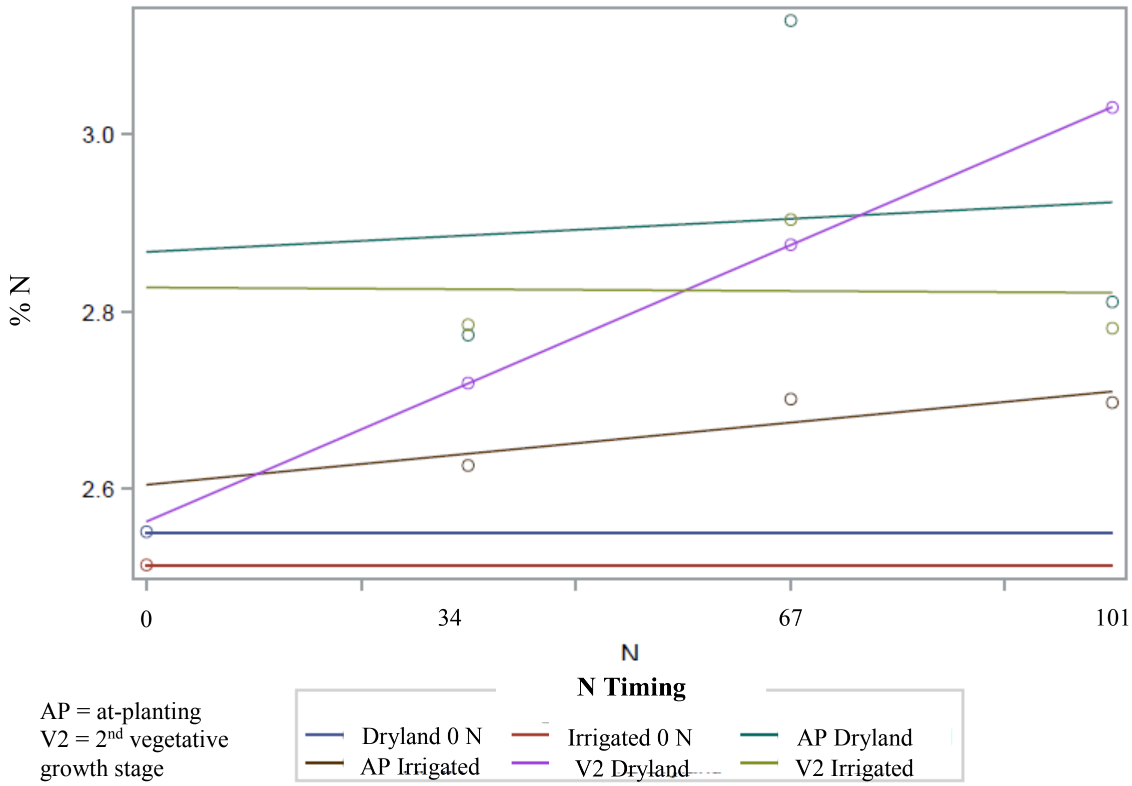


Figure 7. The effect of Agrotain Ultra™ treated urea rates of 0, 34, 67, and 101 kg N ha^{-1} applied at planting, and the V2 growth stage on early season whole plant N.

AP Dryland	AP Irrigated	V2 Dryland	V2 Irrigated
$Y = 2.867 + 0.0006x$	$Y = 2.604 + 0.0011x$	$Y = 2.563 + 0.0052x$	$Y = 2.827 - 0.00007x$
$R^2 = 0.0092$	$R^2 = 0.7087$	$R^2 = 1.00$	$R^2 = 0.0008$
$p = 0.9388$	$p = 0.3629$	$p = <0.0001$	$p = 0.9822$

Dryland 0 N	Irrigated 0 N
$Y = 2.55 + 0.00x$	$Y = 2.51 + 0.00x$
$R^2 = 0.7700$	$R^2 = 0.7700$
$p = <0.0001$	$p = <0.0001$

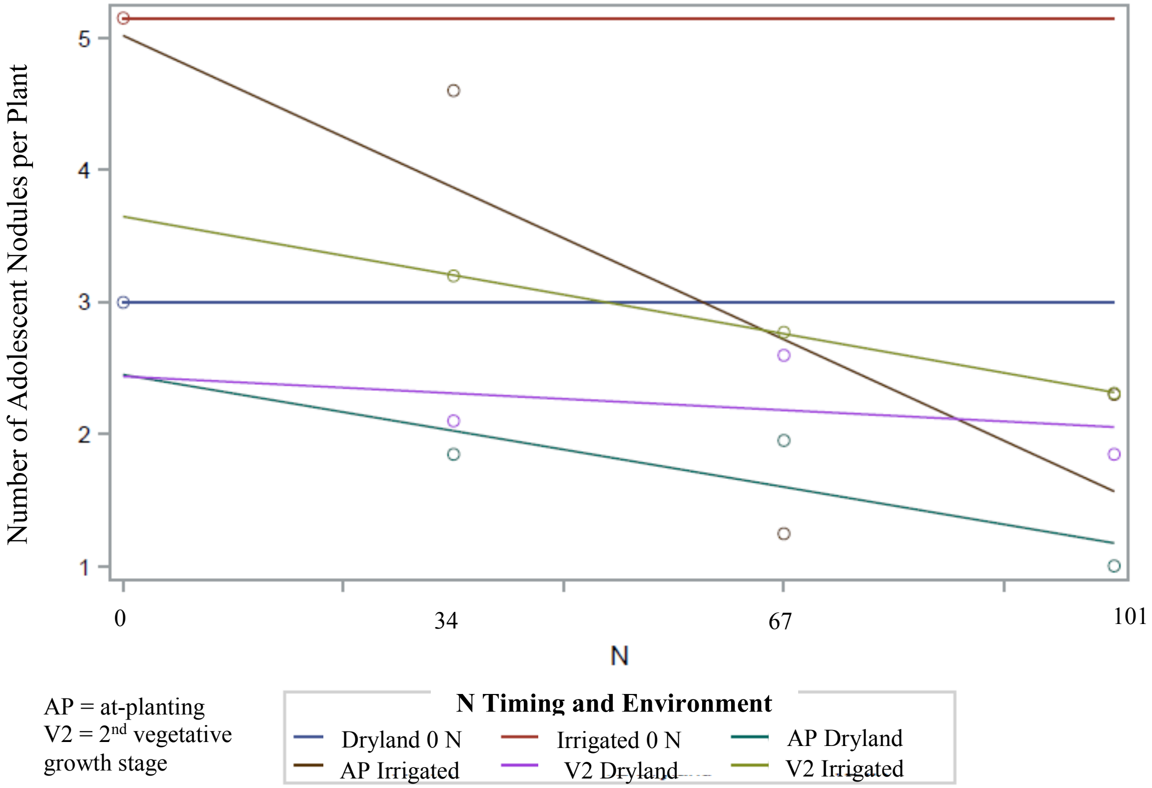


Figure 8. The effect of Agrotain Ultra™ treated urea rates of 0, 34, 67, and 101 kg N ha^{-1} applied at planting, and the V2 growth stage on early season adolescent nodule.

AP Dryland	AP IR	V2 Dryland	V2 IR
Y = 2.45 – 0.0142x	Y = 5.02 – 0.0383x	Y = 2.437 – 0.0043x	Y = 3.6472 – 0.015x
$R^2 = 0.6628$	$R^2 = 0.4505$	$R^2 = 0.1105$	$R^2 = 0.9998$
p = 0.3944	p = 0.5316	p = 0.7842	p = 0.0086

Dryland 0 N	Irrigated 0 N
Y = 3.00 + 0.00x	Y = 5.15 + 0.00x
$R^2 = 0.7888$	$R^2 = 0.7888$
P = 0.0352	p = 0.0058

Part III.

Effect of Sulfur Rate on Soybean (*Glycine max L.*) and Corn (*Zea mays L.*) Yield

Abstract

Sulfur (S) is becoming a more important limiting nutrient in production due to higher yielding crops, fewer S impurities in modern phosphate fertilizers, soil organic matter levels that are too low to provide enough S, and Tennessee's use of conservation tillage (no-tillage) reducing the rate of decomposition of organic matter.

Field studies were conducted in a silt loam soil using ammonium sulfate to evaluate S for soybean (*Glycine max*) and corn (*Zea mays*) yield optimization from 2015 to 2016 in Milan, TN (35.9198° N, 88.7589° W). S was broadcast-applied at planting at 0, 11, 23, and 34 kg ha^{-1} in a randomized complete block design with four replicates. Soybean S leaf tissue levels at R1 were similar to the zero S control; but nutrient concentrations of iron, manganese, and copper were reduced, and zinc was increased. These effects increased with S rate. Corn leaf tissue S levels increased with S rate at V6 and R1, while iron, manganese, and copper decreased in leaf tissue as S rate increased, similar to the soybean experiment. Soybean seed S was significantly increased with increased S rates, however seed N was not affected by the S applications. Soybean yield and 100 seed weight were not affected by S treatment. However, S applications significantly increased corn yield on average by 1,511.25 kg ha^{-1} (16%). Overall, results indicate that S fertilizer at 11 kg S ha^{-1} may improve corn yields in an S deficient soil, while S applications to a soybean crop affect the early season concentration of some micronutrients as well as level of seed S, but may not be economical if yield is not increased.

Introduction

Sulfur (S) is among the 17 essential nutrients for healthy plant growth and is a requirement for all crops (Barden et al., 1987). With N, phosphorus, and potassium being primary macronutrients, S is a secondary macronutrient. In order to achieve maximum plant growth and high yield, nutrients must be at adequate levels. With the majority of S in the soil being found in organic matter, S exists as organic compounds, sulfides (S^{2-}), elemental S (S), and sulfate (SO_4^{2-}). Similar to nitrogen (N), S in soil organic matter is not readily available for plant uptake. In warm, well aerated soils, organic S slowly goes through a process known as mineralization to form sulfate S which is available to plants (Place et al., 2007).

According to Davidson (2015), S plays a critical role in protein synthesis and is crucial for various plant processes since it is a key component of amino acids, proteins, and peptides. In addition, S is essential for the development of chlorophyll by being a dominant component of one of the enzymes needed for the formation of the chlorophyll molecule (Sela, 2017). Also S is necessary for the success of nodulation and N fixation in legumes such as soybean.

Development of vegetative growth would be impossible without chlorophyll production; and without S, chlorophyll production would be impossible. Sulfur is a key ingredient of ferredoxin, which is an iron-S protein found in chloroplasts. Further, ferredoxin also contributes to the metabolic role in both N fixation and sulfate reduction and the consumption of the N by the rhizobacteria living in the root nodules (Davidson, 2015). Sulfur and iron cofactors are components of root nodules, which are high in protein and the enzyme nitrogenase. Thus, being an integral component of two amino acids, S deficiency can limit N fixation and, eventually, yield (Davidson, 2014).

Sulfur can be depleted from the soil profile by plant uptake, leaching, and volatilization;

and these processes can increase in tilled soil (Place et al., 2007). Consistent tillage discourages overall soil heath by depleting soil aggregates, infiltration, and soil tilth. Also, tillage promotes soil erosion, which is a main reason for nutrient loss in a cropping system.

Plants that are deficient in S or N may display similar symptoms. Both appear as interveinal chlorosis and stunted plant growth. Sulfur is a less mobile plant nutrient than N. When a deficiency occurs, the plant cannot easily move S to younger tissue; therefore, deficiency symptoms will be seen in the younger tissue first. In contrast, N is very mobile in the plant tissue, resulting in symptoms being observed in the older tissue before the younger. An optimal N:S ratio of 15:1 (Camberato et al., 2012; Iowa, 2012) assures optimum N use efficiency, plant vigor, water use efficiency, phosphate use, carbohydrate production and utilization, rate of grain fill, and maturity (AgriSolutions, 2011). Therefore, this N:S ratio basically emulates the correlative relationship that N and S have in producing key plant proteins (AgriSolution, 2011).

In recent years, S has become a more limiting nutrient in crop production for various reasons, including higher crop yields that require more S, minimal S amounts in modern phosphorus fertilizers, less use of S containing pesticides, reduced S emissions to the atmosphere, and soil organic matter levels that are too low to provide enough S (Davidson, 2014). According to Morrison (2009), less than half the amount of S reached the soil as acid rain in recent years compared to in the 1980s. Therefore, crop responses to S fertilizer application could become more common. The Clean Air Act in 1970 reduced S emissions significantly, causing a reduction in S deposition in many areas (Place et al., 2007). Other conditions that can cause S deficiency in the soil are cold temperatures and water-logged soils (Further Agriculture Solutions, 2012), where low oxygen conditions reduce available sulfate S into sulfide, which is unavailable for plant uptake. The sulfide will not convert back to a plant available form until it combines with oxygen in warm soil. Tennessee's switch to conservation tillage (no-till) in the 1970's might have an inhibiting effect on S availability in the spring, due to cooler soil temperatures slowing the rate of S release from soil organic matter, which is the main sink of soil sulfate (Morrison, 2009). Therefore, industry changes and conservation tillage may increase row crop S deficiencies, increasing the need to identify an S rate that will allow Tennessee producers to optimize their soybean and corn crops.

Material and Methods

Sulfur rate response experiments were conducted in 2015 and 2016 without irrigation at the Milan Research and Education Center in Milan, TN (35.9198° N, 88.7589° W; Collins/Falaya silt loam). Experiments were established in a field that had been used for S rate studies in 2013 and 2014. The plot randomization for S rate was kept the same in all experiments in 2015 and 2016, which allowed an S rate to be applied to the same treatment location within experiments each year. Ammonium sulfate (21-0-0-24S) was the S fertilizer source. The fertilizer was applied in treatments of 0, 11, 23, and 34 kg S ha^{-1}. Since ammonium sulfate also contains N, to ensure that all plots received the same N rate, ammonium nitrate was applied to the zero S control, blended with ammonium sulfate at an appropriate amount with 11 and 23 kg ha^{-1} rate, and excluded from the highest S rate treatment. Also, in 2015 and 2016, phosphorus (P) and potassium (K) were added to each plot at a rate of 67 kg ha^{-1} due to soil analysis indicating low levels of P and K. Pre plant soil samples were collected from each plot at 0-15 cm prior to treatment application for both soybean and corn experiments in 2015 and 2016, and S level was analyzed using Mehlich 3 extraction (Waypoint labs, Memphis, TN).

All experiments employed a randomized complete block design with 6 replicates utilizing separate plots consisting of four rows measuring 3m wide and 9m long, with a row spacing of 76 centimeters. The previous crop before soybean each year was corn, and the previous crop before corn each year was soybean.

Soybean Experiment

Asgrow 4632 (Monsanto Company; St. Louis, Mo.) was the tested soy variety. Soybeans were planted on May 7, 2015 and May 24, 2016 at a depth of 2.5 centimeters and at a population of 345,800 seeds ha^{-1} in both years. Ammonium sulfate at rates of 0, 55, 110, and 34 kg actual sulfate ha^{-1} were evenly broadcast applied by hand to the soybean experiment on May 8, 2015 and May 24, 2016. Weeds, insects, and diseases were controlled by following University of Tennessee recommendations (Steckel, et.al, 2016; Stewart and McClure, 2016).

Soybean Data Collection

In 2015, pre-plant soil S level, in-season leaf tissue, plant height, normalized difference vegetation index (NDVI), harvest moisture, 100 seed weight, seed S and N content, grain yield, and post-harvest soil samples were collected. In 2016, only pre-plant soil samples, 100 seed weight, seed S and N content data, and seed yield were collected.

Leaf tissue samples were collected from two non-harvest (border) rows of each plot using the youngest fully developed trifoliates at early bloom stage (R1) which follows SAAESD recommended procedures for nutrient sampling in soybean (SAAESD Southern Cooperative Bulletin, 2000). Tissue samples were analyzed by a commercial lab (Brookside Laboratories, Inc., New Bremen, OH) using nitric acid and hydrogen peroxide digestion in a CEM MARS Express microwave system. The digested sample was then analyzed on a Thermo 6500 Dou ICP, and S and other nutrients were reported as mg kg^{-1}. Plant height was measured on July 29, 2015 and July 8, 2016 from the ground to the youngest developed trifoliate with a fiberglass telescoping measuring rod. Visual differences in leaf color were observed among treatments at late full pod stage (R4) in 2015; therefore, NDVI readings were collected from two center rows of each plot to measure the S treatments' effect on the photosynthetic activity of the soy crop. There were no visual differences in canopy color in 2016; therefore, NDVI readings were not taken.

Plots were harvested with a Kincaid plot combine on October 7 in 2015 and October 5 in 2016. Plot weights were adjusted to 13% seed moisture and converted to yield as Mg ha^{-1}. At harvest, 100 seeds were collected randomly from the harvest subsample and weighed to the nearest 0.1 grams. A 0.45 kg seed sample was sent to Brookside Laboratories for analysis of seed S and N by being digested with nitric acid and hydrogen peroxide in a CEM MARS Express microwave system. The digested sample was then analyzed on a Thermo 6500 Dou ICP.

Corn Experiment

In 2015, the corn hybrid tested was Dekalb 66-97 (Monsanto Company; St. Louis, Mo.). In 2016, Dekalb 66-87 (Monsanto Company, St. Louis, MO) was planted because Dekalb 66-97 was not available for use. Corn was planted on April 28, 2015 and April 8, 2016 (Table 23) at a depth of 5 centimeters and at a population of 83,980 seeds ha^{-1} both years. Ammonium sulfate at rates of, 0, 55, 110, and 34 kg actual sulfate ha^{-1} was evenly broadcast applied by hand to corn plots on April 28, 2015 and April 8, 2016. Applications of UAN, which is a solution of urea and ammonium nitrate in water, was side dressed applied to the corn plots at a rate of 157 kg ha^{-1} between the V5-V6 growth stages. Weeds were controlled using University of Tennessee recommendations (Steckel, et al, 2016).

In 2015, to measure the effects of the S applications in the corn experiment, in-season leaf tissue samples, plant height, harvest moisture, test weight, 100 seed weight, seed S and N content, and grain yield were collected. In 2016, because of dry weather impact on ear fill and yield and reduced seed fill, seed S and N content was not analyzed.

Leaf tissue samples were collected from two non-harvested rows of each plot, using the youngest fully developed leaf at the sixth leaf stage (V6) and the ear leaf at R1 which followed SAAESD recommended procedures for tissue sampling in field corn (SAAESD Southern Cooperative Bulletin, 2000). Tissue samples were sent to a commercial lab (Brookside Laboratories, Inc. New Bremen, OH) to evaluate S and other nutrient levels. Leaf tissue was digested with nitric acid and hydrogen peroxide in a CEM MARS Express microwave system. The digested sample was then analyzed on a Thermo 6500 Dou ICP. Height was measured at two separate times, once from the ground to the youngest developed leaf at the fifth leaf stage (V5) and once from the ground to the tip of the tassel, with a fiberglass telescoping measuring rod.

Corn plots were harvested on September 17, 2015 and September 9, 2016 with a Kincaid plot combine. Plot seed weights were converted to 155 g kg^{-1} moisture, and yields were calculated as Mg ha^{-1}. At harvest, 100 seeds were collected randomly from the harvest subsample and then weighed to the nearest 0.1 grams. In 2015, a 0.45 kg seed sample from each plot was sent to Brookside Laboratories for analysis of seed S and N using a nitric acid and hydrogen peroxide digestion in a CEM MARS Express microwave system. The digested sample was then analyzed on a Thermo 6500 Dou ICP.

Data Analysis

Data were analyzed with SAS 9.4 (ver. 9.4; SAS Institute; Cary, NC) using the GLIMMIX procedure. Type III statistics were used to test all fixed effects and the interactions of the fixed effects. All data were considered fixed effects except for replication, which was categorized as a random effect. The least square means were based on an alpha of 0.05 and utilized for mean separations. The DANDA.sas, developed by Dr. Arnold Saxton in 2013, is a design and analysis macro that was used to build the GLIMMIX procedures and convert the mean separations to letter groupings. Data were analyzed across years where appropriate for mean separation and contrasts among treatments.

Results and Discussion

Pre-plant soil S analysis indicated the sites used for corn and soybean experiments were S deficient at the 0-15 cm level at the time of fertilizer application in the spring, based on a commercial lab recommendation to apply S when soil levels are below <22 ppm sulfate (Table 21). In 2015, adding S at-planting increased post-harvest S levels in soil samples at some depths (Table 21). Overall, the analysis of post-harvest soil samples indicated that S levels deeper in the soil profile were higher following S treatments compared to the upper soil profile. Higher concentrations of S at deeper soil depths exhibit the characteristics of the negatively charged sulfate ion (SO42-). Sulfate is extremely vulnerable to leaching due to it being water soluble and an extremely mobile nutrient in the soil solution. Therefore, little residual S in the rooting zone will be leftover for the next growing season on this Collins/Falaya silt loam. However, as roots reach the 15-31 cm and 31-61 cm levels of the soil profile later in the growing season, residual S will be available for plant uptake at those depths.

Corn and soybean leaf tissue was analyzed for S content and results were compared to the standard critical values for nutrient sufficiency stated in the Southern Cooperative Series Bulletin: Reference Sufficiency Ranges for Plant Analysis in the Southern Region of the United States (2000). Soybean leaf tissue S at flowering was within the sufficiency range of 0.25-0.60%, regardless of treatment, but all tissue levels following S treatments were at the lower end of the range (0.27-0.29%). Tissue samples were collected twice during the growing season from the corn experiment at V6 and VT. Corn V6 tissue S for most S treatments were within the sufficiency range of 0.15-0.40%, including the zero control, but all 2016 S treatments exceeded this range. The excess tissue S in 2016 could be attributed to the drought conditions the corn experienced during the growing season in Milan, TN. Adequate rainfall was received during the very early season, but became intensely untimely as the growing season continued. Similarly, VT S levels were within the sufficient values of 0.15-0.60% S in both years with the lowest being 0.17% (zero control) and highest being 0.22% (34 kg S ha^{-1}).

Results of Soybean Experiment

The main effects of S rate on R1 soybean tissue nutrient levels, NDVI values, or plant height measurements in 2015 are summarized in Table 22. Sulfur treatment significantly affected R1 tissue levels of magnesium, boron, iron, manganese, copper, and zinc but did not affect early season tissue levels of S, phosphorus, calcium, aluminum, or sodium. Sulfur treatment affected NDVI measurement; however, soybean height was not influenced by S treatment (Table 23). Treatment means by S rate for significant measurements are described in Table 5. All S rates significantly increased NDVI mid-season "greenness" of the soybean plants compared to the zero added S control, but differences among S rates were not detected (Table 23). Sulfur is a key component in chlorophyll production, and the increase in S availability to the plants may have increased chlorophyll production and improved canopy color.

In R1 soybean leaf tissue, concentrations of magnesium, boron, iron, manganese, and copper were reduced below the zero added S control at some S rates, while zinc level increased at all S rates (Table 23). Boron and iron concentrations were the most consistently reduced, affected by as little as 11 kg ha^{-1} S. Manganese and copper levels were significantly decreased from the zero added S control at only the highest S rate of 34 kg ha^{-1}. R1 leaf tissue S, phosphorus, and calcium were not significantly affected by the S additions. The anion sulfate may have out competed the cation nutrient forms of magnesium, iron, manganese, and copper causing the reduction, which are all vital nutrients to overall plant health and reproduction.

The main effect of S treatment, year, and interaction of main effects with seed S content, seed N content, 100 seed weight, and yield are summarized in Table 24. Sulfur treatment increased seed S level across years but did not impact seed N level, 100 seed weight, or soybean yield. Sfredo and Moreira (2015) in an experiment with 0, 25, 50, 75, and 100 kg S ha^{-1} on a Typic Haplortox and Eutrotox soil in southern Brazil also reported no S effect on seed weight but an increase in soybean yield from S treatment that was not related to seed weight.

Seed S level increased with as little as 11 kg ha^{-1} added S. Sulfur rates of 23 to 34 kg ha^{-1} produced higher but similar seed S levels (Table 25). Because soybean is an oil crop that is high in protein, S is an essential ingredient in the production of enzymes used to produce the oils and proteins that compose large parts of the soybean seed. Therefore, the increase of S probably enabled the plant to increase its oil and protein production, which are vital for increasing the nutritional value of the seed. Kaiser and Kim (2013) also reported significantly greater seed S levels following several S treatment combinations on three different soil sites in Minnesota.

The 100 seed weights and yield did not increase with the application of S, and a year difference was detected. Seeds were heavier in 2015 than 2016 (data not shown), and average soybean yield was higher in 2016 than 2015 (data not shown). Higher yields in 2016 can probably be credited to more timely rainfall events that occurred during critical reproduction stages than in 2015 (Table 24). A lack of significant yield response due to S was reported by Lawson (2012) in an Iowa study where all rates of calcium sulfate were found to not have significantly increased soybean yield on a Fruitland coarse sand soil with 1 percent organic matter and 25 kg ha^{-1} of S in the topsoil before planting. However, these results conflict with Boem et al. (2007) whose experiment in Argentina resulted in a significant increase in soybean yield on a Typic Argiudoll silt loam soil with 31 mg g^{-1} of organic matter and 17.3 µg g^{-1} of soil sulfate from gypsum and ammonium sulfate applications. The yield response to S fertilization ranged from 160 to 500 kg ha^{-1}, and the seed yields of the control treatments were from 6 to 14% lower than the fertilized ones, suggesting that the unfertilized soybean underwent S deficiency (Boem et al., 2007). The response differences between these two studies could be attributed to Argentina having higher organic matter levels and warmer temperatures than Iowa. Lack of yield response with S treatment in our experiment may be due to the reduced uptake of essential nutrients such as boron.

Results of Corn Experiment

The main effects of year, treatment, and the interaction among main effects with plant tissue measurements at V6 and R1are summarized in Tables 9 and 10, respectively. Sulfur treatments significantly affected V6 tissue S, V6 tissue iron, V6 tissue manganese, and V6 tissue copper (Table 26). Additionally, S treatments affected R1 tissue S, R1 tissue manganese, and R1 tissue copper (Table 27), but had no impact on early or late season levels of the macronutrients N, phosphorus, or potassium or micronutrients magnesium, calcium, iron, boron, or zinc.

Sulfur tissue concentration at V6 was significantly increased over the zero S control by S fertilizer in both 2015 and 2016, with higher leaf S concentrations following 23 to 34 kg ha^{-1} S (Table 28). However, tissue concentrations were larger in 2016 than in 2015. Drought conditions in 2016 could possibly have resulted in the higher accumulation in the leaf tissue in response to the water stress. Sulfur leaf tissue concentrations at R1 also increased with all S treatments, with no significant increase at rates above 11 kg ha^{-1} (Table 29). Results are in agreement with findings by O'Leary and Rehm (1990) who observed ear leaf S tissue increased with increasing rate of S at six of 10 sites on three different silt loam and one sandy loam soil from 11, 23, and 45 kg S ha^{-1}. Iron, manganese, copper, aluminum, and zinc were the only micronutrients affected by the S treatments (Table 30).

Iron, manganese, and copper V6 tissue levels were reduced in corn following the 34 kg ha^{-1} S fertilizer treatment, but were not significantly impacted at lower fertilizer rates. R1 tissue levels of manganese were reduced with all S rates, while copper levels declined at S rates of 23 to 34 kg ha^{-1} S (Table 30). The reduction of copper and manganese at V6 and R1 may be due to the saturation of S in the soil solution, which creates complexes between copper-S and manganese-S which are not plant available. Aluminum and zinc concentrations increased in 2016 tissue with increased S rate (Table 31). The extra accumulation of iron, aluminum, and zinc as S rate increased is probably a reaction to the drought stress experienced in 2016.

The main effects of S treatment on plant height, seed weight, seed S, and yield are included in Table 34. Sulfur application affected corn height at VT, seed S, and yield, but did not have a significant impact on 100 seed weight (Table 32). All rates of S increased corn height compared to the zero added S control; however, only corn treated with S at 11 to 23 kg ha^{-1} was consistently taller (Table 33). Sulfur is a main cofactor of chlorophyll with N. Therefore, more S available to the plant may have resulted in higher production of chlorophyll which correlates with more height and vegetation. With significantly increased tissue S resulted in significantly increased seed S. All S rates increased seed S compared to the zero S control, and the lowest S rate of 11 kg ha^{-1} produced similar seed S concentrations at higher S rates (Table 33).

Sulfur applications increased yield in an adequate rainfall year (2015) and a drought year (2016). All S rates increased corn yield, and the lowest S rate of 11 kg ha^{-1} produced similar yields at higher S rates (Table 33). More drought stress occurred during 2016 than 2015, causing the corn's reproduction to suffer. However, plots fertilized with S out produced the zero added S control in spite of drought conditions. On average, yields across all treatments and years were increased with S applications by 1,511.25 kg ha^{-1} (16%) in both adequate rainfall and drought years. Wortmann et al. (2009) reported no yield increase with S over a three year period, experiment from 20 kg ha^{-1} on either loamy sand, sandy loam, silt loam, or silty clay loam soils. It was concluded that S fertilization was likely to not increase corn yields on medium or fine-textured soils or on sandy soils with more than 10 mg kg^{-1} soil organic matter (Wortmann et al., 2009). However, our results are similar to Sawyer et al. (2011), where S applications at-planting as gypsum at a rate of 11 kg S ha^{-1} to a fine textured soil increased corn yields by 1,021 kg ha $^{-1}$.

Conclusion

In the soybean experiment, S applications decreased early season uptake of most macro and micronutrients, which may have impacted yield. However, the treatments did increase soybean seed S level, which contributes to a more nutritional, protein packed seed. S fertilizer significantly increased NDVI readings in one year where visual canopy color differences were observed, probably contributing to higher chlorophyll production and overall plant health. Sulfur treatments did not increase seed N content, 100 seed weight or yield, and the year difference for seed weight and year that was detected is attributed to more timely rainfall in 2015 than in 2016. Ultimately, this study found that S additions to a soybean crop in an S deficient soil is not economical for a Tennessee producer to implement into their system.

In corn, S fertilizer did not appear to affect the leaf tissue concentration of as many micronutrients as it did in soybean. Also, S fertilizer increased the seed percent S, which contributes to a more nutritional grain. Corn treated with S produced more height/vegetation in both a good rainfall year and a drought year, which probably resulted in the significant yield increase that was observed. Therefore, this study found that in S deficient soils, the addition of S to corn at a rate as low as 11 kg ha^{-1} may be economically advantageous for Tennessee producers.

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Appendix

Tables & Figures

Table 20. Ammonium sulfate application rates, planting dates, and timings to Dekalb 66-97 and 66-87 corn hybrids and Asgrow 4632 soybean variety experiments

Crop	**Variety**[a]	**Planting and Application Date**	**Application Timing**	**Rate (kg S ha^{-1})**			
Soybean	Asgrow 4632	5/8/2015	Planting	0	11	23	3
		5/24/2016	Planting	0	11	23	3
Corn	Dekalb 66-97	4/28/2015	Planting	0	11	23	3
	Dekalb 66-87	4/9/2016	Planting	0	11	23	3

[a] Monsanto Company, 800 N. Lindbergh Boulevard, St. Louis, MO 63137.

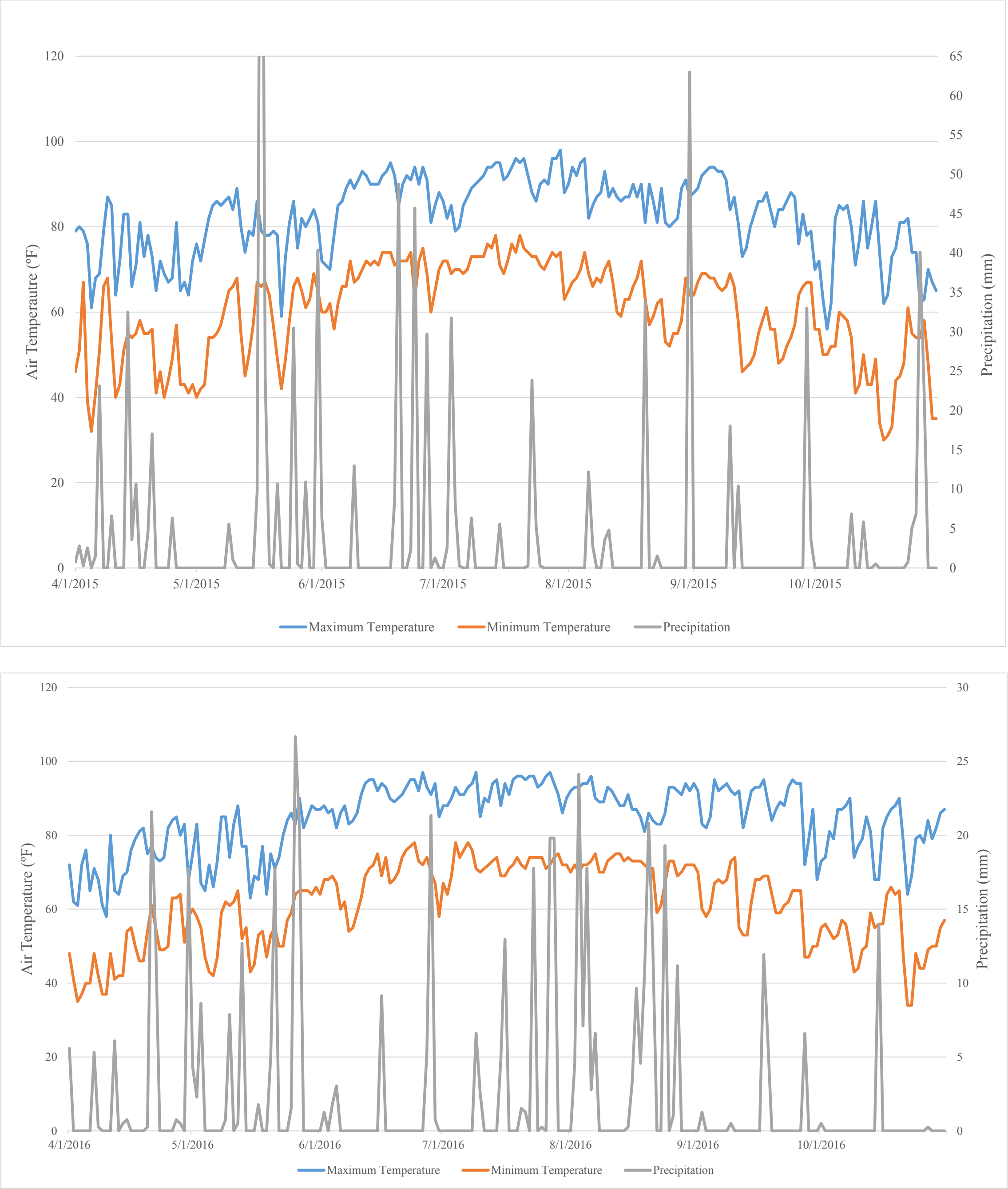

Figure 9. Weather conditions for 2015 & 2016 (maximum, minimum air temperature, and precipitation)

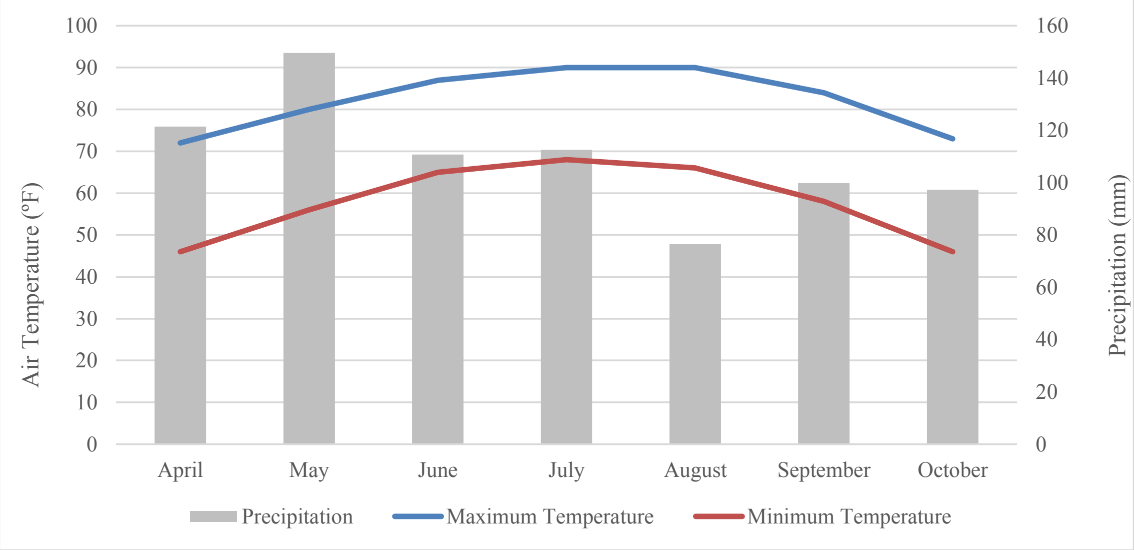

Figure 10. 30 year observed climatic normals for Milan, TN

Table 21. 2015 & 2016 Pre-plant soil SO_4^{2-}-S levels at 0-15 cm and 2015 post-harvest soil SO_4^{2-}-S levels at 0-15 cm, 15-31 cm, and 31-61 cm in corn and soybean experiments treatment means

	2015 Corn Pre-Plant	**2016 Corn Pre-Plant**	**2015 Soybean Pre-Plant**	**2016 Soybean Pre-Plant**	**2015 Corn Post-Harvest**			**2015 Soybean Post-Harvest**		
Treatment[a]	**0-15 cm**	**0-15 cm**	**0-15 cm**	**0-15cm**	**0-15 cm**	**15-31 cm**	**31-61cm**	**0-15 cm**	**15-31 cm**	**31-61 cm**
(kg S/ha)	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*
0	3.99 a	10.00 a	4.50 a	8.53 a	6.80 a	5.85 c	5.21 b	6.33 b	5.67 c	5.17 b
11	2.67 a	9.00 a	4.83 a	10.33 a	7.17 a	6.17 bc	6.50 ba	7.00 ba	6.83 ba	6.83 a
23	3.17 a	9.33 a	4.83 a	8.67 a	7.33 a	7.00 ba	9.17 a	7.17 a	6.33 bc	7.33 a
34	4.00 a	10.00 a	4.17 a	9.00 a	7.33 a	7.50 a	9.00 a	7.50 a	7.33 a	7.67 a
trt	p = 0.7638	p = 0.8441	p = 0.9796	p = 0.5612	p = 0.5316	p = ***0.0192***	p = ***0.035***	p =	p = ***0.0039***	p = ***0.0147***

[a]ammonium sulfate was applied at 11, 23, and 34 kg ha^{-1}in 2013 and 2014 prior to the 2015 sampling

Table 22. Significance of the main effects of S treatments on soybean R1 tissue sulfur, phosphorus, potassium, calcium, boron, iron, manganese, copper, zinc, aluminum, and sodium concentrations, normalized difference vegetation index readings, and mature height in Milan, TN in 2015

	R1[b] Tissue Analysis													
	df	**S**	**P**	**Mg**	**Ca**	**Bo**	**Fe**	**Mn**	**Cu**	**Zn**	**Al**	**Na**	**NDVI[c]**	**Height[d]**
Treatment	3	0.129	0.12	***0.017***	0.515	***<0.0001***	***<0.0001***	***0.0129***	***0.0477***	***0.0013***	0.4172	0.2354	***0.0193***	0.7087

[a] Treatment consisted of ammonium sulfate applied at-planting at rates of 0, 11, 23, and 34 kg S ha^{-1}

[b] Soybean leaves were sampled at R1 growth stage and nutrients were measured in part per million

[c] Normalized Difference Vegetation Index ranges from +1.0 to -1.0 and measured at R5

[d] Plant height was measured in centimeters at R4 growth stage

Table 23. Soybean R1 tissue sulfur, magnesium, boron, iron, manganese, copper, and zinc concentrations and normalized difference vegetation index readings treatment means in Milan, TN in 2015

R1[b] Tissue Analysis								
Treatment[a]	**R1 S**	**R1 Mg**	**R1 Bo**	**R1 Fe**	**R1 Mn**	**R1 Cu**	**R1 Zn**	**NDVI[c]**
(kg S/ha)	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*(mg kg^{-1})*	*0.0 to 1.0*
0	2763.33ab	3856.67a	31.25 a	101.08a	96.97a	8.72a	28.20b	0.75b
11	2745b	3520.00b	28.07b	88.28b	95.95a	8.53a	37.48a	0.77a
23	2801.67ab	3696.67ab	25.73c	79.12c	91.83ab	8.37ab	35.35a	0.76a
34	2856.67a	3605.00b	25.15c	73.58c	85.28b	7.63b	34.38a	0.76a
trt	p = 0.1285	p = ***0.0167***	p = ***<0.0001***	p = ***<0.0001***	p = ***0.0129***	p = ***0.0477***	p = ***0.0013***	p = ***0.0193***

[a] Treatment consisted of ammonium sulfate applied at-planting at 0, 11, 23, and 34 kg ha^{-1}
[b] Soybean leaves were samples at R1 growth stage and nutrients were measured in part per million
[c] Normalized Difference Vegetation Index readings ranges from +1.0 to -1.0 and measured at R5

Table 24. Significance of the main effects of S treatments on soybean seed percent sulfur, percent nitrogen, 100 seed weight, and yield in Milan, TN in 2015 and 2016

Effect[a]	**df**	**Seed Sulfur[b]**	**Seed Nitrogen[c]**	**100 Seed Weight[d]**	**Yield[e]**
Year	1	0.423	0.3864	***<0.0001***	***<0.0001***
Treatment	3	***<0.0001***	0.1219	0.0781	0.3885
Year*Treatment	3	0.5503	0.0711	0.6123	0.5342

[a] Treatment consisted of ammonium sulfate applied at-planting at 0, 11, 23, and 34 kg ha^{-1}
[b] Seed sulfur was measured in percent after harvest
[c] Seed nitrogen was measured in percent after harvest
[d] Seed weight was measured in grams per hundred seed at harvest
[e] Yield consisted of soybean yield (kg ha^{-1}) adjusted to 13% moisture

Table 25. Soybean seed percent sulfur treatment means at Milan, TN across 2015 and 2016

Treatment[a]	**Seed Sulfur [b]**
(kg S ha^{-1})	*g kg^{-1}*
0	2.29 c
11	2.74 b
23	2.88 a
34	2.91 a
trt	p = ***<0.0001***

[a] Treatment consisted of ammonium sulfate applied at-planting at 0, 11, 23, and 34 kg ha^{-1}

[b] Seed sulfur was measured in percent after harvest

Table 26. Significance of the main effects of S treatments and the interaction among the main effects on corn V6 tissue sulfur, nitrogen, phosphorus, magnesium, calcium, iron, potassium, manganese, copper, boron, zinc, and aluminum concentrations (mg kg^{-1})

		V6[b] Tissue											
			2016										
Effect[a]	**df**	**S**	**N**	**P**	**K**	**Mg**	**Ca**	**Fe**	**Mn**	**Cu**	**B**	**Zn**	**Al**
Year	1	***<0.0001***	.	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	0.6451	0.0694	***<0.0001***	***<0.0001***	0.629	***<0.0001***
Treatment	3	***<0.0001***	0.8735	0.216	0.1136	0.6565	0.1499	***0.0286***	***0.0475***	***0.0064***	0.4069	0.9072	0.3618
Year*Treatment	3	***0.0048***	.	0.6316	0.4734	0.5289	0.2989	0.5264	0.5064	0.9213	0.6111	0.8787	0.4479

[a] Treatment consisted of ammonium sulfate applied at-planting at 0, 11, 23, and 34 kg ha^{-1}

[b] Corn leaves were sampled at V6 growth stage and nutrients were measured in part per million

Table 27. Significance of the main effects S treatments and the interaction among the main effects on corn R1 tissue sulfur, nitrogen, phosphorus, magnesium, calcium, iron, manganese, copper, boron, zinc, and aluminum concentrations (mg kg^{-1}) in Milan, TN across 2015 and 2016

		R1[b] Tissue Analysis											
			2016										
Effect[a]	**df**	**S**	**N**	**P**	**K**	**Mg**	**Ca**	**Fe**	**Mn**	**Cu**	**B**	**Zn**	**Al**
Year	1	***<0.0001***	.	***<0.0001***	***0.0182***	0.4758	***<0.0001***	0.2505	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
Treatment	3	***<0.0001***	0.4002	0.4107	0.2459	0.3635	0.8125	0.1167	***0.0013***	***0.0129***	0.1995	0.7183	0.1441
Year*Treatment	3	0.5871	.	0.9477	0.2708	0.3318	0.763	***0.02***	0.1711	0.1184	0.0901	0.0335	***0.0111***

[a] Treatment consisted of ammonium sulfate applied at-planting at 0, 11, 23, and 34 kg ha^{-1}

[b] Corn leaves were sampled at R1 growth stage and nutrients were measured in part per million

Table 28. V6 tissue sulfur concentrations (g kg^{-1}) year*treatments means in Milan, TN in 2015 and 2016

Year	**Treatment[a]**	**S Tissue[b]**	**V6 Sufficient Tissue S Range**
2015	0	2.50 c	1.50 - 4.0
	11	3.09 b	
	23	3.36 ab	
	34	3.71 a	
2016	0	2.75 c	
	11	4.28 b	
	23	4.73 a	
	34	4.77 a	

[a] Treatment consisted of ammonium sulfate applied at-planting at 0, 11, 23, and 34 kg ha^{-1}

[b] Corn tissue was sampled at V6 growth stage and sulfur was measured in part per million

Table 29. Corn R1 tissue sulfur concentrations (g kg^{-1}) treatment means in Milan, TN across 2015 and 2016

Treatment[a]	**S Tissue**[b]	**R1 Tissue S Sufficiency Range**
0	1.71 b	1.5 - 6.0
11	2.06 a	
23	2.05 a	
34	2.16 a	

[a] Treatment consisted of ammonium sulfate applied at-planting at 0, 11, 23, and 34 kg ha^{-1}

[b] Corn tissue was sampled at R1 growth stage and sulfur was measured in part per million

Table 30. Corn V6 and R1 tissue iron, manganese, and copper concentrations (ppm) treatment means in Milan, TN across 2015 and 2016

	V6 Tissue Analysis[b]			**R1 Tissue Analysis**[c]	
Treatment[a]	**Fe**	**Mn**	**Cu**	**Mn**	**Cu**
(kg S ha^{-1})	*mg kg^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*
0	447.75 a	152.68 ab	18.85 a	145.56 a	13.89 a
11	435.92 a	159.75 a	18.71 a	125.90 cb	12.84 a
23	444.50 a	159.58 a	18.18 a	130.22 b	12.48 ba
34	321.00 b	130.36 b	15.26 b	112.88 c	11.32 b
trt	p = ***0.0286***	p = ***0.0475***	p = ***0.0064***	p = ***0.0013***	p = ***0.0129***

[a] Treatment consisted of ammonium sulfate applied at-planting at 0, 11, 23, and 34 kg ha^{-1}

[b] Corn tissue was sampled at V6 growth stage and sulfur was measured in part per million

[c] Corn tissue was sampled at R1 growth stage and sulfur was measured in part per million

Table 31. Corn R1 tissue iron, aluminum, and zinc concentrations (mg kg^{-1}) year*treatment means

			R1 Tissue Analysis[b]	
Year	**Treatment**[a]	**Fe**	**Al**	**Zn**
2015	0	245.71 a	52.33 a	12.76 a
	11	180.00 ab	46.72 a	12.73 a
	23	242.33 a	45.40 a	11.95 b
	34	139.67 b	45.92 a	13.10 a
2016	0	197.33 b	62.27 b	9.95 a
	11	279.83 a	72.17 a	10.80 a
	23	211.00 b	72.48 a	11.13 a
	34	205.83 b	61.22 b	10.27 a

[a] Treatment consisted of ammonium sulfate applied at-planting at 0, 11, 23, and 34 kg ha^{-1}

[b] Corn tissue was sampled at R1 growth stage and sulfur was measured in part per million

Table 32. Significance of the main effects S treatments and the interaction among the main effects on corn mature height and 100 seed weight and yield in Milan, TN across 2015 and 2016

Effect[a]	**Mature Height**[b]	**Seed S**[c]	**100 Seed Wt.**[d]	**Yield**[e]
Year	***0.0104***	.	***<0.0001***	***<0.0001***
Treatment	***0.0032***	***0.0018***	0.3503	***0.0046***
Year*Treatment	0.4679	.	0.4235	0.4599

[a] Treatment consisted of ammonium sulfate applied at-planting at 0, 11, 23, and 34 kg ha-1

[b] Corn height was measured at R1 growth stage in centimeters

[c] Seed sulfur was measured in grams per kilogram

[d] Seed weight was measured in grams per hundred seed at harvest

[e] Yield consisted of corn yield (kg ha-1) adjusted to 15.5% moisture

Table 33. Corn mature height and yield (mg ha-1) treatment differences in Milan, TN across 2015 and 2016

Treatment[a]	**Mature Height**[b]	**Seed S**[c]	**Yield**[d]
(kg S ha^{-1})	*(cm)*	*g kg^{-1}*	(*Mg ha^{-1}*)
0	111.10 b	0.858 b	9.11 b
11	116.12 a	1.1 a	10.31 a
23	114.72 a	1.08 a	10.63 a
34	113.46 ba	1.09 a	10.81a
trt	p = ***0.0032***	p = ***0.0018***	p = ***0.0046***

[a] Treatment consisted of ammonium sulfate applied at-planting at 0, 11, 23, and 34 kg ha-1

[b] Corn height was measured at R1 growth stage in centimeters

[c] Seed sulfur was measured in grams per kilogram

[d] Yield consisted of corn yield (kg ha-1) adjusted to 15.5% moisture

Part IV.

Conclusions

The objective of the first part of this research was to evaluate various N rates and application timings for soybean yield optimization under field conditions. The objective of the second part of this research was to evaluate various S rates for soybean and corn yield optimization under field conditions.

Part II.

Soybean Nodulation

Based on the analysis of early and mid-season nodulation data, overall nodulation was not affected by the N treatments across the two years and environments. Although, in both years the irrigated environment had less nodules than the dryland. However, N treatments did significantly decrease nodule size and maturity numbers. Inhibition of active and adolescent nodules were the result of added N compared to the zero control when applied at-planting and the V2 growth stage. Nevertheless, 34 kg ha^{-1} applied at-planting had the least negative effect on active and adolescent nodules compared to the other treatments.

Yield and 100 seed weight

One hundred seed weights and yield were not significantly affected by the N treatments; although, dryland tended to have larger seeds and higher yields. Lodging in the irrigated environment was a severe issue in 2015 and 2016, which was presumed to have jeopardized yield numbers, whereas, the dryland had better plants at harvest. This result was probably due to extra moisture entering the irrigated environment, N promoting more vegetation, and the soybean variety used; consequently setting up a good environment for lodging. Therefore, more research is needed to correct this issue to achieve better yield data and overall plant health in an irrigated environment. As of now, the study indicates that it is not economical for a Tennessee producer to incorporate N applications into their soybean production systems.

Part III.

Soybean yield and other harvest measurements

Sulfur applications did not significantly increase seed N, 100 seed weight, or yield. Sulfur applications decreased early uptake of most macro and micronutrients, which was assumed to have potentially held back yield numbers. However, S treatments did significantly increase seed S, which contributes to a more nutritional soybean. Ultimately, this study revealed that S applications to a soybean crop is not economical for a Tennessee producer to incorporate into their production systems.

Corn yield and other harvest measurements

Nutrient uptake was not as strongly impacted in the corn experiment as it was in soybean. Therefore, with less limiting factors, more height/vegetation was produced, which probably resulted in the significant yield data. Also, treatments significantly increased seed S levels, which is a key factor to producing a more nutritional grain. Thus, this experiment disclosed that S fertilizer applications at a rate as low as 11 kg ha^{-1} can be profitable for Tennessee producers to include into their production systems by increasing yields by 1, 511.25 (16%)

Vita

Kacey Cannon was born January 24, 1993 in Memphis, TN, the oldest child of Randy and Melissa Cannon of Somerville, TN. She graduated from Rossville Christian Academy in May of 2011, after which she accepted a softball scholarship to Jackson State Community College (JSCC), where she received an Associate of Science degree in Agriculture with a concentration in Plant Science in December 2013. After graduating from JSCC, she continued her education at the University of Tennessee at Martin and received a Bachelor of Science degree in Agriculture with a concentration in Crop and Soil Management in December 2015. After graduation, she accepted a position at the University of Tennessee as a Graduate Research Assistant in the Plant Sciences Department with Dr. Angela McClure, UT Extension Corn and Soybean Specialist. She was awarded a Master of Science degree in Plant Science with a concentration in Crop Science in May 2017. Upon graduation, Kacey accepted a position with an independent crop consulting company as a crop consultant/research and development support for west Tennessee.