The Evaluation of Winter Wheat Response to Nutrient Sources of Sulfur and Application Timing 2020 – 2023

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ABSTRACT

Enhanced efficiency fertilizers (EEFs) have gained considerable interest in recent years as human population trends demand greater productivity from cropping systems while minimizing human health and environmental concerns from nutrient loss. Previous research has shown EEFs to be beneficial in diverse cropping systems, but research into their use in winter wheat development has been limited. This study assessed the effects of Sulfur (S) sources derived from three EEF products in comparison to a commonly used commercial product (Ammonium Sulfate) and a control (no S) as well as the effect of application timing of S on the production of tillers and grain yield of winter wheat. Field trials were conducted over a 3-year period in Warsaw, VA and Westmoreland County, VA. During the early growing season of winter wheat, the tissue samples and aerial normalized difference vegetation index (NDVI) values of before and after the mid-winter application indicated that there was some response to application timing of S, but with the exception of the Warsaw 2021 second tissue sampling S percentage analysis, there was no significant response from the sources of S tested. As the growing season progressed, NDVI values measuring tiller density showed no significant difference, which later corresponded with the end of the growing season, as there was no grain yield response to source of S or application timing of S. Overall, the S additives from the EEFs tested did not consistently impact wheat tiller development or grain yield and are therefore cost prohibitive.

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Introduction

Tiller development of winter wheat (*Triticum aestivum* L.) is a major component of yield potential and relies heavily on environmental conditions, variety selection, and management practices (Tilley et al., 2019). One key element of winter wheat management is nutrient application. The production of winter wheat demands high nitrogen (N) inputs, but in order to maximize N response, sufficient levels of sulfur (S) must be present (Hu and Sparks, 1992). Sulfur plays an important role in the formation of proteins, is a key ingredient in the formation of chlorophyll, and is a significant component of N metabolism enzymes such as nitrate reductase and glutamine synthetase (Duke and Reisenaue, 1986; Tao et al., 2018). Without adequate S, N use efficiency (NUE) may be reduced and the crop cannot reach its full potential in yield or protein content (Tao et al., 2018).

While S requirements were once met through wet and dry deposition of S compounds and release from organic matter, S deficiency of agricultural land has become more problematic due to the gradual replacement of S-containing fertilizers with high purity N fertilizers, the introduction of high yielding crop cultivars with increased S demand, and, since the 1970s, strict restrictions on sulfur dioxide emissions to reduce greenhouse gases (Naeem, 2008). The National Atmospheric Deposition Program's 2021 Annual Summary reported that Virginia received an average 3 kg ha^{-1} of sulfate as SO_4^{2-} through rainfall that year, which is considerably less than the wheat crop requirement of 15-20 kg ha^{-1} (Zhao et al., 1999). For the eastern United States, decreases in wet deposition of sulfate averaged more than 68 percent between the 1989 to 1991 and 2014 to 2016 observation periods (USEPA, 2022). With an increasing prevalence of S deficiency in agricultural soils, a growing demand for research of S fertilization has been evident. Two aspects of S management that must be considered are timing of application and source. Girma et al. (2005) concluded that the form of S applied is equally important as the rate of S applied in obtaining a significant response. The study revealed that significantly higher yields were obtained when calcium sulfate ($CaSO_4$) was applied as opposed to elemental S due to the immediate availability of sulfate in $CaSO_4$ (Girma et al., 2005). During year 2 and 3 of the study, reports of high rainfall during the growing season presumably caused $CaSO_4$ to leach out and caused a lower forage yield, while the slow transformation of elemental S made S available to the crop (Girma et al., 2005). In general, S sources other than elemental S are known to increase growth and yield of wheat, as sources containing sulfate provide S in a readily usable form, whereas elemental S must be oxidized by soil bacteria in order for plants to utilize it and creates temporary acidity in the rhizosphere, potentially reducing wheat yields (Kulczycki, 2021; Girma et al., 2005). In a more recent study by Khan et al. (2019), researchers found that the source of S and time of application had an effect on grain yield. The highest grain yield was achieved from sulfate of potash (potassium sulfate) followed by ammonium sulfate, gypsum, and elemental S, revealing an increase of 63%, 45%, 40%, and 38% in grain yield over the control (no S) respectively (Khan et al., 2019). This study also found that applying S at sowing maximized grain production compared to early application of S (15 days) before sowing of the wheat crop (Khan et al., 2019). Understanding timing of S application and the source of S is important for certain soil types and climatic conditions. Sulfate is relatively mobile in most soils because it has a double negative charge and is repelled by the negative charge of the soil, and although sulfate can bind to iron and aluminum in the soil, these elements are more likely to bind to phosphate at the exclusion of sulfate, causing sulfate to leach from soils, particularly sandy soils (Camberato and Casteel, 2017). The availability of S is critical during the period of incompatibility of conditions where rapid plant growth occurs in early spring, while the rate of S release from soil organic matter is slow (Girma et al., 2005).

While a number of fertilizers and byproducts can serve as good sources of S for field crop production, enhanced efficiency fertilizers (EEFs) containing S could be an effective way to deliver N and S in a single application. EEFs have gained considerable interest in recent years as a growing human population demands greater productivity from cropping systems while mitigating human and environmental health effects from nutrient losses to the environment (Li et al., 2018). By either slowing the rate of nutrient release or delaying the transformation processes with inhibitors or coating materials, EEFs aim to achieve better synchronicity between nutrient release and crop uptake (Dimkpa et al., 2020; Li et al., 2018). EEFs are continuously being developed to improve uptake and utilization of nutrients by plants. The global EEF market is anticipated to gain a compound annual growth rate (CAGR) of 6.11 percent throughout the forecast period of 2020-2028 (Research Nester, 2020). The two basic classifications of EEFs include controlled-release fertilizers and stabilized fertilizer products (Adams et al., 2018). Controlled-release fertilizers include materials of sparing solubility and coated materials, including inorganic-, bio-, or organic-coated, whereas stabilized fertilizers include materials treated with nitrification and/or urease inhibitors (Adams et al., 2018; Dimkpa et al., 2020). In a comprehensive analysis of worldwide studies published between 1980 and 2016, Li et al. (2018) evaluated four major types of EEFs (polymer-coated fertilizers PCF, nitrification inhibitors NI, urease inhibitors UI, and double inhibitors DI, i.e. urease and nitrification inhibitors combined), for their effectiveness in increasing yield, improving NUE, and reducing N losses across three different cropping systems (paddy, grassland, and dryland systems). For wheat and maize systems, Li et al. (2018) found that UI increased yield 3%, improved NUE by 14% and reduced N-loss by 28%; NI had comparable benefits in improving productivity, but marginal reduction in aggregated N-loss; DI was ineffective except for a marginal yield increase (<3%); and PCF offered little to negative effects on productivity. Li et al. (2018) concluded that EEF efficacies in wheat and maize systems were more complicated and generally less effective, but that there are potential benefits of EEFs when a need is created, such as downward adjusting N application from a conventional rate.

The objective of this study was to assess the effects of S sources derived from three EEF products in comparison to a market standard product (Ammonium Sulfate) and a control (no S) as well as the effect of S application timing on the production of tillers and grain yield of winter wheat.

Materials and Methods

Site Description and Experimental Design

Field experiments were conducted from 2020 to 2023 and maintained under rainfed conditions. During the 2020-2021 and 2022-2023 growing seasons, the study was conducted at Warsaw, VA, located in Richmond County (Kempsville loam—fine-loamy, siliceous, subactive, thermic Typic Hapludults). During the 2021-2022 growing season, the study was conducted at two locations; Warsaw, VA (Richmond County) and Westmoreland County, VA (Suffolk sandy loam—fine-loamy, siliceous, semiactive, thermic Typic Hapludults). Weather data from Warsaw, VA for each growing season is provided in Table 1.

For each trial, the cultivar 'Liberty 5658' was planted with a Hege 1000, 7-row grain drill (Hege Equipment Inc.; Colwich, KS) at 22 seeds per row meter in 19 cm rows, with individual plots measuring 24.4 m^2 before spray back and 13.7 m^2 after spray back. Seed was treated with the pesticides CruiserMaxx® Vibrance® Cereals (Sedaxane, Difenoconazole, Mefenoxam, and Thiamethoxam; Syngenta Crop Protection, LLC; Greensboro, NC) and Cruiser® 5FS (Thiamethoxam; Syngenta Crop Protection, LLC; Greensboro, NC). Plots were planted in conventionally tilled fields and arranged in a randomized complete block design with ten treatments and four replications. Three EEF products (SymTRX 10S, SymTRX 20S, SymTRX 10S + Comp1-MES10) and Ammonium Sulfate (AMS) were tested, representing various sources of S, and compared to a control (no S). SymTRX 10S contains 140g N kg^{-1}, 240g P_2O_5 kg^{-1}, and 100g S kg^{-1} (14-24-0-10S). It is derived from anhydrous ammonia, sulfuric acid, phosphoric acid, manure digestate protein hydrolysate, food digestated protein hydrolysate, and biosolids protein hydrolysate (Anuvia Plant Nutrients, 2020). SymTRX 20S contains 160g N kg^{-1}, 10g P_2O_5 kg^{-1}, and 200g S kg^{-1} (16-1-0-20S). It is derived from anhydrous ammonia, sulfuric acid, aluminum sulfate, manure digestate protein hydrolysate, food digestate protein hydrolysate, and biosolids protein hydrolysate (Anuvia Plant Nutrients, 2020). SymTRX 10S + Comp1-MES10 contains 130g N kg^{-1}, 320g P_2O_5 kg^{-1}, and 100g S kg^{-1} (13-32-0-10S). It is similarly an enhanced efficiency homogenous multi-nutrient fertilizer containing 16 percent organics. AMS is a commercial product derived from the reaction of sulfuric acid with heated anhydrous ammonia to create $(NH_4)_2SO_4$ and contains 210g N kg^{-1} and 100g S kg^{-1} (21-0-0-23S) (USEPA, 1992). The EEF materials were obtained from Anuvia Plant Nutrients (Plant City, FL), while the AMS and supplemental materials were obtained from Nutrien Ag Solutions (Montross, VA). Commercial products of 11-52-0-0S, 0-0-60-0S, and 44-0-0-0S were supplemented to achieve equal amounts of N, Phosphorous (P), and Potassium (K) in each treatment and application date. The second variable, application timing, was tested by applying S either at planting, during mid-winter (December/January), or a split-application (half the rate at plant and half the rate in mid-winter). The at-plant application of S was tested with S sources of AMS, SymTRX 10S, SymTRX 20S, and SymTRX 10S + Comp1-MES10. The mid-winter (December/January) application of S was tested with the S sources AMS, SymTRX 10S, and SymTRX 20S, and there was one split application of S using the S source SymTRX 10S. Phosphorous was delivered during the application timing of S for all treatments because the EEF products cannot be separated from their P content. Refer to Table 2 for the list of treatments, the corresponding nutrient ratios, and the nutrient rate calculations of each treatment applied at plant and in mid-winter (December/January).

In year 2021, plots were harvested using a Wintersteiger Classic plot combine (Wintersteiger AG; Ried, Austria). Moisture, grain weight, and test weight were obtained from a Grain Analysis Computer (DICKEY-john, TSI Inc.; Auburn, IL) shortly after harvest. In 2022 and 2023, plots were harvested using a Zürn 150 plot combine (Zürn Harvesting GmbH & Co. KG; Schöntal-Westernhausen, Germany) equipped with a HarvestMaster GrainGage (Juniper Systems Inc.; Logan, UT) to record moisture, grain weight, and test weight. Cultural practices for each site-year, including planting dates, fertility and pesticide applications, and harvest dates are provided in Table 3.

Data Collection

Tissue samples were collected during the 2020-2021 and 2021-2022 growing seasons. Samples were obtained on three separate occasions for each site-year to evaluate nutrient status after the first fertility treatment, after the second fertility treatment, and at Zadoks growth stage 30 (GS 30). Refer to Table 4 for tissue collection dates. Plant samples were taken from the portion of the experimental plot that would later be sprayed back to harvest length as to not impact yield data. The entire above-ground portion of the plants were removed with scissors at the base of the plant and sent to Waypoint Analytical Virginia, Inc. to undergo acid digestion for analysis. Soil sampling was conducted at the beginning of the 2021-2022 growing season to analyze whether S uptake was occurring once roots elongated to reach subsoils containing significant quantities of sulfate. Soil samples were taken from 0 to 15 cm and 15 to 30 cm of the soil profile from each location (Warsaw, VA and Westmoreland, VA) using an AMS Soil Recovery Probe (AMS, Inc.; American Falls, ID). Analytical methods of SMP buffer pH, Mehlich 3, loss of ignition, and water pH were used to analyze the soil samples. Results of the soil analysis can be found in Table 5.

Since research shows that aerial NDVI is a reliable predictor of tiller density (Lee and Oakes, 2022), NDVI was collected at all site-years as a proxy for tiller density. NDVI data was obtained using a MicaSense RedEdge multispectral sensor affixed to a DJI Matrice 100 Quadcopter (SZ DJI Technology Co., Ltd.; Nanshan District, Shenzhen, China). A MicaSense Calibrated Reflectance Panel (AgEagle Aerial Systems Inc.; Wichita, KS) was used to calibrate NDVI before each flight. Images were collected at an altitude of 50 meters with a 75% overlap; the Atlas Flight app was used for flight planning and navigation. The UAV used its built-in GPS to navigate, acquire, and store the images. After each flight, images were merged into orthomosaics in Pix4D software. Using the index calculator in Pix4D, NDVI was extracted from each individual plot using the formula:

$$(NIR - R) / (NIR + R)$$

in which NDVI is calculated as a ratio between the red (R) and near infrared (NIR) values to obtain an average NDVI reading for the entire plot (Landsat Missions, n.d.). Table 6 lists NDVI collection dates for each site-year.

Harvest data (percent moisture and grain weight) was used to calculate grain yield in Microsoft Excel. The grain yield calculation protocol produced by the Ohio State University Soil Fertility Lab (2021) was used as a guide for calculations. Grain moisture was adjusted to a standard percentage by calculating the coefficient ratio of measured moisture versus standard biomass without moisture, using the formula:

$$(100 - \text{measured moisture}) / (100 - 13.5\ \%\ \text{standard moisture})$$

Then, the total plot weight was multiplied by the biomass ratio above to provide pounds of grain at a standardized moisture. This value was then divided by the area of the harvested plot and converted to megagrams per hectare for scientific reporting purposes.

Statistical Procedure

The Proc GLM and Proc Mixed procedures in SAS 9.4 (SAS Institute, Cary, NC) was used to determine if there were differences in NDVI (tiller density), tissue sample content, and yield among the treatments. The rep effect was considered random. A *p* level of 0.05 was used to determine statistical difference. When differences were detected, Fisher's protected LSD was used to separate the means.

Results and Discussion

Tissue Sample Analysis

The comparison between tissue samples, measuring the effect of the first fertility treatment, the second fertility treatment, and nutrient status at GS 30 indicated differences in crop nutrient status at each sampling date during the growing season. The first tissue sample was collected at three site-years (Warsaw 2021, Warsaw 2022, Westmoreland 2022) to measure the effects of the first fertility application, in which at-plant treatments received S and P, the split application received half the rate of S and P, and all treatments received the same amount of N and K. Analysis of variance for the first tissue sample indicated a treatment main effect for N percentage, P percentage, and S percentage, with S percentage also having a location by treatment interaction (Table 7). Site-year data for N percentage and P percentage tissue samples were analyzed together because there was no location by treatment interaction. Since S percentage had a location by treatment interaction, the three site-years were analyzed separately (Table 8). In the first tissue sample, N percentage was greater for the at-plant applications of AMS (4.81%) and SymTRX 20S (4.75%) compared to the at-plant control (4.50%), and the mid-winter applications of the control (4.45%), AMS (4.41%), SymTRX 20S (4.44%) and SymTRX 10S (4.46%). Likewise, the at-plant application of SymTrx 10S (4.71%), the SymTrx 10S split application (4.61%), and the SymTrx10S + Comp1-MES10 at plant (4.65%) were not different from the AMS and SymTrx20S at-plant. This may be the effect of the role of S in maximizing N response (Hu and Sparks, 1992). The first tissue sample S content of the Warsaw 2021 trial corresponded with S applications, as the at-plant application treatments of AMS (0.36%), SymTRX 20S (0.36%), SymTRX 10S (0.35%), SymTRX 10S + Comp1-MES10 (0.35%), and the SymTrx20S split application (0.33%) were significantly greater than the controls (0.28%). The treatments that received a full at-plant rate also had significantly greater S percentage than the mid-winter applications. The first tissue sample S percentage of the Warsaw 2022 trial also showed at-plant application of S treatments, with the addition of the split application of S, to have greater S percentage than the mid-winter application treatments. The Westmoreland 2022 first tissue sample S percentage only had the AMS at-plant application of S (0.42%) to be greater than the mid-winter application treatments of AMS (0.37%) and SymTRX 20S (0.37%). First tissue sample P content corresponded with timing of nutrient application as all at-plant application treatments and the split application of SymTrx 10S were significantly greater in P content than plant tissues of treatments that received P during mid-winter application.

The second tissue sample was collected to measure the effects of the second fertility application, in which mid-winter treatments received S and P, the split application received another half rate of S and P, and all treatments received the same amount of N. Analysis of variance for the second tissue sample indicated a treatment main effect for N percentage, P percentage, K percentage and S percentage, with S percentage and P percentage also having a location by treatment interaction (Table 7). N percentage and K percentage values allowed the site-year data to be combined and analyzed together because there was no location by treatment interaction, and S percentage and P percentage were analyzed separately due to the location by treatment interaction (Table 9). In the second tissue sample, N content was significantly greater for the mid-winter applications of AMS (4.51%), SymTRX 20S (4.7%), and SymTRX 10S (4.59%) compared to the SymTRX 10S split application (4.27%), and at-plant applications of control (4.00%), AMS (4.01%), SymTRX 20S (4.04%), SymTRX 10S (4.09%), and

SymTrx 10S + Comp1-MES10 (4.07%). Again, this this may be due to the effect of the role of S in maximizing N response (Hu and Sparks, 1992). The second tissue sample S content of the Warsaw 2021 trial corresponded with S applications, as the mid-winter application treatments of AMS (0.43%), SymTRX 20S (0.38%) and SymTRX 10S (0.38%) were greater than the controls (at plant, 0.22%; mid-winter, 0.27%), SymTRX 10S split application (0.29%), and at-plant application treatments of AMS (0.22%), SymTRX 20S (0.22%), SymTRX 10S (0.30%), and SymTRX 10S + Comp1-MES10 (0.23%). This site-year tissue sample had a response based on source of S, as the SymTRX 10S at plant application S content (0.30%) was greater than the at-plant application treatments of control (0.22%), AMS (0.22%), SymTRX 20S (0.22%), and SymTRX 10S + Comp1-MES10 (0.23%). The Warsaw 2022 and the Westmoreland 2022 S content tissue data was not significantly different at LSD $p<0.05$. The second tissue sample P content corresponded with timing of nutrient application again for the Westmoreland 2022 site-year, in which all mid-winter application treatments received P during that time and were significantly greater in P content than the at-plant application treatments and split application treatment. The Warsaw 2022 P content data was not significantly different at LSD $p<0.05$. The Warsaw 2021 P content showed the AMS mid-winter application treatment (0.40%) as significantly greater than the at-plant application treatments of control (0.33%), AMS (0.33%), SymTRX 20S (0.32%), and SymTRX 10S + Comp1-MES10 (0.31%), and the SymTRX 20S mid-winter application treatment (0.38%) as significantly greater than the SymTRX 10S + Comp1-MES10 at-plant application treatment. The treatment main effect for the second tissue sample K content showed the mid-winter application treatments of AMS (4.08%), SymTRX 20S (3.96%), and SymTRX 10S (3.94%)

to be significantly greater than the at-plant application treatments of control (3.52%), AMS (3.71%), SymTRX 20S (3.60%), SymTRX 10S (3.62%), and SymTRX 10S + Comp1-MES10 (3.53%).

The third tissue sample collection occurred at each site-year during GS 30, where wheat plants shift from vegetative to reproductive growth, all meaningful tillering has been completed, and the main stem and tillers start to synchronize (Larsen et al., 2008). Analysis of variance for the third tissue sample indicated only a treatment main effect for N content (Table 7). Nitrogen percentage was significantly greater for the mid-winter application treatments of AMS (4.05%) and SymTRX 20S (4.08%) compared to the at-plant applications of control (3.85%), AMS (3.82%), SymTRX 10S (3.82%), SymTRX 10S + Comp1-MES10 (3.82%), and mid-winter application control (3.86%) (Table 10). The N content of the SymTRX 10S mid-winter application treatment (4.01%) was also significantly greater than the at-plant application treatments of AMS, SymTRX 10S, and SymTRX 10S + Comp1-MES10.

NDVI

Aerial NDVI is a reliable predictor of tiller density (Lee and Oakes, 2022), which is a major determinant of yield potential (Tilley et al., 2019). NDVI values were analyzed for each site-year before the mid-winter application, after the mid-winter application, and at GS 30. Analysis of variance indicated a treatment main effect and location by treatment interaction for NDVI values before the mid-winter application, a treatment main effect and location by treatment interaction for NDVI values after the mid-winter application, and no treatment main effect or location by treatment interaction for the NDVI values at GS 30 (Table 11). Because there was a location by treatment interaction for NDVI values of the before and after mid-winter application, NDVI values for each site-year are provided in Table 12.

The Warsaw 2021 before mid-winter application NDVI values indicated a correlation between application timing and tiller density, as treatments with the at-plant application of S and the split application of S had significantly higher NDVI values compared to the treatments with the mid-winter application of S. The Warsaw 2022 before mid-winter application NDVI values were not significantly different at LSD $p<0.05$. The Warsaw 2023 before mid-winter application NDVI values showed more overlap between treatments, but followed a similar pattern as the Warsaw 2021 before mid-winter application NDVI values; the SymTRX 10S at-plant application NDVI value was significantly greater than the treatments with mid-winter applications of S, and the SymTRX 10S split application and remaining at-plant application treatments were also significantly greater than mid-winter applications, with the exception of the SymTRX 20S mid-winter application. Additionally, the SymTRX 20S mid-winter application treatment NDVI value was significantly greater than the SymTRX 10S mid-winter application treatment, but this cannot be attributed to fertilizer source as both treatments received the same supplemental nutrients at this point, and have yet to receive the EEF material. It must also be noted that there was not a Westmoreland 2022 NDVI reading for the before mid-winter application comparison due to mechanical difficulties.

The NDVI values of after mid-winter application during the Warsaw 2021 trial showed that the SymTRX 10S mid-winter application (0.80) and the split application of SymTRX 10S (0.81) were the only treatments to have a significantly greater NDVI value than the at-plant control (0.76). During the Warsaw 2022 trial, the AMS at-plant NDVI value (0.46) was significantly greater than the controls, the mid-winter application treatments, and the SymTRX 10S split application treatment. The NDVI values of the at-plant application treatments of SymTRX 20S (0.45) and SymTRX 10S (0.45) were also significantly greater than the mid-winter application treatments of control (0.41), AMS (0.39), and SymTRX 20S (0.41). Lastly, the NDVI values of the at-plant control (0.43), the at-plant SymTRX 10S + Comp1-MES10 (0.44), and the split application of SymTRX 10S (0.43) were significantly greater than the AMS mid-winter application (0.39). The Westmoreland 2022 trial NDVI values of after mid-winter application showed that the at-plant application treatments of AMS (0.38), SymTRX 20S (0.38), SymTRX 10S (0.39), and SymTRX 10S + Comp1-MES10 (0.39) and the split application of SymTRX 10S (0.38) were significantly greater than the mid-winter application treatments, and that the at-plant control NDVI value (0.37) was also significantly greater than the mid-winter application treatments of SymTRX 20S (0.32) and SymTRX 10S (0.33). During the Warsaw 2023 trial, the at-plant application treatment SymTRX 10S NDVI value (0.83) was significantly greater than the mid-winter application treatments of control (0.79), SymTRX 20S (0.79), and SymTRX 10S (0.78). Additionally, the at-plant application treatments of control (0.82), AMS (0.81), SymTRX 10S + Comp1-MES10 (0.82), and the split application of SymTRX 10S (0.82) were significantly greater than the SymTRX 10S mid-winter application treatment NDVI value (0.78). Even though there were site-years where application timing of S showed significant differences in NDVI values, the totality of these results indicates that source of S did not have an effect on NDVI values before or after the mid-winter application, and therefore did not increase tiller density or plant growth. NDVI values for all site-years at GS 30, indicated no significant difference across treatments at LSD $p<0.05$, which corresponds with combined site-year data of the mean grain yield response.

Grain yield

Mean grain yields ranged from 6.49 Mg ha^{-1} to 7.05 Mg ha^{-1}. Significant differences in grain yield were not observed over the course of this study. One explanation for this is that plant tissue analysis for all treatments at each sampling period were within the sufficient range for N percentage (3% - 4.25%), P percentage (0.15% - 0.5%), K percentage (2% - 3.25%), and S percentage (0.1% - 0.35%) (Waypoint Analytical Virginia, Inc., 2022). Soil test results also showed 12 mg kg^{-1} of plant available S present within the first 30cm of the soil profile at both locations during the 2021-2022 growing season (Table 5). This value equates to 27 kg ha^{-1} ((12 mg kg^{-1}*2) *(1.121)) which is greater than the wheat crop requirement of 15-20 kg ha^{-1} (Zhao et al., 1999), concluding sufficient levels of plant available S were available for uptake.

Summary and Conclusion

This study showed that during the early growing season of winter wheat there is a response to application timing of S, indicated by the tissue samples and NDVI values before and after the mid-winter application. The Warsaw 2021 second tissue sampling S percentage analysis was the only measurement taken across site-years to indicate a response from the sources of S tested, but this did not affect yield or tiller density as the growing season progressed. For all site-years, NDVI values showed no significant difference at GS 30. This later corresponded with the end of the growing season, as there was no grain yield response to S source or application time. This is most likely due to sufficient levels of nutrients, confirmed through tissue analysis, and adequate sulfur availability from the soil. Further research could consider evaluating protein content and its effect on the improvement of baking quality of hard red wheat varieties or soft red varieties with low protein content. In general, EEFs have been proven to be beneficial in diverse cropping systems by improving soil health, reducing nutrient losses, and increasing productivity, but their application into a winter wheat production system has not been fully justified by this study. The S additives from the EEFs tested did not consistently impact wheat tiller development or grain yield and are therefore cost prohibitive.

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Table 1. Weather data of each trial in Warsaw, VA. Total Rainfall (mm) and average high and low temperature (°C) by month of growing season.

	2020-2021			2021-2022			2022-2023		
	Rainfall	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall	Temperature (°C)	
Month	(mm)	Avg. High	Avg. Low	(mm)	Avg. High	Avg. Low	(mm)	Avg. High	Avg. Low
October	177.0	21.6	10.7	99.8	23.2	12.8	90.9	19.4	7.6
November	142.7	18.4	5.8	11.9	14.2	1.3	75.7	17.1	5.0
December	152.1	10.3	0.2	26.4	13.9	2.5	105.9	9.7	-1.4
January	70.9	7.7	-1.2	95.3	7.1	-4.0	50.8	12.6	2.2
February	59.4	6.6	-0.3	27.7	12.6	-0.9	42.7	13.9	2.2
March	115.6	16.4	3.9	59.7	16.4	3.6	29.2	15.1	2.9
April	61.5	20.5	7.1	39.1	19.9	6.6	176.8	22.8	9.0
May	123.7	24.8	11.1	123.4	24.7	14.1	97.8	23.0	11.4
June (to harvest)	162.8	28.4	18.3	27.7	29.6	18.1	19.6	27.7	14.9

Table 2. Treatments, N-P_2O_5-K_2O-S ratios, and nutrient rate calculations of each treatment applied at plant and mid-winter.

	At Plant Application			Mid-Winter (Dec./Jan.) Application		
Treatment	**N-P_2O_5-K_2O-S Applied**	**Product (N-P_2O_5-K_2O-S)**	**Rate (kg ha^{-1})**	**N-P_2O_5-K_2O-S Applied**	**Product (N-P_2O_5-K_2O-S)**	**Rate (kg ha^{-1})**
(1) Control (No S) - At Plant Application	35-60-60-0	11-52-0-0	129.3	35-0-0-0	44-0-0-0	89.1
		0-0-60-0	112.0			
		44-0-0-0	56.8			
(2) Ammonium Sulfate (21-0-0-23S) – At Plant Application	35-60-60-25	21-0-0-23	116.5	35-0-0-0	44-0-0-0	89.1
		11-52-0-0	129.2			
		0-0-60-0	112.0			
(3) SymTRX 20S (16-1-0-20S) – At Plant Application	35-60-60-25	16-1-0-20	140.0	35-0-0-0	44-0-0-0	89.1
		11-52-0-0	126.6			
		0-0-60-0	112.0			
		44-0-0-0	6.6			
(4) SymTRX 10S (14-24-0-10S) – At Plant Application	35-60-60-25	14-24-0-10	280.0	35-0-0-0	44-0-0-0	89.1
		0-0-60-0	112.0			
(5) Control (No S) – Mid-winter Application	35-0-60-0	0-0-60-0	112.0	35-60-0-0	11-52-0-0	129.2
		44-0-0-0	89.1		44-0-0-0	56.8
(6) Ammonium Sulfate (21-0-0-23S) – Mid-winter Application	35-0-60-0	0-0-60-0	112.0	35-60-0-25	21-0-0-23	116.5
		44-0-0-0	89.1		11-52-0-0	129.2
(7) SymTRX 20S (16-1-0-20S) – Mid-winter Application	35-0-60-0	0-0-60-0	112.0	35-60-0-25	16-1-0-20	140.0
		44-0-0-0	89.1		11-52-0-0	126.6
					44-0-0-0	6.6
(8) SymTrx 10S (14-24-0-10S) – Mid-winter Application	35-0-60-0	0-0-60-0	112.0	35-60-0-25	14-24-0-10	280.0
		44-0-0-0	89.1			
(9) SymTRX 10S (14-24-0-10S) – Split application, ½ at plant and ½ in mid-winter	35-30-60-12.5	14-24-0-10	140.0	35-30-0-12.5	14-24-0-10	140.0
		0-0-60-0	112.0		44-0-0-0	44.5
		44-0-0-0	44.5			
(10) SymTRX 10S + Comp1-MES10 (13-32-0-10S) - At Plant Application	35-60-60-25	21-0-0-23	28.9	35-0-0-0	44-0-0-0	89.1
		13-32-0-10	210.6			
		0-0-60-0	112.0			
		44-0-0-0	13.0			

Table 3. Cultural practices for each site-year.

2020-2021		2021-2022				2022-2023	
Warsaw		**Warsaw**		**Westmoreland**		**Warsaw**	
22-Oct-20	Plant	11-Nov-21	Plant	21-Oct-21	3 T ha^{-1} Lime	21-Oct-22	3.7 T ha^{-1} Lime
22-Oct-20	At-Plant Fertilizer Treatment	11-Nov-21	At-Plant Fertilizer Treatment	19-Nov-21	Plant	31-Oct-22	Plant
3-Dec-20	Mid-Winter Fertilizer Treatment	29-Dec-21	Mid-Winter Fertilizer Treatment	19-Nov-21	At-Plant Fertilizer Treatment	2-Nov-22	At-Plant Fertilizer Treatment
11-Dec-20	0.07 L ha^{-1} Harmony Extra SG Herbicide + 2Q Liberate Surfactant Per 100 Gal. of Water	15-Feb-22	28 kg ha^{-1} 12-0-0-1.5	14-Jan-21	Mid-Winter Fertilizer Treatment	13-Dec-22	Mid-Winter Fertilizer Treatment
9-Mar-21	0.06 L ha^{-1} Quelex Herbicide + 1.9L Liberate Surfactant Per 379L of Water	16-Feb-22	0.06 L ha^{-1} Quelex Herbicide + 1L Liberate Surfactant per 379L of Water	15-Feb-22	28 kg ha^{-1} 12-0-0-1.5	20-Mar-23	0.04 L ha^{-1} Harmony Extra SG + 0.06 L ha^{-1} Quelex + 1L Liberate Surfactant per 379L of Water
27-Mar-21	67.2 kg ha^{-1} 24-0-0-3	8-Mar-22	0.3 L ha^{-1} Fitness Fungicide + 1L Liberate Surfactant per 379L of Water	23-Mar-22	67.2 kg ha^{-1} 24-0-0-3	21-Mar-23	61.6 kg ha^{-1} 24-0-0-3
16-Jun-21	Harvest	21-Mar-22	67.2 kg ha^{-1} 24-0-0-3	2-Apr-22	2.3 L ha^{-1} BOROSOL 10 & 1.2 L ha^{-1} NUTRISYNC Copper	29-Mar-23	0.3 L ha^{-1} Fitness Fungicide
		2-Apr-22	2.3 L ha^{-1} BOROSOL 10 & 1.2 L ha^{-1} NUTRISYNC Copper	11-Apr-22	0.3 L ha^{-1} Fitness Fungicide + 1L Induce Surfactant per 379L of Water	25-Apr-23	1 L ha^{-1} Miravis Ace Fungicide + 1L Liberate Surfactant per 379L of Water
		11-Apr-22	0.3 L ha^{-1} Fitness Fungicide + 1L Induce Surfactant per 379L of Water	4-May-22	1 L ha^{-1} Miravis Ace Fungicide	20-Jun-23	Harvest
		4-May-22	1 L ha^{-1} Miravis Ace Fungicide	20-Jun-22	Harvest		
		18-Jun-22	Harvest				

Table 4. Tissue collection dates for 2020-2021 and 2021-2022 growing seasons.

Warsaw 2020-2021	Warsaw 2021-2022	Westmoreland 2021-2022
2-Dec-20	28-Dec-21	14-Jan-22
24-Feb-21	3-Mar-22	3-Mar-22
29-Mar-21	30-Mar-22	30-Mar-22

Table 5. Soil Analysis of 0 to 15 centimeters and 15 to 30 centimeters of soil profile at Warsaw location and Westmoreland location during the 2021-2022 growing season.

	OM		pH	ENR	P		K		S	
Sample ID	%	Rating		kg ha^{-1}	mg kg^{-1}	Rating	mg kg^{-1}	Rating	mg kg^{-1}	Rating
Warsaw Location 0 - 15 cm	0.7	VL	6.8	66.1	72	H	120	VH	5	VL
Warsaw Location 15 - 30 cm	1.2	L	6.7	77.3	52	H	114	VH	7	VL
Westmoreland Location 0 - 15 cm	1.6	L	7.0	86.2	18	L	81	M	6	VL
Westmoreland Location 15 - 30 cm	0.9	VL	6.8	71.7	17	L	85	M	6	VL

Values on this report represent the plant available nutrients in the soil. Rating after each value: VL (Very Low), L (Low), M (Medium), H (High), VH (Very High). ENR - Estimated Nitrogen Release.

Table 6. NDVI collection dates for each site-year.

Warsaw 2020-2021	Warsaw 2021-2022	Westmoreland 2021-2022	Warsaw 2022-2023
13-Nov-20	29-Dec-21	27-Jan-22	8-Dec-22
3-Dec-20	27-Jan-22	11-Feb-22	6-Jan-23
23-Feb-21	21-Feb-22	21-Feb-22	30-Jan-23
23-Mar-21	30-Mar-22	20-Apr-22	1-Mar-23
16-Apr-21	20-Apr-22	3-Jun-22	30-Mar-23
	3-Jun-22		

Table 7. Analysis of variance table for tissue sample analysis of the first fertility treatment, second fertility treatment, and Zadoks growth stage 30 (GS 30).

Source of variation		First Tissue Sample Measuring Effects of First Fertility Treatment				Second Tissue Sample Measuring Effects of Second Fertility Treatment				Third Tissue Sample at GS 30			
	df	N%	P%	K%	S%	N%	P%	K%	S%	N%	P%	K%	S%
Location	2	***	***	**	***	***	***	***	***	***	***	***	***
Treatment	9	***	***	NS	***	***	***	***	***	**	NS	NS	NS
Location x treatment	18	NS†	NS	NS	***	NS	**	NS	***	NS	NS	NS	NS

** Significant at 0.01 probability level.

*** Significant at <0.001 probability level.

† NS, not significant.

Table 8. First fertility treatment tissue sample analysis of nutrients with a treatment main effect and/or location x treatment interaction.

Treatment No.	Treatment	N%*	P%*	S%**		
				Warsaw 2021	Warsaw 2022	Westmoreland 2022
1	Control - At Plant Application	4.50 bcd†	0.58 a	0.28 c	0.48 b	0.38 ab
2	AMS - At Plant Application	4.81 a	0.60 a	0.36 a	0.54 a	0.42 a
3	SymTrx 20S - At Plant Application	4.75 a	0.61 a	0.36 a	0.56 a	0.41 ab
4	SymTrx 10S - At Plant Application	4.71 ab	0.61 a	0.35 a	0.56 a	0.41 ab
5	Control - Mid-winter Application	4.45 cd	0.50 b	0.28 c	0.40 c	0.38 ab
6	AMS - Mid-winter Application	4.41 d	0.49 b	0.28 c	0.40 c	0.37 b
7	SymTrx 20S - Mid-winter Application	4.44 d	0.48 b	0.30 bc	0.38 c	0.37 b
8	SymTrx 10S - Mid-winter Application	4.46 cd	0.49 b	0.28 c	0.41 c	0.37 ab
9	SymTrx 10S - Split Application	4.61 abcd	0.56 a	0.33 ab	0.54 a	0.40 ab
10	SymTrx 10S + Comp1-MES10 - At Plant Application	4.65 abc	0.59 a	0.35 a	0.56 a	0.40 ab

* Tissue sample nutrient with a treatment main effect, but no location x treatment interaction allowed site-year data to be combined.

** Tissue sample nutrient with a treatment main effect and location x treatment interaction. Each site-year provided.

† Values followed by the same letter within each nutrient column are not significantly different at LSD $p < 0.05$.

Table 9. Second fertility treatment tissue sample analysis of nutrients with a treatment main effect and/or location x treatment interaction.

Treatment No.	Treatment	N%*	K%*	P%**			S%**		
				Warsaw 2021	Warsaw 2022	Westmoreland 2022	Warsaw 2021	Warsaw 2022	Westmoreland 2022
1	Control - At Plant Application	4.00 e†	3.52 f	0.33 bc	0.65 NS‡	0.57 bc	0.22 d	0.47 NS	0.50 NS
2	AMS - At Plant Application	4.01 e	3.71 def	0.33 bc	0.65	0.59 b	0.22 d	0.47	0.45
3	SymTrx 20S - At Plant Application	4.04 e	3.60 def	0.32 bc	0.68	0.53 c	0.22 d	0.47	0.46
4	SymTrx 10S - At Plant Application	4.09 de	3.62 def	0.36 abc	0.66	0.54 bc	0.30 b	0.46	0.50
5	Control - Mid-winter Application	4.38 bc	3.76 bcde	0.36 abc	0.69	0.67 a	0.27 bcd	0.46	0.47
6	AMS - Mid-winter Application	4.51 ab	4.08 a	0.40 a	0.67	0.71 a	0.43 a	0.46	0.49
7	SymTrx 20S - Mid-winter Application	4.70 a	3.96 ab	0.38 ab	0.67	0.67 a	0.38 a	0.46	0.51
8	SymTrx 10S - Mid-winter Application	4.59 ab	3.94 abc	0.35 abc	0.71	0.69 a	0.38 a	0.50	0.50
9	SymTrx 10S - Split Application	4.27 cd	3.82 bcd	0.36 abc	0.68	0.61 b	0.29 bc	0.49	0.49
10	SymTrx 10S + Comp1-MES10 - At Plant Application	4.07 de	3.53 ef	0.31 c	0.67	0.57 bc	0.23 cd	0.47	0.47

* Tissue sample nutrient with a treatment main effect, but no location x treatment interaction allowed site-year data to be combined.

** Tissue sample nutrient with a treatment main effect and location x treatment interaction. Each site-year provided.

† Values followed by the same letter within each nutrient column are not significantly different at LSD $p < 0.05$.

‡NS, not significant.

Table 10. Zadoks growth stage 30 (GS 30) tissue sample analysis of nitrogen percentage.

Treatment No.	Treatment	N%*
1	Control - At Plant Application	3.85 bc†
2	AMS - At Plant Application	3.82 c
3	SymTrx 20S - At Plant Application	3.92 abc
4	SymTrx 10S - At Plant Application	3.82 c
5	Control - Mid-winter Application	3.86 bc
6	AMS - Mid-winter Application	4.05 a
7	SymTrx 20S - Mid-winter Application	4.08 a
8	SymTrx 10S - Mid-winter Application	4.01 ab
9	SymTrx 10S - Split Application	3.98 abc
10	SymTrx 10S + Comp1-MES10 - At Plant Application	3.82 c

* Tissue sample nutrient with a treatment main effect, but no location x treatment interaction allowed site-year data to be combined.

† Values followed by the same letter are not significantly different at LSD $p<0.05$.

Table 11. Analysis of variance table for aerial NDVI values before the mid-winter application, after the mid-winter application, and at Zadoks growth stage 30 (GS 30).

	NDVI Values Before Mid-Winter Application		**NDVI Values After Mid-Winter Application**		**NDVI Values at GS 30**	
Source of variation	df		df		df	
Location	2	***	3	***	2	***
Treatment	9	***	9	***	9	NS†
Location x treatment	18	***	27	**	18	NS

** Significant at 0.01 probability level.

*** Significant at <0.001 probability level.

†NS, not significant.

Table 12. Aerial NDVI values of before and after mid-winter application for each site-year.

		NDVI Values **Before** Mid-Winter Application			NDVI Values **After** Mid-Winter Application			
Treatment No.	**Treatment**	**Warsaw 2021**	**Warsaw 2022**	**Warsaw 2023**	**Warsaw 2021**	**Warsaw 2022**	**Westmoreland 2022**	**Warsaw 2023**
1	Control - At Plant Application	0.71 a†	0.34 NS‡	0.66 ab	0.76 b	0.43 bc	0.37 ab	0.82 ab
2	AMS - At Plant Application	0.73 a	0.35	0.65 abc	0.79 ab	0.46 a	0.38 a	0.81 ab
3	SymTrx 20S - At Plant Application	0.73 a	0.36	0.66 ab	0.80 ab	0.45 ab	0.38 a	0.81 abc
4	SymTrx 10S - At Plant Application	0.72 a	0.35	0.67 a	0.78 ab	0.45 ab	0.39 a	0.83 a
5	Control - Mid-winter Application	0.63 b	0.33	0.61 cd	0.79 ab	0.41 cd	0.34 bc	0.79 bc
6	AMS - Mid-winter Application	0.63 b	0.33	0.63 cd	0.79 ab	0.39 d	0.34 bc	0.80 abc
7	SymTrx 20S - Mid-winter Application	0.62 b	0.33	0.64 bc	0.79 ab	0.41 cd	0.32 c	0.79 bc
8	SymTrx 10S - Mid-winter Application	0.63 b	0.33	0.61 d	0.80 a	0.40 bcd	0.33 c	0.78 c
9	SymTrx 10S - Split Application	0.70 a	0.34	0.66 ab	0.81 a	0.43 bc	0.38 a	0.82 ab
10	SymTrx 10S + Comp1-MES10 - At Plant Application	0.73 a	0.34	0.66 ab	0.78 ab	0.44 abc	0.39 a	0.82 ab

†Values followed by the same letters within each site-year are not significantly different at LSD $p<0.05$.

‡NS, not significant.