

EVALUATION OF SECONDARY AND MICRONUTRIENTS IN KANSAS

by

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Abstract

The limitation of an essential nutrient for plant growth can affect crop yield. Research has been focused mainly on macronutrients, nevertheless micronutrients are equally important. This thesis is divided into three studies, which had the purpose of assessing frequent questions that producers have about micronutrient fertilizers and their effect on several crops in Kansas. The objective of the first study was to summarize and analyze results from studies since 1962 until 2015 to verify responses to zinc (Zn) and sulfur (S) fertilization in corn (*Zea mays*), sorghum (*Sorghum bicolor* (L.) Moench), wheat (*Triticum aestivum*) and soybean (*Glycine max* (L.) Merr). The treatments evaluated consisted of fertilizer Zn or S application versus their respective unfertilized treatments. Zinc fertilization significantly increased corn yield; no significant response was found for sorghum, wheat and soybean. Sulfur fertilization did not increase yields on corn and wheat. The objectives of the second study were: (i) to evaluate soybean response to S and micronutrients boron (B), copper (Cu), manganese (Mn), and Zn fertilizer application and to assess soil test and soybean seed and tissue nutrient concentration with fertilization. Treatments consisted of an unfertilized control, micronutrient fertilizer as individual nutrient for B, Cu, Mn, S and Zn applied broadcast pre-plant, in addition to a blend of these nutrients using two different placements (broadcast and band). Secondary and micronutrient fertilization showed no significant effect on soybean yield at any of the ten locations. Zinc fertilization showed significant effects on soybean tissue and seed Zn concentration. The objective of the third study was to evaluate soybean tissue nutrient response to micronutrient fertilizers in field strips with high variability in soil properties in the area evaluated. The study consisted of two strips (with and without fertilizer) and replicated three times. The treatment with fertilizer included a blend of Cu, Mn and Zn at a rate of 11.2 kg ha⁻¹ and B at a rate of 2.8 kg ha⁻¹. Initial soil tests B, Cu,

Mn and Zn were not good indicators of soybean tissue response. Within-field variability of soybean Zn and B tissue content were affected by soil pH and organic matter; and these factors may be used to help explain field variability in plant availability. The micronutrient blend treatment showed higher tissue Zn and B values compared to the control. When pH ranged from 5.5 to 7.6, B in soybean tissue was higher on the control than the micronutrient blend treatment. Copper concentration in soybean tissue did not show significant difference between treatments at any location, regardless of pH and organic matter levels.

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“The good teacher explains. The superior teacher demonstrates.

The great teacher inspires.” William Arthur Ward

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Dedication

For all agronomists with whom we share the same passion, especially to my parents and older brother, who introduced me to this path and had a constant and undefeatable faith on me.

For the motor of my life and success: my family.

Chapter 1 - Introduction and Thesis Organization

Achieving maximum yield of a particular crop is determined by limiting factors affecting yield potential during the growing season. One of the limiting factors can be related to nutrient supply and the way to avoid yield reduction is to guarantee good nutrient supply to the plant. Research on crop nutrition has emphasized macronutrients with limited work related to micronutrients; this is attributed to the higher amounts of macronutrients required by the plant.

Deficiency or toxicity of S and micronutrients can severely affect an adequate plant growth. The limited or null amount of data regarding B, Cu, Mn and Zn is an obstacle to provide answers to all the concerns and questions for crop production in Kansas. Sulfur deficiencies have become common all over the state of Kansas due to intensive crop systems and higher yielding varieties. Another cause of S deficiencies is attributed to the decrease of atmospheric $\text{SO}_4\text{-S}$ deposition over the years (Lamond, 1997). The United States Environmental Protection Agency imposed regulations to reduce SO_2 emissions through the Clean Air Act. From 1980 to 2014, SO_2 levels in the air have decreased 80% (USEPA, 2010).

Secondary and micronutrients have the potential to contribute in maximizing yields. Past studies suggest potential trends of plant nutrient uptake in response to secondary and micronutrients. Nutrients evaluated in the studies presented here include B, Cu, Mn, S and Zn. The main function of B in the plant is to the synthesis of the cell wall material. Furthermore, flower retention, pollen formation, germination and seed and grain production can be reduced when B is deficient. Boron can also affect lignin formation (Pilbeam and Kirkby, 1983). Copper is involved in the lignin synthesis, carbohydrate metabolism and is necessary for photosynthesis and respiration (Havlin et al., 2012). Manganese is involved in the activation of enzymes, nitrogen metabolism and photosynthesis (Fageria et al., 2002). Sulfur is required for the

synthesis of chlorophyll, and components of protein. Functions of Zn in the plant involve carbohydrate metabolism, protein synthesis, growth regulation and the activation of a large number of enzymes (Römheld and Marschner, 1991).

Soil conditions must be taken in consideration when evaluating micronutrients. Organic matter plays an essential role and is the main source of most micronutrients, especially for Zn and Cu. It is not unusual to observe secondary and micronutrient deficiencies on soils with low organic matter. Soil pH influences the bioavailability of micronutrients in the soil. Availability of B, Cu, Fe, and Zn tends to decrease as pH increase (Essington, 2004). Soil texture can also affect the availability of micronutrients; coarse texture soils have the tendency to be low on B concentration. On the other hand, soils with poor aeration are more likely to have Fe, Zn and Mn deficiencies (Voss, 1998).

Thesis Organization

The overall objective of this thesis is to evaluate the response of crops to S and micronutrient fertilizers in Kansas. Chapter 2 is a review and summary of all the studies that involve S and Zn for the main crops in Kansas: Corn (*Zea mays*), sorghum (*Sorghum bicolor* (L.) Moench), wheat (*Triticum aestivum*) and soybean (*Glycine max* (L.) Merr). The review contains data since the 1962 until 2015 and is focused on Zn and S. Chapter 3 evaluates soybean response to secondary and micronutrients fertilizers applied as individual nutrients and blends. In this study, 10 locations were established in 2013 and 2014 in eastern Kansas. The variables analyzed were yield, soybean tissue and nutrient concentration in the seed. Soil samples were collected before planting and post-harvest to evaluate soil test changes with the application of micronutrients. The nutrients of interest were B, Cu, Mn, S and Zn. Chapter 4 assesses soybean response to a blend of secondary and micronutrients broadcasted versus unfertilized using on-

farm strip trials. Three locations were established in collaboration with local producers. The objective was to analyze Cu, Mn and Zn concentration in soybean tissue as affected by soil pH and organic matter.

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Chapter 2 - A Summary and Evaluation of Zinc and Sulfur Fertilization in Kansas Since 1962

Abstract

The relevance of zinc (Zn) and sulfur (S) for crop production in Kansas has incremented along the decades. The objective was to find, summarize and analyze data since 1962 until 2015 to verify responses to Zn and S fertilization for corn (*Zea mays*), soybean ([*Glycine max (L.) Merr.*]), sorghum [*Sorghum bicolor (L.) Moench*] and wheat (*Triticum aestivum*) in Kansas. The sources used were the Kansas Fertilizer Research Reports, ACCESS Digital Library and K-REX (K-State Research Exchange). The results of this review identified knowledge gaps on research related to Zn and S fertilization, mainly for soybean production. Overall, corn was the crop with the highest amount of observations for both Zn and S fertilizer research. The statistical analysis showed no effect of Zn fertilization on sorghum, wheat and soybean yield, although Zn tended to increase yields above the unfertilized controls. Zinc fertilization showed an increase of approximately 25% on corn yield above the untreated plots. No significant differences were found between S fertilized and unfertilized on corn and wheat. The analysis for soybean and sorghum involving S fertilization was excluded due to the few amount of data found. The majority of the papers reviewed do not report soil test information for the nutrients analyzed, except for Zn research in corn and sorghum. A poor relationship was found between soil test Zn and relative yield for corn and sorghum; indicating that the fertilization based on initial soil test Zn alone is not the best predictor for yield responses of corn and wheat. Results from this review indicate a limited amount of research for Zn and S for the main crops in different regions and soils of Kansas.

Introduction

An overview of the past six decades indicates a trending increase on the United States' production for corn soybean and wheat. Since 1948 to 2011, soybean production has been duplicated and corn yields have quadruplicated (Wang et al., 2015). Kansas' lands are mainly devoted for agriculture. High production and grain nutrient removal require optimum inorganic source applications.

Zinc and S are key nutrients for optimum crop production with soils and cropping systems of Kansas. According to Fixen et al., (2010), 13% of the soils in Kansas have less than 3 mg kg⁻¹ of S and 75% are lower than 1 mg kg⁻¹ of Zn concentration; these levels may be a limiting factor for optimum crop production. Cropping systems intensification, degraded soils and lack of soil conservation practices are factors that facilitate the deficiencies of nutrients.

In the past, S deficiencies were most likely to appear in irrigated corn on coarse textured soils with low organic matter. Nowadays the need for S inputs in the whole state has increased due to the higher yields, intensive cropping systems, decreasing organic matter content and limited amount of atmospheric S deposition. Lamond (1997) found positive yield responses accredited to S fertilization in corn, wheat and grain sorghum. The application of S fertilization increased corn yields, mainly on locations prone to suffer S deficiency and with coarse textured soil (Sawyer, 2011). Increased S deficiencies in Argentina generated multiple researches assessing S fertilization through the years; soybeans showed an increase on seed yield with the application of S fertilizers (Gutierrez, 2007). In Kansas, the recommended soil test for S is the Ca(PO₄)₂ extractable sulfate at a soil sampling depth of 0 to 60 cm, due to the mobility of S in soil. Leikam et al. (2003) suggest that yield goal (YG), crop factor (CF), organic matter sulfur (OM S), supply of S by manure (Man S) and irrigation (H₂O S) and soil test S (STS) should be taken in consideration for S fertilization. The following equation is used to develop S

fertilization recommendations in Kansas: $S \text{ Rec} = (YG \times CF) - OM \text{ S} - H_2O \text{ S} - Man \text{ S} - ST \text{ S}$

Crop factors for the main crops of Kansas include: Corn and grain sorghum: 0.2 mg S cm^3 ;

wheat: 0.60 mg S cm^3 ; soybean: 0.40 mg S cm^3 .

A critical level of Zn in soil has not been established in Kansas. Leikam et al. (2003) recommend applying Zn fertilizer if DTPA- Zn contain less than 1 mg kg^{-1} in order to build Zn soil test level and to correct soil deficiency on corn, sorghum and soybean. The recommended calculation to obtain Zn rate to be applied is: $11.5 - (11.25 \times \text{ppm DTPA Zn})$. According to Sims and Johnson (1991), the range in critical level of Zn in soil lies between 0.2 to 2.0 mg kg^{-1} ; Mallarino (2013) refers that Zn levels below 1 mg kg^{-1} should be considered to be marginal. When low Zn soil levels are found, corn and sorghum are the most responsive crops to Zn, meanwhile soybean has presented medium response (IPNI, 2015). In India, Takkar et al. (1992) and in Turkey, Kalayci (1999) reported wheat production decreases due to Zn deficiency. On corn production, Orabi et al. (1981) attributed a 22% of yield increase with the application of Zn fertilizers; contrasting response was found by Wang et al. (2012).

The increasing deficiencies of Zn and S demand a major focus; by reviewing the history of Zn and S in Kansas, a better approach will provided for future researches. The objective of this study was to summarize and analyze data since 1962 until 2015 to verify responses to Zn and S fertilization for corn, soybean, sorghum and wheat in Kansas.

Materials and Methods

This study contains a summary of published and unpublished research containing data on Zn and S fertilization in Kansas since 1962. The sources used were the Kansas Fertilizer Research Reports, ACCESS Digital Library and K-REX (K-State Research Exchange). A total of 72 papers on micronutrient fertilization for corn, soybean, sorghum and wheat were reviewed.

Greenhouse and growth chamber studies were excluded, only data from experimental fields was utilized. Due to the limited amount of data, the analysis focused only for S and Zn. A total of 36 papers were used for Zn and 14 papers for S. A database was generated with information of the study's year, location, treatments, fertilizer rate, and method of application. The response variables were yield, soil and tissue analysis. Not all three response variables were found for all papers, and only yield was found in all papers. The number of locations for Zn research includes a total of 18 for corn, 6 for soybean, 20 for sorghum and 7 for wheat. The number of locations for S research was 10 for corn and 20 for wheat. The scarce amount of observations (less than 20 observations) for experiments with S in soybean and sorghum lead to the exclusion of these crops. The criterion of selection consisted that the paper would include yield data, a soil fertilizer applied treatment and a corresponding control. Only research made in Kansas was taken in consideration. A lack of soil test for the Zn was found in the majority of the reviewed papers, except for Zn research in corn and sorghum. The soil test method evaluated for Zn was the DTPA. Sulfur soil test information was not sufficient for analysis.

Statistical Analysis

The statistical analysis was completed using the GLIMMIX procedure in SAS (SAS, 2006). The statistical analysis for fertilizer response on yield evaluated only individual nutrient application versus unfertilized control. Locations were considered as random factor in the model. Relative yield was calculated by subtracting the mean of the control from the mean of the fertilized treatments by each location which contained soil analysis for Zn and S. If the paper analyzed had less than five locations with soil test it was not taken into consideration. A linear regression completed to evaluate the relationship between initial soil Zn versus relative yield. The REG procedure in SAS (SAS, 2006) was used for the regression model. The coefficient of

determination (R^2) was analyzed to evaluate the relationship between variables. Statistical differences were established at the 0.10 probability level. A statistical analysis was not made for the descriptive data classified by years or zones due to the unbalanced amount of observations obtained when classifying data.

Results and Discussion

Previous studies suggested that there has been a few increase of new research concerning Zn. The papers found were ranged from the year 1962 until 2012. Most of the papers found are from 1962 to 1979 (36 papers), and fewer papers found from 1990 until 2012 (14 papers) (Appendix). Less research regarding Zn on soybean and wheat has been completed compared to the numerous amounts of corn and sorghum in Kansas. Zinc experiments since 1962 for soybean had 23 observations; and wheat had 33 observations (Table 2-1). Zinc fertilization had the tendency of having higher yield values over unfertilized treatments on the four analyzed crops but significant differences were only found in corn yield. Zinc fertilization had a positive effect on corn yields by increasing $1,732 \text{ kg ha}^{-1}$ above the unfertilized (Table 2-1). Wheat, sorghum and soybean showed no significant yield increase with the application of Zn fertilizer (Table 2-1). Corn and sorghum are expected to have a high response to Zn fertilizers and a low to medium response for wheat and soybean (Prasad and Power 1997; Martens and Westerrman, 1991; Loue, 1986; Follet et al. 1981). These results are in agreement for the yield response to Zn fertilizers on corn, wheat and soybean but contradictory results were found for sorghum. From the few data found on soil Zn, the regression could only be made for corn and sorghum (Figure 2-1). For corn analysis, 21 locations contained soil Zn data from 1964 to 1987; papers about sorghum contained 10 locations with soil Zn data since 1968 until 1994. Poor relationship between relative yield and soil Zn concentration was found for both corn and sorghum with R^2 of 0.01 and 0.03,

respectively (Figure 2-1). Low R^2 between soil Zn and crop yields, for corn and sorghum, indicates that the model explains none of the variability of yield response. Soil Zn was not a strong indicator of yield response on the studies evaluated in our review.

The amount of observations analyzed for corn and wheat regarding S research were 166 and 147, respectively (Table 2-2). No significant differences were found between S fertilization versus control on corn or wheat. According to Hoefl et al. (1985), positive significant crop response to S in the Midwestern states have been inconsistent. In Indiana no significant corn yield response was found at any of 24 locations evaluated with similar results in Illinois and Missouri (Hanson, 1979). Recent corn studies have shown that 6 of 11 strip trials responded positively to broadcast application of S (Sawyer, 2009). Kim et al. (2013) found no relation between yield and soil SO_4 -S test. Soil test for S was not found consistently through the papers reviewed; therefore the relationship of yield and soil SO_4 -S test could not be evaluated. For, Kansas it would be expected that on coarse textured soils with low organic matter and low supply of SO_4 , it would be most likely to find a positive yield response (Lamond, 1997).

Descriptive data analysis

Due to the variability that might be presented through the state of Kansas, yield data collected for corn, sorghum, soybean and wheat was classified in three regions of origin: east, central and west. An analysis was also made comparing the fertilized studies with Zn or S versus the unfertilized by the year when the research were done.

Zinc fertilization presented higher corn yield relative to the unfertilized on low yielding zones, which are the west and central regions of Kansas. On the eastern region, which has better conditions such as higher amount of precipitation and deeper soils, the fertilization of Zn had no effect on corn yield (Figure 2-2). Sorghum yield tended to be higher by the unfertilized

treatments than by Zn fertilization at the three evaluated regions (Figure 2-2). Data for soybean yield involving Zn fertilization was only found for eastern and western region, no data for the central region (Figure 2-2). Wheat yield varied among regions, being the east region with highest yields, but no marked differences were seen between treatments (Figure 2-2).

As analyzed by years of research, it is evident that most of data found for corn and sorghum pertains to the decade of 1960 and 1970, no recent studies have been reported evaluating Zn on these two crops (Figure 2-3). Corn yield tended to be higher than the unfertilized in 83% of the years analyzed. Sorghum yield presented the highest values with Zn fertilization in 8 of 10 years (Figure 2-3). Soybean data was only from the decade of 1970 and only 3 years of research. On wheat yield across the years, a clear tendency could not be denoted between the evaluated treatments (Figure 2-4).

Slight differences between S fertilized and unfertilized (less than 300 kg ha⁻¹) were found on corn yield for the west and central regions; S fertilized was higher than the unfertilized (Figure 2-5). On the east region, sulfur fertilization had lower corn yield values than the control. For wheat yield in the east and west regions, S fertilization was found to be higher than the unfertilized; same results were not found on the central region (Figure 2-5). Sulfur fertilization had positive effect on 66% of the 9 years of research analyzed (Figure 2-6). Sulfur fertilization was found to have higher wheat yield than the unfertilized treatment on 4 of 6 years (Figure 2-6).

Conclusion

Zinc fertilization in Kansas tended to affect positively yield of corn, soybean, sorghum and wheat. However, statistically significant differences were only observed on corn with Zn fertilization. Most published and unpublished papers found were from 1960 to 1970 (36 papers), few were found from the 1990 to 2005 (14 papers). In order to provide current information for

producers more studies must be completed considering changes in some conditions including soil degradation in recent years. Research regarding Zn and S in Kansas, has been mainly focused on corn but very limited attention has been given to soybean. No relationship was found between crop yield response and soil test Zn, indicating that soil test at the range evaluated in these studies might not be the best indicator for potential response to fertilizer application. New research should focus in the search of critical soil test levels for these nutrients for the main crops in Kansas. Valuable data has been accumulated from year to year, this review is an example of the importance of data recollection from previous years in order to proceed with new research, and improve Zn and S management.

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Table 2-1. Minimum, maximum, mean and standards error for yield response to Zn fertilization and unfertilized treatments across locations in Kansas.

Treatments	n [†]	Minimum ----- kg ha ⁻¹ -----	Maximum	Mean	Standard Error
<u>Corn</u>					
Zn Fertilized	422	6107	7493	6802 a [‡]	420
Unfertilized		4308	5831	5071 b	463
<u>Soybean</u>					
Zn Fertilized	28	1878	2436	2156	162
Unfertilized		1557	2289	1922	213
<u>Sorghum</u>					
Zn Fertilized	156	4398	5934	5167	464
Unfertilized		3948	5602	4776	500
<u>Wheat</u>					
Zn Fertilized	40	2227	4637	3432	710
Unfertilized		2146	4557	3351	710

[†] n: number of observations analyzed

[‡] Different letters indicate statistically significant differences at the P < 0.10.

Table 2-2. Minimum and maximum values that represent the highest and lowest corn and wheat yielding measurement and the standard error for S fertilization and unfertilized treatments across locations in Kansas.

Treatments	n [†]	Minimum	Maximum	Mean	Standard Error
		----- kg ha ⁻¹ -----			
		<u>Corn</u>			
S Fertilized	166	6561	8953	7757	723
Unfertilized		6566	9009	7787	738
		<u>Wheat</u>			
S Fertilized	147	2647	3460	3022	262
Unfertilized		2473	3420	2947	286

[†] n: number of observations analyzed

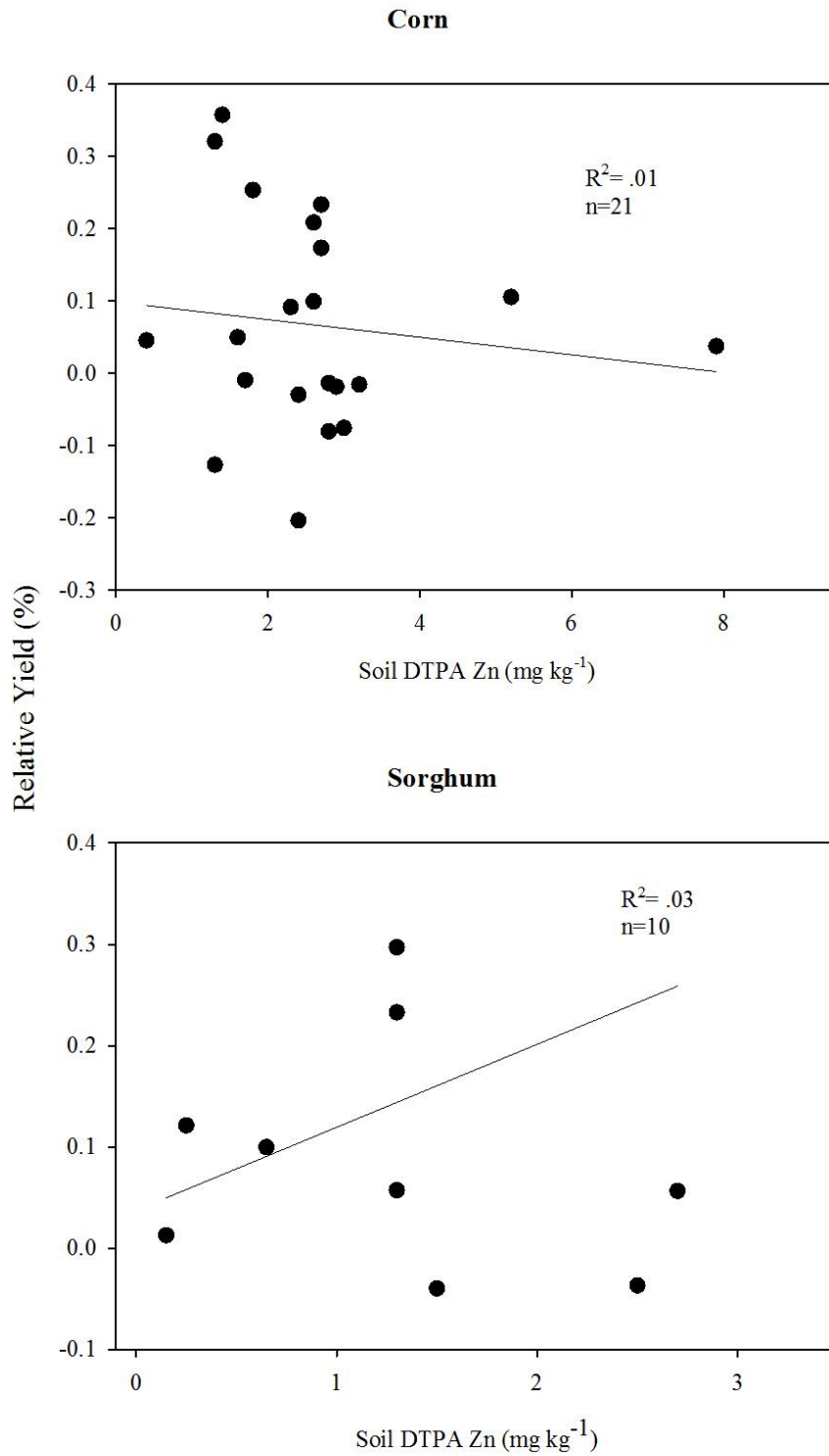


Figure 2-1. Corn and sorghum relative yield response to Zn application as related to soil DTPA Zn. Data shown include only the observations that contained soil test values.

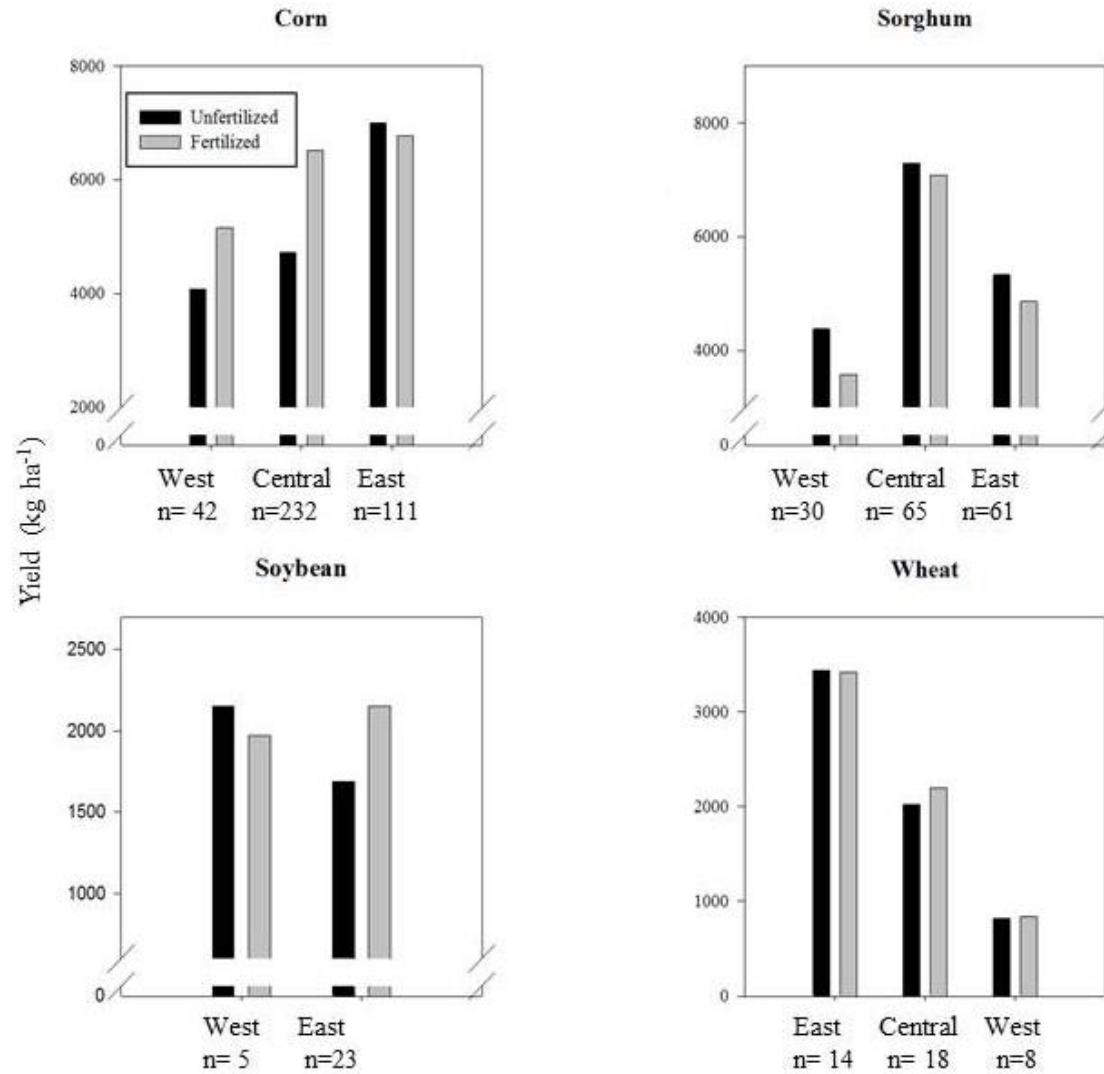


Figure 2-2. Descriptive data of Zn fertilized treatments versus unfertilized for corn, sorghum, soybean and wheat yield classified by regions in Kansas from 1962 to 2012.

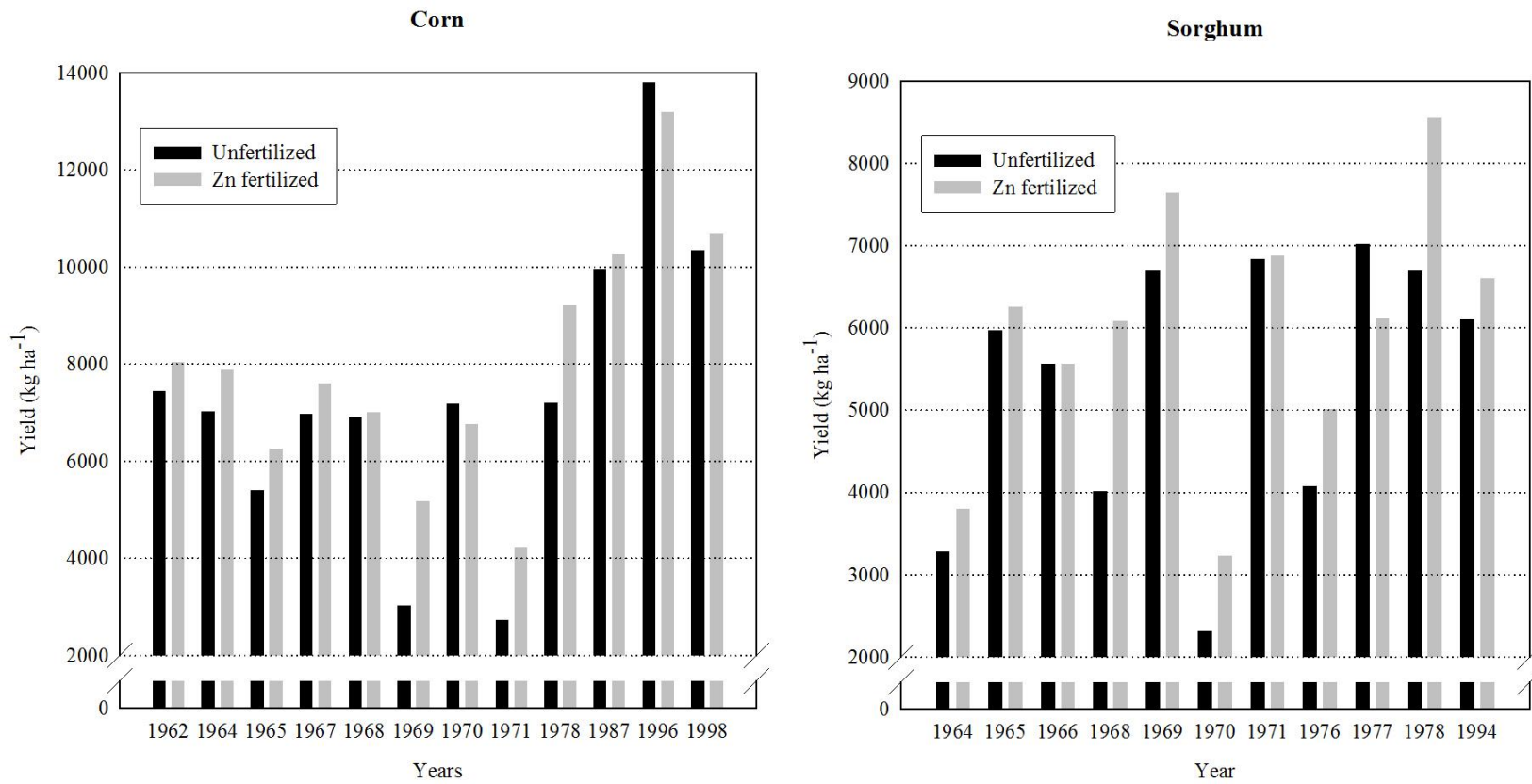


Figure 2-3. Descriptive yield data of Zn fertilized treatments versus unfertilized for corn and sorghum from 1962 to 1994.

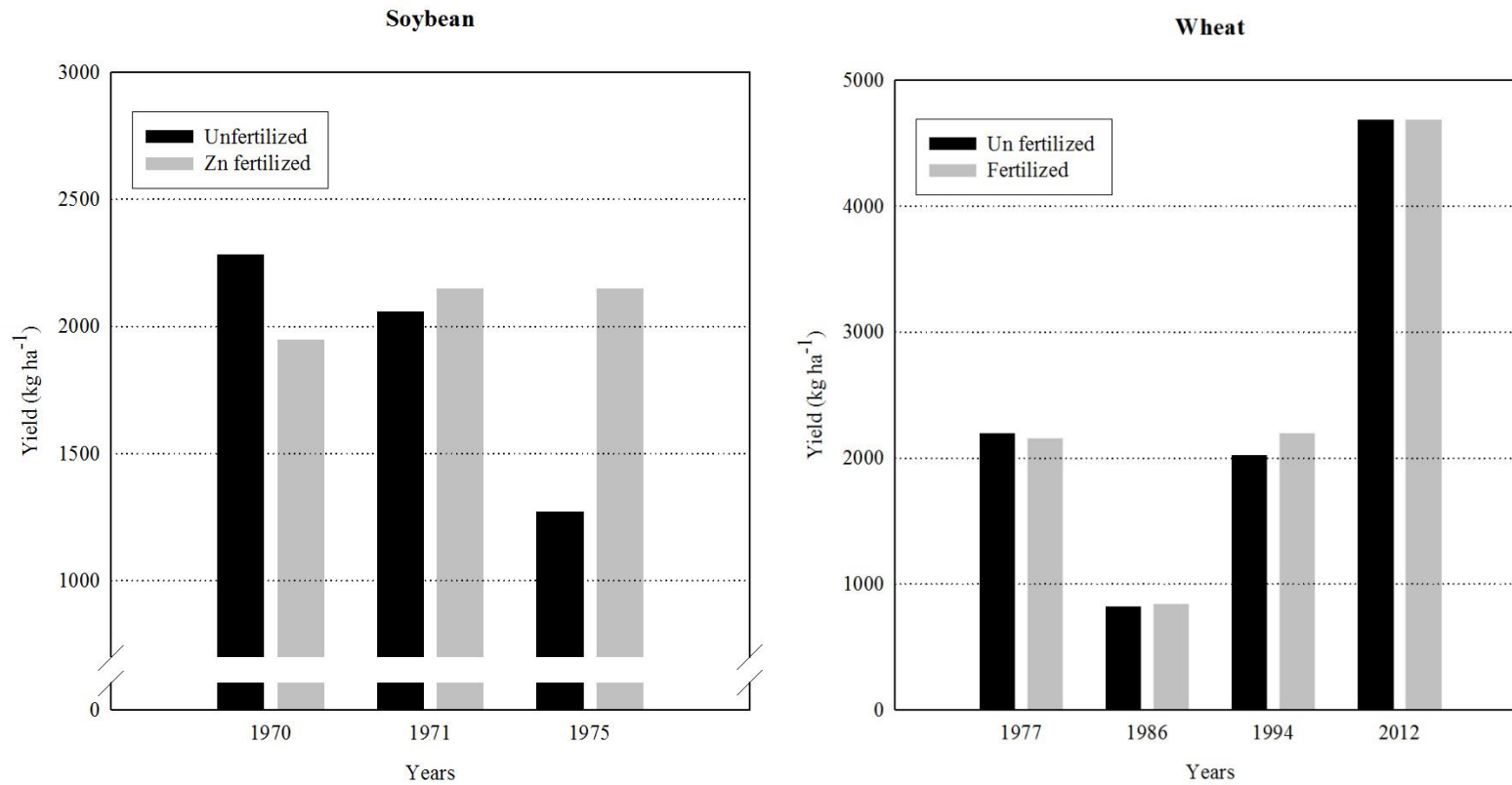


Figure 2-4. Descriptive yield data of Zn fertilized treatments versus unfertilized for soybean and wheat from 1970 to 2012.

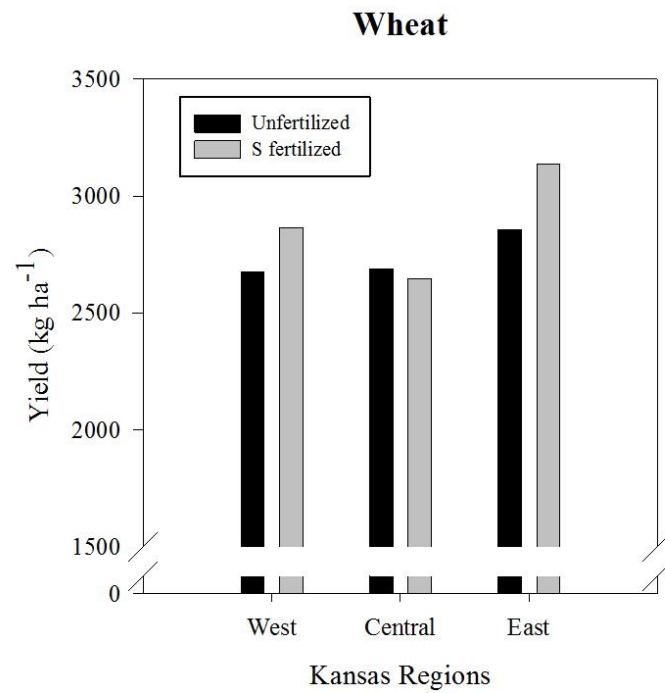
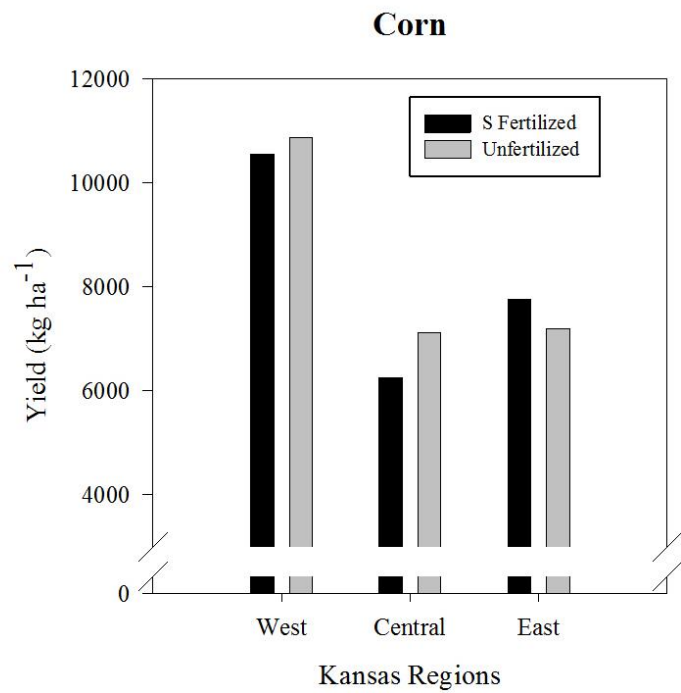


Figure 2-5. Descriptive yield data of S fertilized treatments versus unfertilized for corn and wheat classified by regions in Kansas from 1962 to 2012.

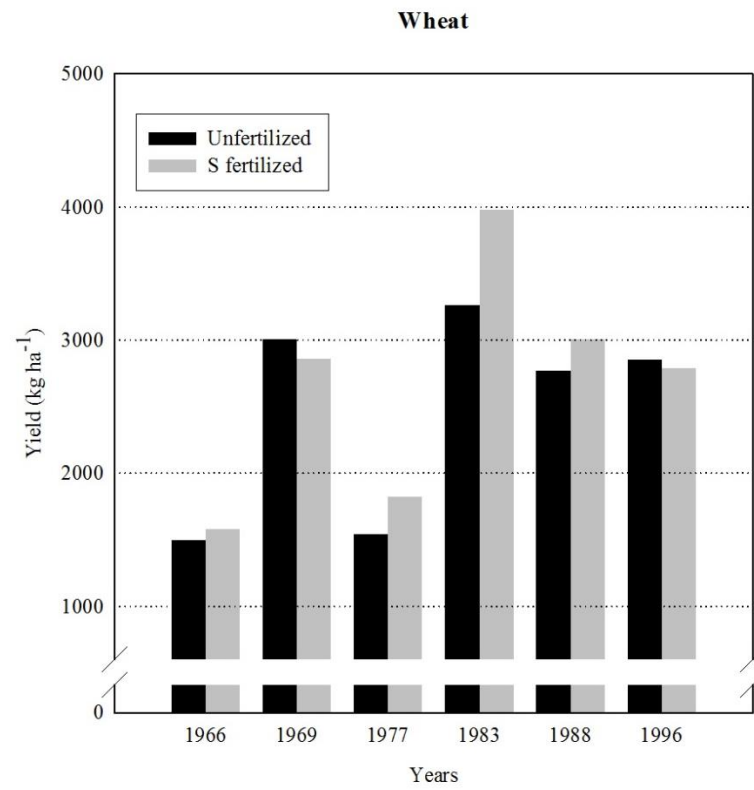
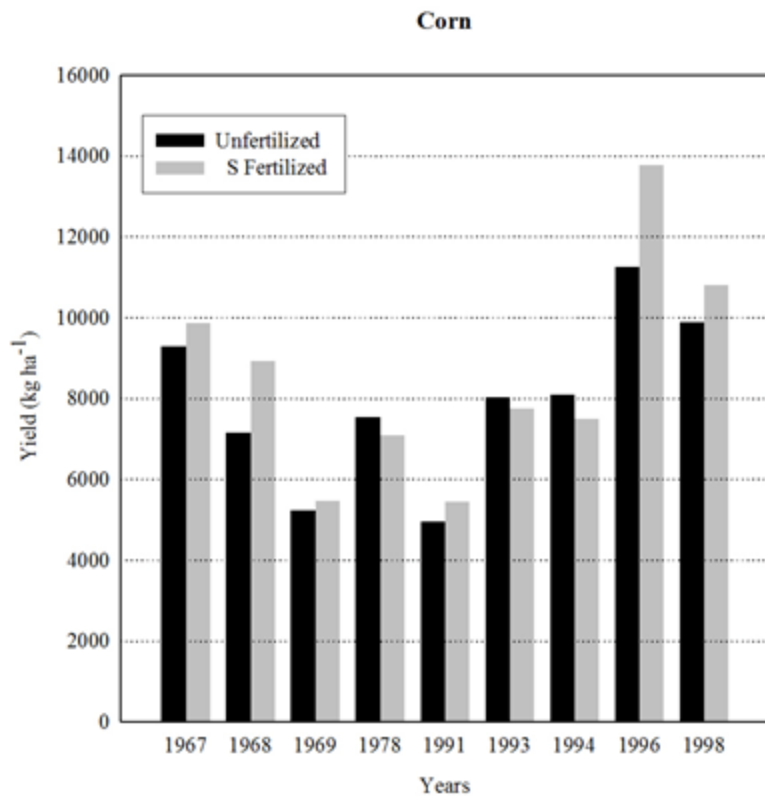


Figure 2-6. Descriptive yield data of S fertilized treatments versus unfertilized of corn and wheat from 1967 to 1996.

Chapter 3 - Evaluation of soybean response to soil-applied micronutrient fertilizers

Abstract

Research involving secondary and micronutrients for soybean (*Glycine max (L.) Merr.*) production is limited. The objective of this study was to evaluate soybean yield and tissue concentration to boron (B), copper (Cu), manganese (Mn), sulfur (S) and zinc (Zn). A randomized complete-block design with four replications was established at ten locations during 2013 and 2014. Treatments consisted of an unfertilized control, micronutrient fertilizer as individual nutrient for B, Cu, Mn, S and Zn applied broadcast pre-plant, in addition to a blend of these nutrients using two different placements (broadcast and band). All fertilizer sources were dry and sulfate-based, except for liquid fertilizer applied as band placement. Soil samples were collected from each plot prior to planting and after harvest. Trifoliolate samples were collected at full bloom (R2) to beginning of pod(R3) growth stage and analyzed for the micronutrients evaluated in this study. At harvest, seed was weighed for yield and a subsample was collected for nutrient analysis. Sulfur, B, Cu, Mn and Zn fertilization showed no significant effect on soybean yield at any of the evaluated locations. Zinc fertilization showed significant effects on soybean tissue and seed Zn concentration. Across locations, the broadcasted blend showed the highest Cu and Zn soil test concentration for the post-harvest samples compared to the individual nutrient, band blend, and control. The decision for the application of micronutrient fertilization should be inherent to certain soil properties like coarse textured and low organic matter soils. Results show that it was the location with the previous characteristics the one that show an average yield increase.

Introduction

Intensified production systems typically involve a greater demand for commercial fertilizers to secure maximum yield of a particular crop. This need for incrementing commercial nutrient inputs raises questions about the role of secondary and micronutrient fertilizers for boosting yields. Since early 1960's, the consumption of secondary and micronutrient fertilizer has incremented by more than 4% (USDA-ERS, 2012). The records indicate that Zn and S products have been the most utilized. The most common inorganic sources of micronutrients are sulfate-based (ZnSO_4 , MnSO_4 , CuSO_4), which are water soluble and are rapidly available to the plant after applying to the soil.

Nowadays, secondary and micronutrient deficiencies are becoming common. A recent study revealed that out of 1.4 million soil samples analyzed, 37% of the soils in United States are under 1 mg kg^{-1} DTPA equivalent for Zn (Fixen et al., 2010). The western Corn Belt and Central Great Plains presented low S levels (Fixen et al., 2010). In Kansas soils, Zn, Fe and S are the most likely to be deficient; Cu and Mn deficiencies have not been registered in the region (Leikam et al., 2003). Therefore, higher probability of yield increases for corn (*Zea mays*) and soybean with Zn among the micronutrients (Mueller, 2012).

The recommended test method for soil test Cu (STCu), soil test Mn (STMn) and soil test Zn (STZn) is the DTPA-extractable (diethylenetriamine-pentaacetic acid) at a depth of 0 to 15 cm (Leikam et al., 2003). Soil test B (STB) method used is the hot-water soluble extraction (Berger and Troug, 1939) (Sims and Johnson, 1991). It is well known that soil test for some micronutrient has a complex interpretation and has shown limited success as diagnostic tool as compared to macronutrients and pH (Jones, 1991; Sims et al., 1991). Therefore complementing soil test information with tissue testing may provide an improved diagnosis (Lohry, 2007). In order to understand the plant nutrition status of certain crop, tissue test values of specific parts of

plants at certain stages are compared to published and established Nutrient Sufficiency Ranges (NSR). The first published NSR for soybeans took the uppermost trifoliates without petiole when the plant was on initial bloom (R1) to podset (R3) (Jones, 1967). A NSR can be broad, and may vary according to soybean varieties and environment. Several published for soybean NSRs involving micronutrients have been made over time, nevertheless only slight or no changes have been adapted (Adriano, 1986) (Bell, 1995) (Mills and Jones, 1996). Kansas lacks of accurate soybean NSR for B, Cu, Mn and Zn.

Soybean is considered relatively tolerant to B and Cu deficiencies, and more sensitive to Fe, Zn and Mn deficiency (Martens et al., 1991). Although micronutrients are required in small quantities, deficiency may represent a major barrier to achieve maximum yield. Despite the lack of visual deficiency symptoms, some level of deficiency of secondary and micronutrients can result in yield reduction (Viets and Lindsay, 1973). The yield response of soybean to micronutrient application varies. Some studies in Kansas have shown significant yield increases with Mn application (Loecker et. al 2010). Widmar (2013) applied on topdress: B as boric acid, S as gypsum, Cu as oxysulfate, and sulfate based Fe, Mn, and Zn on wheat; none of these nutrients applied individually or as a blend had significant effect on wheat yield. In the North Central region of the US a number of research studies on micronutrients have been established in recent years (Mallarino et al. 2015). The application of banded ZnSO_4 at a rate of $5.6 \text{ kg Zn ha}^{-1}$ showed found no corn yield responses in most of the locations; initial STZn was not a good predictor of yield responses to the application of Zn (Bickel and Killorn, 2001). Locations with STS ranging from 2 to 10 mg kg^{-1} (soil sample depth: 30-60 cm), showed that fertilization increased yield at 2 out of 3 locations but the responses had no relationship to STS (Kim and Kaiser, 2013). Broadcast fertilization at a rate of $44.8 \text{ kg Cu ha}^{-1}$, a granular blend of Cu_2O and

elemental Cu, showed significant effect on soybean and corn yield on sandy loam and high organic matter soils (Oplinger et al., 1974).

The concentration of nutrients in the seed may help reveal how soils differ in their ability to supply nutrients for plant uptake and accumulation (Rashid and Fox, 1992). Worldwide, micronutrient malnutrition, especially Fe and Zn, affects over 2 billion people. Micronutrient fertilizers can also provide improved food quality through agronomic biofortification, and in consequence, potentially contribute to increase human health (IPNI and IFA, 2012). Certain findings lead to promising results with micronutrient fertilization but studies have focused mainly in Fe and Zn and very limited number of studies on soybeans. Cakmak (2010) found that a 3-fold increase in grain Zn concentration could be reached by the combination of soil applied ZnSO₄ at a rate of 50 kg ha⁻¹ and foliar Zn at a rate of 0.4 kg ZnSO₄ ha⁻¹. Previous studies show that soils with low organic matter and pH > 7, annual fertilization of B, Cu and Zn for six years tended to increase B and Zn concentration in seed whereas Cu remained irresponsive (Martens et al., 1974).

The objective of this study was (i) to evaluate soybean response to S and micronutrients (B, Cu, Mn, Zn) fertilizer application on soil, (ii) to assess changes in soybean seed and tissue nutrient concentration with fertilization; and (iii) evaluate soil test changes with soil-applied micronutrient fertilizers.

Materials and Methods

Experimental Design

This project was completed at ten locations in Kansas during 2013 and 2014. The locations were selected to represent different soil types and yield potential for soybean, with no recent history of micronutrient deficiency. The size of individual plots was 3 m wide and 8.2 m

long. Row spacing was 76 cm for all locations except for one location located in Clay County (Table 3-1), with a row spacing of 38 cm. Soybean varieties were those selected by cooperating producers and K-State experimental stations (Table 3-1). The experimental design was a randomized complete block design with four replications with 8 fertilizer treatments plus a control. Treatments consisted of micronutrient fertilizer applied as individual nutrient for B, Cu, Mn, S and Zn, in addition to a blend of these nutrients using two different placements (broadcast and band application). Micronutrients fertilizer compositions were: Mn: (2% N, 20% Mn, 12% S) Derived from: Urea, MgSO₄, CaSO₄, Fe SO₄, MnSO₄, MnO; Zn: (2% N, 20% Zn, 14% S) derived from: Urea, CaSO₄, FeSO₄, MgSO₄, ZnSO₄, ZnO; Cu: (2% N, 12% Cu, 6% Zn, 13% S) Derived from: CaSO₄, Cu (I) and (II) Oxide, CuSO₄, FeSO₄, ZnO, ZnSO₄. Gypsum (22 kg S ha⁻¹) was the source used for the S treatment. Boron was not sulfate based and was composed of 10% B, 10% Ca, 5% Mg, and 1.5 % S; derived from: MgO, Ulexite, CaSO₄, Colemanite. The rates for Cu, Mn, S, and Zn applied as an individual nutrient were broadcast applied at 11.2 kg ha⁻¹ and 2.8 kg ha⁻¹ for B. The broadcasted blend was at same rate for each B, Cu, Mn, S, and Zn. The sources mixed for the liquid blend were ZnSO₄ (35.5% Zn, 17.5% S) and MnSO₄ (32% Mn, 19% S) diluted in water, Cu-EDTA(7.5%Cu) and boric acid (10% B). Zinc, Mn, and Cu at 1.1 kg ha⁻¹ and B at 0.6 kg ha⁻¹). The band blend was applied with dribble placement over the row at planting.

Field sampling and chemical analysis

Soil samples were collected from each individual plot prior to treatment application and at post-harvest at a depth of 0-15 cm. A composite of ten cores with a diameter of 1.8 cm was collected randomly within each plot. Soil samples were oven dried at a temperature of 40°C, ground to pass a 2 mm sieve and analyzed. Soil pH was determined on 1:1 (soil: water). Soil test

phosphorus was determined by Mehlich3-extraction colorimetric method (Frank et al., 1988) and K by ammonium acetate ICP Spectrometer (Warncke and Brown, 1998). Copper, Mn and Zn were analyzed by DTPA-extraction using ICP (inductively coupled plasma) spectrometer (Whitney, 1998) and B by the hot water method (Watson, 1998). Additional soil samples were collected by block for soil organic matter analysis and analyzed by the method of Walkley-Black (Combs and Nathan, 1998). Soil texture was analyzed using the hydrometer method (Bouyoucos, 1962).

Tissue samples were collected at full bloom (R2) to beginning of pod(R3) growth stage (Ritchie et al., 1997), taking 30 uppermost fully-expanded trifoliolate (without the petiole) from the two middle rows. Tissue samples were analyzed for total P, K, S, Cu, Mn and Zn. Samples were oven-dried at 65°C then ground to pass through a 2 mm screen to finally be analyzed using the Nitric-Perchloric digest method (Gieseking et al. 1935; Donohue, 1992). The two middle rows were harvested with a plot combine, grain samples were weighed to calculate yield and adjusted to 130 g kg⁻¹ moisture. Test weight and moisture was determined by using a seed analysis computer (GAC 2100, Dickey John). Seed samples were oven dried at 65°C and ground to pass through a 2 mm screen. Analysis of Zn, Fe, Cu Mn and SO₄ from Nitric-Perchloric digest was done by an ICP Spectrometer (Gieseking et al. 1935).

Statistical Analysis

The GLIMMIX procedure of SAS 9.3 was used for anova analysis by location and using blocks as random in the model (SAS, 2006). Statistical differences were established at a 0.10 probability level. For the analysis across locations, the model utilized locations and blocks as random factors.

Results and Discussion

Location characteristics

Soil pH ranged from 5.2 to 7.1 for all ten locations (Table 3-2). Six out of the ten locations had a pH mean ≥ 6.5 , at this pH levels deficiencies are most likely to occur for B (Batey, 1971) and Mn (Voss, 1998). Mengel (1980) states that soil with high pH (<7) have higher potential to increase Zn deficiencies: locations 1 and 9 presented pH means <7 . Locations 1, 4 and 9 represented the highest percentages of coarse textured soils and the lowest values of the ten locations for organic matter (Table 3-2).

Soil test phosphorus varied from 5 to 59 mg kg⁻¹, four locations out of 10 (locations 2, 8, 9, and 10) can be considered below the critical level of 20 mg kg⁻¹ (Leikam et al., 2003). The ranges of soil potassium ranged from 134 to 501 mg kg⁻¹ (Table 3-2). Boron in soil was found to be below 1.8 mg kg⁻¹ for all locations. Critical soil test values have been poorly defined for B. According to Sim and Johnson (1991), critical values for Cu using DTPA method range from 0.1 to 2.5 mg Cu kg⁻¹; all evaluated locations had STCu values which ranged from 0.2 to 2.1 mg kg⁻¹. Where there is more clay content, a higher amount of STCu can be found (Fagbami et al., 1985); The locations 2, 5,6,7,8 and 10 presented values above 20% of clay and STCu was found to be above 1.1 mg kg⁻¹, this data is in agreement with Fagbami et al. (1985) who stated that soil with higher clay content typically contain higher levels of STCu. Locations 1, 3, 4, and 9 had above 22% of sand presented values of STCu below 1 mg kg⁻¹. Three locations had STZn levels below 1 mg kg⁻¹, this value is considered to be marginal or deficient (Mallarino, 2013; Leikam et al., 2003). Initial STMn varied from 11 to 45 mg kg⁻¹ (Table 3-2).

Yield Analysis

No significant yield response was found at any of the ten locations or across locations evaluated (Table 3-4). This result is similar to previous studies using soil-applied micronutrients in soybean (Mallarino et al. 2015; Sutradhar et al. 2015). Specific soil conditions, such as coarse textured soils, low in organic matter, calcareous or eroded are traditionally expected to be conducive to S or micronutrient deficiency. Even though in this study certain of those conditions were identified (sandy soils in location 1 and locations 1, 3, 4 5, 9 with pH above 6.5) no response was found for soybean yield as affected by S or/and B, Cu, Mn and Zn fertilization. It was expected that at least the three locations with marginal STZn would have significant yield response over the control but this expected results were not found. Mallarino et al. (2015) stated that in the North Central region of the United States, soils suffice the demand of micronutrients for corn and soybean production. Due to the lack of yield response critical levels of soil micronutrients could not be assessed for this study.

Seed nutrient concentrations

Copper

Seed nutrient concentrations across locations there were no significant differences for Cu (Table 3-5). Copper is perhaps the most immobile of the micronutrients in the soil (Moraghan, 1991), therefore expectations of increase plant uptake were minor with the broadcasted treatments. Copper concentration in the seed was significantly different in locations 1 and 4 which had some of the highest percentages of sand (81% and 37%, respectively) and the lowest values of organic matter from all ten locations (11 g kg⁻¹ and 15 g kg⁻¹). In location 1, individually applied Cu and broadcast blend, were significantly higher than the unfertilized

control (Table 3-5). The broadcast blend was $0.9 \text{ mg Cu kg}^{-1}$ significantly above the control in location 4.

Manganese

No significant differences were found across locations. Manganese fertilization had no significant effect in any of the six locations evaluated by Sutradhar (2015), soil Mn ranged 7.3 to 57 mg kg^{-1} . For the significant differences found in Mn contents in seeds, there was no clear tendency of one treatment making the difference. In location 7, the individual application of S had the highest Mn content in seed compared to all treatments. In site 10, both blends were significantly lower than Mn applied individually; unfavorable interactions between micronutrients may have given these results.

Sulfur

Sulfur content in seed in coarse textured soil (location 1) showed that S individually applied and band blend had the same results as in the control; the broadcast blend had the lowest values of seed S concentrations (Table 3-6). Location 1 had a coarse textured soil which is prone for S leaching and low in S supply (Lamond, 1997); the fertilization had positive effects under this soil condition. In location 8, significant differences were found, being the broadcast blend the treatment with the highest S concentration in seed. No significant differences were found between treatments across locations.

Zinc

Statistical difference across locations was only found Zn seed nutrient ($P < 0.001$). Across locations, the broadcasted blend followed by the individual Zn applied treatment postulated the maximum amounts of Zn in the seed compared to unfertilized control (Table 3-6). Broadcasted and granular Zn fertilizer improved Zn seed concentration over the fluid banded Zn fertilizer

input. Four out of the ten locations demonstrated significant differences in soybean seed Zn concentration; in three locations (2, 4 and 6), the application of Zn fertilization (broadcast blend and Zn applied alone) was significantly higher than the control (Table 3-6). The results presented are in agreement with Martens et al. (1974) who found responses that Zn fertilization increased Zn in soybean seed. Across locations, the band blend was significantly lower than the broadcast blend and individual Zn, meaning that the rate applied for the band blend may have not suffice for an increase in Zn seed content.

Tissue nutrient concentration

Copper

Significant differences in Cu tissue concentration were found only in locations 1 and 8 (Table 3-7). These results are in agreement with the findings of Payne (1986) where Cu fertilization increased a 38% to 58% Cu tissue in soybean. Location 1 had 81% of sand; hence the application of Cu fertilization in coarse textured soils increases Cu concentration in tissue. The broadcasted blend treatment showed higher significant values of Cu concentration in tissue compared to the control in locations 1 and 8 (Table 3-7). Copper concentrations in all locations (except locations 3 and 10) were below the sufficient value of 10 mg kg^{-1} (Jones, 1967; Adriano, 1986). Therefore these sufficiency ranges may need to be evaluated in future studies for Cu. Copper deficiencies have not been reported in Kansas.

Manganese

The range of Mn concentration was 39.3 to 124 mg kg^{-1} among the ten locations. Significant differences for Mn concentration were found at four locations (1, 4, 6 and 8) but no significant difference across locations. In location 4 and 6 the control showed higher values than the band applied blend (Table 3-7). These results show that when applying micronutrients as a

blend, unfavorable interactions may occur; Zn fertilization may reduce Mn availability for plant uptake (Voss, 1998). As example, Giordano et al., (1974) and Moraghan et al. (1991) reported interactions involving Zn and Mn. Another reason can be the fertilizer source: In the band blend, the chelated source was used for Cu (EDTA, Ethylenediaminetetraacetic acid). Soil-applied MnEDTA is not as effective as MnSO₄ fertilizer to correct Mn deficiency in soybean (Shuman et al., 1979; Voth and Christenson, 1980). Voss (1998) states that band Mn in chelated forms may intensify Mn deficiency, this due to Fe-Mn imbalance, where Fe makes Mn less available for plant uptake.

Sulfur

Sulfur concentration was not significantly affected by fertilizer application at any location or across locations (Table 3-8). Oil seeds like soybeans demand high quantities of S (IPNI, 2012). Nowadays, S deficiencies across Kansas are common, especially on coarse textured soils with low organic matter; it was expected to find positive responses on location 1, but S fertilization had no effect on S concentration in tissue. Inconsistent results of crop response to S fertilization have been found in Kansas (Hoeft et al. 1986).

Zinc

Eight of the ten locations showed significant differences between treatments for Zn concentration on the leaf. Across locations, broadcasted blend had the tendency to increase the Zn concentration compared to control, band blend and Zn individually applied treatment (Table 3-8). The band blend had a lower rate of Zn fertilizer compared to the individually applied Zn, yet both were statistically the same for tissue Zn concentration. Zinc concentration in soybean

tissue for the all plots was above of the critical level 15 to 21 mg kg⁻¹ published by Bell et al. (1995).

Post-harvest soil analysis

Boron

Across locations, individual B application had no statistical difference from the broadcast blend but both treatments were significantly higher than the unfertilized plots and band blend (Table 3-9). The individually applied B and broadcast blend duplicated STB concentration at post-harvest over the control. STB in the control across locations had a mean of 0.6 mg kg⁻¹ and the broadcast blend had a mean of 1.1 mg kg⁻¹, even though STB was significantly increased by fertilization, the means are still in the published range of critical level (0.1-2.0 mg kg⁻¹) (Sims and Johnson, 1991). Significant differences in post-harvest STB were found in 8 of 9 locations. The only location where no significant difference was found was location 8; since this was the only locations which did not follow the tendency of results, it may be implied that the effects could have be attributed to the soil sampling in spots with low B content.

Copper

Significant differences were found across locations for STCu (Table 3-9) Broadcasted blend had the highest soil test values after harvest across locations with of 5.31 Cu mg kg⁻¹; this was significantly different from all other treatments (Table 3-9). Individual Cu treatment showed being significant lower than broadcast blend with 4.36 Cu mg kg⁻¹, but significantly higher than control and band blend treatments with 1.26 and 1.45 Cu mg kg⁻¹, respectively, without significant different between them. The broadcast blend quadruplicated Cu concentration in soil as compared to the untreated control.

Manganese

In location 4, STMn was significantly higher by the broadcast blend over the individual Mn, control and band blend. Manganese fertilization through the individually applied Mn fertilizer or micronutrient blends had no significant increase over the control across locations. Same results were found on wheat production in Kansas (Widmar, 2013). A response was less likely expected because STMn was found to be high in most of the locations; above 40 mg kg⁻¹ across site.

Zinc

Broadcasted blend had the highest soil test values after harvest across locations with of 8.75 Zn mg kg⁻¹; this is 6.88 Zn mg kg⁻¹ higher than control treatment. Individual Zn treatment showed being significant lower than broadcast blend with 7.34 Zn mg kg⁻¹, but significantly higher than control and band blend treatments with 1.87 and 2.08 Zn mg kg⁻¹, respectively, without significant difference between them. Broadcast blend was the treatments with the highest significant response and it may be due to the additional Zn contained in the other micronutrient fertilizers; the broadcast blend had an additional amount of 521 grams of Zn per plot and a total rate of 13 kg Zn ha⁻¹. More nutrient concentration was found in soil with the broadcasted blend than by individual Cu or Zn treatments, this may be due to the higher volume of granular fertilizer contained in the blend which lead to better application uniformity. Martens and Westermann (1991) affirmed that greater application efficiency could be found with the incorporation of banded ZnSO₄ compared to broadcast this due to lower surface contact area and fast availability for root uptake.

Conclusion

Micronutrient fertilization had no effect on soybean yield at any location or across locations, and therefore soil test as diagnostic tool for micronutrient fertilization cannot be evaluated in this study.

Copper, Mn and S seed content did not show consistent response across locations. There was significant increase in seed Zn content at four locations. It is important to highlight that Zn was applied in higher concentrations in the broadcasted blend because the source for Cu contained 6% of Zn, being the final Zn in the broadcast blend at a rate of 13 kg ha⁻¹.

Sulfur fertilization showed no effect on tissue at any location or across locations. Manganese and Cu had several responsive locations for tissue and seed nutrient concentration, but was not consistent across locations. Manganese concentrations on seed and tissue seemed affected at some locations where the band blend was applied; unfavorable interactions between Zn and Mn in the blend could have suppressed the availability of Mn for plant uptake. Therefore, if micronutrients were to be applied as a blend, the broadcast blend may attribute better results for Mn uptake by the plant.

The post-harvest soil analysis showed that fertilizing with B, Cu and Zn can help build nutrient content for the next crop, nevertheless the total amount may not be in available forms for future plant uptake. Under the evaluated conditions, Zn was the only micronutrient that had a significant increase with the fertilization across locations, with the broadcast blend treatment showing the highest values in Zn tissue, seed and post-harvest soil.

Results showed no yield increase by any of the nutrients or the combination of the nutrients; hence the application of B, Cu, Mn, S and Zn is not economically feasible for producers in Kansas. The application of the broadcast blend improved the concentration of Zn in seed and therefore a potential benefit for bio fortification. The findings of this study are an

important approach to agronomic biofortification and for future considerations about remunerations for seed quality improvement. The application of micronutrient fertilization is inherent to certain soil properties like coarse textured and low organic matter soils. Tissue, seed and post-harvest soil tests were effective diagnostic tools to understand output that micronutrient fertilizers may produce.

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Table 3-1. Description of the ten locations evaluated on 2013 and 2014.

Location	County	Soil Series	Soil Sub- Group	Precipitation (mm) †		Soybean Variety
				30-yr avg	1-yr avg	
<u>2013</u>						
1	Reno	Ost LS‡	Udic Argiustolls	787	763	P94Y23§
2	Franklin	Woodsen SIL	Abruptic Argiaquolls	990	734	Prod. 3801
3	Republic	Crete SL	Pachic Argiustolls	690	571	P33D39
4	Shawnee	Eudora SIL	Fluventic Hapludolls	920	773	Prod. 3801
5	Jefferson	Eudora SIL	Aquertic Argiudolls	838	799	H 3826
<u>2014</u>						
6	Clay	Cass SIL	Fluventic Haplustolls	787	683	P39T67R
7	Brown	Chase SIL	Aquertic Argiudolls	889	571	383-2R
8	Franklin	Woodsen S	Abruptic Argiaquolls	990	687	P48T53R
9	Shawnee	Eudora SIL	Fluventic Hapludolls	920	819	NK 39U2
10	Republic	Crete SIL	Pachic Argiustolls	690	482	P33D39

† Thirty year average from 1971 to 2000; annual average January 1st until December 31st of each year; Locations 3,4,9 and 10 received supplemental irrigation.

‡ LS,Loamy Sand; SIL, Silt Loam; S, Silt.

§ P,Pioneer; Prod,Producers; NK, Syngenta Northrup-King; H, Hoegemeyer

Table 3-2. Initial soil chemical properties of ten locations in 2013 and 2014.

Location	pH	Sand	Clay	OM	P	K	B	Cu	Fe	Mn	Zn
		--- - % ----	-----	g kg ⁻¹	----- mg kg ⁻¹ -----						
<u>2013</u>											
1	7.1	81	7	11	27	134	0.6	0.2	14.1	10.9	1.1
2	5.9	12	22	27	9.4	140	1.8	1.3	64.4	40.7	1.2
3	6.5	22	17	21	38	534	0.9	1.0	57.5	43.7	0.8
4	6.7	37	10	15	33	205	0.9	0.8	21.7	19.8	1.3
5	6.9	11	33	32	59	257	1.5	1.2	36.9	24.6	5.0
<u>2014</u>											
6	5.4	13	27	24	28	262	0.5	1.2	63.3	25.5	0.7
7	5.7	12	21	21	57	211	0.4	2.1	85.8	45.3	1.7
8	5.2	14	24	23	5.6	115	0.4	1.4	81.6	40.5	1.3
9	7.0	37	17	20	5.2	211	0.4	0.7	14.9	14.8	0.3
10	5.9	19	22	26	11	501	0.6	1.1	55.2	30.8	1.4

Table 3-3. Significance of F values for treatment effects on yield, seed Cu, Mn, S, Zn concentration and trifoliolate at the R2-R3 growth stage.

Location	Fixed effects								
	Yield	Seed nutrient concentration				Tissue nutrient concentration			
		Cu	Mn	S	Zn	Cu	Mn	S	Zn
	----- P < F -----								
1	0.277	0.040	0.946	0.044	0.437	0.009	0.076	0.118	0.002
2	0.761	0.299	0.923	0.174	<0.001	0.417	0.289	0.555	0.547
3	0.407	0.578	0.509	0.638	0.808	0.545	0.234	0.311	0.002
4	0.281	0.074	0.585	0.306	<0.001	0.597	0.018	0.446	<0.001
5	0.597	0.299	0.746	0.665	0.639	0.184	0.154	0.200	<0.001
6	0.604	0.780	0.895	0.697	<0.001	0.502	0.088	0.134	<0.001
7	0.891	0.132	0.002	0.128	0.118	0.695	0.241	0.171	0.002
8	0.553	0.285	0.133	0.023	0.749	0.057	0.075	0.196	0.005
9	0.681	0.601	0.634	0.929	0.025	0.160	0.687	0.326	0.085
10	0.480	0.479	0.053	0.782	0.519	0.763	0.163	0.328	0.113
Across locations	0.957	0.151	0.452	0.420	<0.001	0.123	0.869	0.544	<0.001

Table 3-4. Average soybean yield by location and across locations as affected by fertilizer treatments.

Location	Treatments							
	Control	B	Cu	Mn	S	Zn	Broadcast Blend	Band Blend
	----- kg ha ⁻¹ -----							
1	1949	1680	2217	2352	2217	2217	2352	1832
2	2620	2553	2486	2620	2486	2553	2486	2585
3	4367	4770	4569	4367	4435	4166	4099	4373
4	3763	4166	3830	3830	3830	4031	4166	4167
5	4166	4435	4099	4166	4166	4099	4569	4292
6	3379	3261	3391	3415	3371	3313	3066	3351
7	5337	5360	5569	5285	5432	5368	5179	5371
8	2619	2773	2598	2732	2511	2517	2572	2725
9	1940	1739	1642	2016	1844	1904	1965	2193
10	4093	4290	4052	3988	4166	4152	4006	3924
Across locations	3433	3507	3445	3485	3453	3438	3453	3481

Table 3-5. Copper and manganese concentration on soybean seed as affected by treatments.

Location	Treatments							
	Control	B	Cu	Mn	S	Zn	Broadcast Blend	Band Blend
----- Cu concentration mg kg ⁻¹ -----								
1	10.6 dc [†]	11 abcd	11.3 a	10.9 abc	10.7 bcd	10.2 d	11.2 ab	10.3 d
2	10.2	10.1	10.5	10.4	10.3	10.3	10.7	10.6
3	11.8	11.0	10.5	11.4	11.5	11.3	11.4	11.8
4	10.7 b	11.5 a	11.0 ab	10.6 b	10.4 b	10.4 b	11.6 a	11.1 ab
5	10.8	10.2	11.7	11.1	10.6	10.6	11.0	10.1
6	13.5	13.6	13.3	13.3	13.2	13.1	13.2	13.3
7	11.8	11.4	12.0	10.9	12.0	11.2	11.7	12.2
8	13.6	13.3	13.1	13.5	13.3	13.6	14.0	13.3
9	11.2	11.0	11.2	11.2	11.0	11.3	11.5	11.9
10	11.6	11.5	12.2	11.7	11.6	11.5	11.2	11.9
Across locations	11.6	11.4	11.7	11.5	11.4	11.4	11.7	11.7
----- Mn concentration mg kg ⁻¹ -----								
1	34.7	34.7	34.7	34.2	34.0	33.4	34.1	34.3
2	22.8	23.0	22.9	22.8	23.9	22.8	22.7	22.9
3	29.5	29.3	26.5	29.0	28.6	28.2	27.7	29.1
4	38.9	38.1	38.4	38.1	38.4	37.3	38.1	36.5
5	19.8	20.6	20.5	19.8	20.0	20.1	20.7	19.4
6	29.8	30.1	29.7	29.5	29.5	30.2	29.8	29.6
7	32.6 d	33.1 cd	34.0 bc	34.7 b	36.0 a	33.2 cd	34.2 bc	33.5 bcd
8	29.4	29.0	29.4	29.4	28.5	28.0	34.3	29.1
9	26.3	27.2	27.0	26.0	27.1	25.3	25.2	25.9
10	33.8 ab	33.8 ab	35.2 a	34.0 a	33.6 abc	34.2 a	32.1 c	32.3 bc
Across locations	29.7	29.9	29.8	29.8	29.9	29.3	29.9	29.3

[†] Numbers followed by different letters between columns indicate statistical difference at $P \leq .10$.

Table 3-6. Sulfur and Zn concentration on soybean seed as affected by treatments.

Location	Treatments							
	Control	B	Cu	Mn	S	Zn	Broadcast Blend	Band Blend
----- S concentration g kg ⁻¹ -----								
1	3.11 a [†]	3.06 a	3.07 a	3.09 a	3.04 a	3.06 a	2.94 b	3.05 a
2	2.03	2.02	2.12	2.07	2.12	2.09	2.16	2.09
3	2.16	2.08	1.96	2.11	2.16	2.11	2.05	2.12
4	2.62	2.72	2.63	2.70	2.66	2.61	2.68	2.65
5	2.18	2.23	2.26	2.14	2.17	2.16	2.21	2.08
6	2.40	2.38	2.41	2.34	2.37	2.37	2.38	2.39
7	2.77	2.76	2.80	2.91	2.96	2.78	2.96	2.78
8	2.83 bc	2.74 c	2.84 bc	2.85 bc	2.85 bc	2.88 b	3.04 a	2.80 bc
9	2.47	2.45	2.47	2.46	2.50	2.45	2.40	2.46
10	2.74	2.72	2.73	2.85	2.76	2.72	2.73	2.73
Across locations	2.42	2.42	2.42	2.45	2.46	2.41	2.45	2.41
----- Zn concentration mg kg ⁻¹ -----								
1	37.8	40.0	41.8	40.8	39.2	38.6	42.2	39.6
2	31.7 cd	30.7 d	32.6 bc	31.7 cd	32.0 c	33.3 b	34.7 a	32.4 bc
3	28.5 ab	24.8 d	25.8 cd	27 bc	27.1 bc	29.0 ab	29.7 a	27.6 bc
4	37.2 dc	37.1 dc	38.8 bc	38.4 c	35.5 d	40.4 b	42.2 a	37.3 dc
5	32.6	32.6	34.8	33.3	32.4	34.4	34.7	32.0
6	36.4 d	36.1 ed	38.1 c	36.1 ed	34.9 e	40.4 ab	41.1 a	39.3 bc
7	43.5	42.6	43.1	45.3	44.2	44.4	48.1	44.0
8	42.9	42.2	43.0	42.8	42.1	43.8	44.1	41.9
9	41.0	41.5	42.7	42.1	42.0	43.0	42.0	40.8
10	35.3	35.7	36.6	36.2	34.7	35.8	35.3	34.8
Across locations	36.6 d	36.3 d	37.7 bc	37.4 c	36.4 d	38.3 b	39.4 a	36.9 cd

[†] Numbers followed by different letters between columns indicate statistical difference at $P \leq .10$.

Table 3-7. Copper and manganese concentration on soybean trifoliate at R2-R3 growth stage as affected by treatments.

Location	Treatments							
	Control	B	Cu	Mn	S	Zn	Broadcast Blend	Band Blend
----- Cu concentration mg kg ⁻¹ -----								
1	5.1 bc [†]	5.55 ab	5.18 bc	5.40 b	4.66 dc	4.50 d	6.06 a	5.35 b
2	7.9	9.03	7.9	8.23	8.40	8.02	8.64	8.43
3	11.8	11.4	11.8	11.8	11.7	12.4	12.3	12.1
4	6.76	6.81	7.01	6.34	6.49	6.82	6.84	6.68
5	7.95	8.27	8.55	8.59	7.63	7.37	8.65	7.74
6	9.47	9.55	9.15	9.26	9.25	9.41	9.68	9.56
7	8.56	8.57	8.38	8.17	8.53	8.37	8.40	8.70
8	7.91 bc	7.66 cd	7.75 bcd	8.16 ab	7.72 bcd	7.50 d	8.42 a	7.88 bcd
9	7.63	7.73	7.45	7.66	7.39	7.50	7.85	8.18
10	13.2	13.3	13.7	13.6	13.8	13.3	13.2	13.9
Across locations	8.63	8.78	8.69	8.72	8.56	8.52	9.00	8.85
----- Mn concentration mg kg ⁻¹ -----								
1	93.9 b	106.9 a	87.4 b	93.3 b	94.9 ab	87.0 b	107.9 a	99.3 ab
2	40.5	45.3	39.3	40.9	45.8	39.5	43.4	40.1
3	115.4	113.3	109.5	124.6	115.5	114.7	120.8	123.9
4	67.2 a	56.4 b	63.1 a	66.7 a	64.5 a	69.8 a	66.1 a	57.1 b
5	67.5	71.3	65.7	73.8	63.9	66.0	68.5	67.4
6	61.3 b	62.7 ab	63.9 ab	63.2 ab	64.9 a	64.1 ab	62.5 ab	58.1 c
7	87.2	95.0	95.8	94.2	88.6	91.4	97.2	84.8
8	50.8 bc	50.6 bc	51.6 ab	51.8 ab	51.6 ab	49.2 c	52.5 ab	53.4 a
9	70.4	70.7	71.2	69.2	69.5	69.2	66.9	69.2
10	95.7	91.9	104.1	87.3	97.6	94.4	92.2	84.4
Across locations	75.0	76.4	75.1	76.5	75.7	74.5	77.8	73.8

[†] Numbers followed by different letters between columns indicate statistical difference at P≤.10.

Table 3-8. Sulfur and Zn concentration on soybean trifoliate at R2-R3 growth stage as affected by treatments.

Location	Treatments							
	Control	B	Cu	Mn	S	Zn	Broadcasted Mix	Band Mix
----- S concentration g kg ⁻¹ -----								
1	2.48	2.54	2.53	2.53	2.41	2.47	2.38	2.40
2	2.40	2.64	2.45	2.53	2.63	2.46	2.60	2.54
3	2.90	2.87	2.73	2.81	2.97	2.80	2.68	2.94
4	2.37	2.16	2.31	2.25	2.29	2.40	2.20	2.22
5	3.43	3.41	3.10	3.30	3.20	3.21	3.60	3.32
6	2.45	2.46	2.49	2.44	2.52	2.51	2.60	2.49
7	3.02	3.15	3.22	3.16	3.13	3.21	3.30	3.12
8	2.45	2.44	2.45	2.46	2.46	2.36	2.57	2.44
9	2.66	2.72	2.68	2.72	2.71	2.66	2.79	2.69
10	3.05	3.04	3.05	3.07	3.15	3.05	3.20	3.10
Across locations	2.72	2.74	2.70	2.73	2.75	2.71	2.79	2.73
----- Zn concentration mg kg ⁻¹ -----								
1	30.5 b	30.7 b	33.0 b	32.9 b	30.2 b	33.5 b	44.5 a	33.0 b
2	40.6	42.9	41.1	39.7	40.5	41.0	44.8	42.2
3	42.7 dc [†]	40.5 d	45.3 bc	44.6 bc	42.8 dc	47.6 ab	50.0 a	45.3 bc
4	30.4 dc	26.9 e	32.0 bc	28.1 d	26.4 e	35.5 a	33.7 ab	28.6 dc
5	44.1 cd	45.9 bcd	46.4 bc	43.3 cd	41.3 d	50.7 ab	55.5 a	45.5 cd
6	29.4 d	32.4 bcd	33.9 b	29.2 d	30.5 cd	34.5 ab	37.6 a	32.8 bc
7	37.1 c	35.9 c	37.8 bc	36.4 c	37.5 bc	40.1 b	43.1 a	39.6 b
8	31.9 c	29.7 d	33.6 abc	33.4 abc	32.6 bc	32.2 bc	35.2 a	34.1 ab
9	34.3 bc	32.8 c	33.3 c	35.6 abc	32.9 c	34.8 abc	37.6 a	36.3 ab
10	44.3	44.6	45.6 c	45.7	44.8	47.7	48.1	46.7
Across locations	36.5 d	36.2 d	38.2 c	36.9 cd	35.9 d	39.8 b	43.0 a	38.4 bc

[†] Numbers followed by different letters between columns indicate statistical difference at P≤.10.

Table 3-9. Post- harvest DTPA-extractable soil test values for B and Cu as affected by treatments containing that same nutrient.

Location	Treatments				P < F
	Control	B	Broadcast Blend	Band Blend	
	----- Soil DTPA B (mg kg ⁻¹) -----				
2	0.46 c [†]	0.98 a	0.78 b	0.44 c	<0.001
3	0.57 c	1.07 b	1.20 a	0.63 c	<0.001
4	0.44 c	0.95 b	1.11 a	0.48 c	<0.001
5	0.76 d	1.73 a	1.41 b	0.93 c	<0.001
6	0.66 c	0.96 b	1.26 a	0.65 c	<0.001
7	0.41 c	0.89 a	0.87 a	0.60 b	<0.001
8	0.66	1.00	1.12	0.97	0.259
9	0.47 b	0.89 a	0.88 a	0.53 b	<0.001
10	0.86 b	1.56 a	1.40 a	0.79 b	<0.001
Across locations	0.59 c	1.12 a	1.11 a	0.67 b	<0.001
	----- Soil DTPA Cu (mg kg ⁻¹) -----				
	Control	Cu	Broadcast Blend	Band Blend	
2	0.93 b	4.00 a	3.07 a	1.02 b	<0.001
3	0.92 b	2.45 b	6.74 a	0.91 b	<0.001
4	0.74 c	3.68 b	8.14 a	0.81 c	<0.001
5	1.01 c	4.28 a	2.73 b	1.11 c	0.002
6	1.44 b	4.37 a	4.73 a	2.00 b	<0.001
7	2.28 c	6.62 a	5.80 b	2.75 c	<0.001
8	1.77 b	4.61 a	5.34 a	1.86 b	<0.001
9	0.92 c	3.63 b	5.89 a	1.05 c	<0.001
10	1.36 b	5.63 a	5.37 a	1.54 b	<0.001
Across locations	1.26 c	4.36 b	5.31 a	1.45 c	<0.001

[†] Numbers followed by different letters between columns indicate statistical difference at P_≤.10

Table 3-10. Post- harvest DTPA-extractable soil test values for Mn and Zn as affected by treatments containing that same nutrient.

Location	Treatments				P < F
	Control	Mn	Broadcast Blend	Band Blend	
	----- Soil DTPA Mn (mg kg ⁻¹) -----				
2	23.1	25.8	23.9	24.8	0.472
3	33.7	37.5	39.2	36.8	0.043
4	14.5 bc [†]	16.9 b	17.2 a	15.4 bc	0.017
5	30.5	37.7	24.2	31.9	0.152
6	42.3	41.8	38.9	57.8	0.632
7	56.9	62.5	63.3	53.4	0.578
8	82.3	89.9	86.7	76.2	0.647
9	28.1	29.5	32.4	28.4	0.130
10	74.6	72.4	70.4	75.9	0.837
Across locations	42.9	45.8	44.0	44.5	0.808
			Broadcasted Blend		
	----- Soil DTPA-Zn (mg kg ⁻¹) -----				
2	0.78 b	3.78 a	3.85 a	0.85 b	<0.001
3	0.70 b	2.29 b	8.76 a	0.71 b	<0.001
4	1.15 c	6.36 b	12.3 a	1.24 c	<0.001
5	5.11c	11.8 a	7.78 b	5.05 c	<0.001
6	1.48 c	8.43 a	8.52 a	2.06 b	<0.001
7	2.73 c	10.4 a	10.5 a	3.22 b	<0.001
8	1.98 b	8.41 a	9.61 a	2.3 b	<0.001
9	0.69 c	6.30 b	8.17 a	0.80 c	<0.001
10	2.28 b	8.26 a	9.20 a	2.51 b	<0.001
Across locations	1.87 c	7.34 b	8.75 a	2.08 c	<0.001

[†] Numbers followed by different letters between columns indicate statistical difference at P_≤.10

Chapter 4 - Soybean Tissue Response to Boron, Copper, Manganese and Zinc Fertilization Using On-farm Strip Trials

Abstract

Limited studies are available evaluating the response to micronutrients particularly using field scale strips. The objective of this study was to evaluate soybean (*Glycine max (L.) Merr.*) tissue response to micronutrient fertilizer application in fields with high soil variability using field strips. Two locations were established in 2014 and one location on 2015. The area consisted of three blocks and each block was divided into two strips. Soil samples were collected at a depth of 0 to 15 cm from the marked points located every 24 m across the length of each block prior to fertilizer application. The treatments included an unfertilized control and a blend of Cu, Mn and Zn at a rate of 11.2 kg ha⁻¹ and B at a rate of 2.8 kg ha⁻¹. When the soybeans reached the stage R2-R3, thirty uppermost trifoliates were collected from the center of each strip every 24 m. For the analysis soil was classified by pH and organic levels above or below the mean of each location. For tissue content of Zn and B, soil pH and organic matter were proper factors to consider for explaining responses to micronutrient fertilization. Micronutrient blend treatment showed higher tissue Zn and B values compared to the control. When pH ranged from 5.5 to 7.6, B in tissue was higher on the control than the micronutrient blend; this result can be attributed to the landscape slope in location 1, the mobility of B in soil and at high pH, B present in soil may be in forms not readily available for the plant. Copper concentration in tissue did not present significant difference between treatments at any location, regardless of pH and organic matter variation. Significant differences were found for manganese due to supply of Mn in the fertilizer blend only in Location 2 when pH was below 5.5 and organic matter below 34 g kg⁻¹. Initial soil

tests of B, Cu, Mn and Zn were not suitable indicators of soybean tissue concentration of these micronutrients.

Introduction

The need for increasing commercial nutrient inputs raises questions about the role of secondary and micronutrient fertilizers. One of the challenges at the field scale is soil variability; determining and classifying the variability of soil parameters may contribute to a better understanding of crop response. Due to the correlation found between soil variability and crop production, location variability must be understood to its fullest capacity in order to create a successful soil fertility management program (Sawchik et al., 2008). When experiments are done on small plots, only small variability of soil properties are exhibited as opposed to larger strips. However, as field variability increases, so does the experimental error that may cover up differences by treatments in the analyzed responses (Nielson, 2010). To obtain a better analysis, the field should be divided in areas according to yield potential, soil characteristics or agronomical practices (Mallarino et al. 2000). Nutrients available for plant growth may vary greatly across the fields.

Out of the seven essential micronutrients for plant growth, research in Kansas has been focused on chloride (Cl) and iron (Fe) and Zn. Few or no information has been found about B, Cu, Mn and Zn on soybean production; therefore the micronutrients for this study were chosen in order to close a gap of information in Kansas research. In the North-Central region, DTPA-extractable (diethylenetriamine-pentaacetic acid) test is recommended for soil test Cu (STCu), soil test Mn (STMn) and soil test Zn (STZn) (Whitney, 1998; Sims and Johnson, 1991). For, soil test B (STB), the hot-water soluble extraction was proposed by Berger and Troug in 1939 and it is still of common use nowadays (Sims and Johnson, 1991). The reliability of micronutrient soil

test is much less than for secondary and macronutrients therefore tissue testing should be considered part of a diagnosis (Jones, 1991). Prior research has shown inconsistent responses of plant nutrient uptake as affected by micronutrient fertilizers (Mallarino et al, 2015). Randall et al. (1975) reported increase of manganese content in leaf with the application of $MnSO_4$ and Mn-EDTA applied to soil or foliage; but Sutradhar (2015) found no impact of fertilizer application on tissue content for these nutrients. Copper fertilizer inputs have shown increases (Oplinger et al., 1974; Ross et al., 2006) on soybeans and no responses across locations by Widmar (2013) on wheat. For soybeans, Zn has medium responsiveness to Zn fertilization (Alloway, 2008) and very low sensitiveness to B deficiencies (Gupta et al., 2008).

The soil characteristics that mainly affect micronutrient availability to plant uptake are pH and organic matter (Havlin et al, 2005). The inclusion of soil pH contributes to a better understanding for the analysis of Zn and Mn plant uptake (Sims, 1986). Copper deficiencies are more likely to be found in acid soils with high organic matter (Mengel, 1980). Reduction of plant available Cu can be attributed to the inner sphere complex that forms between soil organic matter and Cu; organic matter binds Cu more tightly than with any other micronutrient (Schulte and Kelling, 1999) (McBride, 1978). Manganese deficiencies are prone to occur on soils with high organic matter and $pH > 6.5$. Low organic matter is not favorable for B availability; organic matter is an important source of plant available B. At pH above 7.5, $ZnOH^+$ becomes abundant in soil; this form is not readily available for plant uptake (Havlin et al., 2005). The balances between soil properties and availability of micronutrients may complicate. For example: high organic matter may contribute with more B available for plant uptake but may convert Mn to unavailable organic forms (Voss, 1998).

The integration of soil factors and tissue analysis has the potential to generate useful diagnostic tools in order to enhance micronutrient management. Limited studies are available for response to micronutrients and even less in large-scale field strips. The objective of this study was to evaluate tissue response to micronutrient fertilizers in fields with high soil variability.

Materials and Methods

Three strip trials were established through 2014 and 2015. The selected locations were at producer's fields located in the northeast Kansas. Management practices such as variety selection and planting dates were those used by the collaborating producers (Table 4-1). The experimental design consisted of two strips, an unfertilized and fertilized replicated three times (three blocks). The length of each strip was 268.2 m. and the width varied by locations and depending on the equipment used by the producer. The width of the strips for locations 1 and 2 was 12 m; and a width of 10.7 for location 3. The area was divided in three blocks and the sampling scheme was a systematic grid points. Soil samples were collected before fertilizer application at a depth of 15 cm at every 24 m along each block. Composite samples (10-12 cores) were taken in a circumference of about 4.6 m radius. A total of 11 samples per block were collected (33 soil samples per location). The analysis included soil pH, soil test phosphorus, soil test potassium and soil pH, in addition to the micronutrients B, Cu, Mn, and Zn. Soil pH was determined on 1:1 (soil: water). Soil phosphorus was determined by Mehlich3-extraction (Frank et al., 1988) and K by ammonium acetate ICP Spectrometer (Warncke and Brown, 1998). Soil organic matter test was collected per block every 24.2 m and later was analyzed by the method of Walkley-Black (Combs and Nathan, 1998). Copper, Mn and Zn were analyzed by DTPA extraction using inductively coupled plasma spectrometer-optical emission spectroscopy (ICP-OES) (Whitney,

1998). Boron was extracted by hot water method (Watson, 1998) determined via Inductively Coupled Argon Cooled Plasma Spectrometer (ICAP).

After soil sampling, the fertilizer treatment were applied. The treatments included an untreated control and a blend of Cu, Mn and Zn at a rate of 11.2 kg ha⁻¹ and B at a rate of 2.8 kg ha⁻¹. The fertilizer utilized was broadcasted previous to planting.

Every strip was divided into 11 equal grids. When the soybeans reached full bloom (R2) to beginning of pod (R3) growth stage, thirty of the uppermost trifoliates (Ritchie et al., 1997) were taken at every 24.4 m along the strip, on a circumference of a radius of 4.6 m. A total of 11 tissue samples were collected per strip (22 tissue samples per block). Tissue samples were oven dried at 65°C, ground to pass through a 2 mm screen and submitted for analysis. The analysis of tissue samples included total P, K, Cu, Mn and Zn. Samples were digested using the Nitric-Perchloric digest and analyzed using the ICP-OES (Giesecking et al. 1935;Donohue, 1992).

Results and Discussion

Location description

Soil pH and organic matter varied greatly in location 1 as opposed to the other two locations (table 4.1). Soil pH in location 1 ranged from 4.6 to 7.6 with a standard deviation of 0.8 and soil organic matter ranged from 19.2 to 35.2 g kg⁻¹ (Table 4-2). Location 2 had soil pH ranged from 5.2 to 6.5. Location 3 had the lowest ranges of organic matter (15.6 to 27.1 g kg⁻¹).

Boron

DTPA soil B was below 0.7 mg kg⁻¹ for all three locations. About 23% of the sample variation of B concentration in tissue was explained by the initial content of B in the soil (Figure 4-5). The low R² suggest poor relation between of STB and soybean tissue B. Plant uptakes

$B(OH)_3$ which is the form of B present at $pH < 7$; at $pH > 6.5$ B availability for plant uptake decreases (Moraghan et al., 1991) due to lower B solubility at high soil pH. At soil $pH > 7$, $H_2BO_3^-$, HBO_3^{2-} , BO_3^{2-} and $BO_4O_7^{2-}$ are predominant, these anion forms are less readily available for the plant than $B(OH)_3$ (Havlin et al. 2005). Location 1 showed significant differences with higher values for control over the micronutrient blend, however this was only found when pH ranged from 5.5 to 7.6 (Figure 4-1). At location 3, the micronutrient blend was higher than the control when pH ranged from 6.9 – 7.3. Boron is a mobile nutrient in the soil and can be easily leached from the soil surface; location 1 had a slight landscape slope that could have favored the leaching of soil B, consequently reducing its native availability. No significant differences were found when classifying organic matter above or below 29 g kg^{-1} . Significant differences were found on locations 2 and 3.

Copper

Copper levels in the soil varied in location 1, from 0.79 to $2.67 \text{ mg Cu kg}^{-1}$. On locations 2 and 3 DTPA-extractable STCu was below $0.7 \text{ mg Cu kg}^{-1}$, which is considered below the critical range (0.1 - 2.5 mg kg^{-1}) (Sims and Johnson, 1991). The relationship between Cu concentration in the tissue and STCu for the control strips denoted an adjusted R^2 of 0.53 (Figure 4-5). Tissue Cu versus STCu had the highest coefficient of determination when comparing with the other nutrients in this study. The regression analysis indicates that Cu in the tissue and STCu are inversely related to each other. Sims and Johnson (1991) affirm that soil DTPA-Cu is a better diagnostic tool than tissue analysis when reporting deficiencies. Copper concentration in tissue presented no significant difference at any location in any range of pH or organic matter levels (Figure 4-2). Oplinger (1974) applied $44.8 \text{ kg Cu ha}^{-1}$ in locations with less than $5.0 \text{ mg Cu kg}^{-1}$ in soybean leaf tissue and found treatment effect especially on high organic matter soils; these

results differ from the findings of this research. Published sufficiency levels of Cu content in soybean tissue indicate that deficiencies may be observed when tissue test is below 10 mg Cu kg⁻¹ (Jones, 1967; Adriano, 1986). On locations 2 and 3 none of the observations had values above 10 mg Cu kg⁻¹ and location 3 showed values slightly above 10 mg Cu kg⁻¹ in the tissue. Copper deficiencies have not been detected in Kansas and therefore the critical soil test values for Cu should be revised.

Manganese

DTPA-extractable STMn ranged from 10.2 to 76.2 mg kg⁻¹ across the locations, with highest standard deviation for Location 1 (Table 4-2). The simple regression of Mn concentration in tissue and soil has a very scattered set of observations that indicate a slight trend of increasing Mn in tissue as soil Mn increases (Figure 4-5). Manganese content in tissue versus STMn had a R² of 0.23 (Figure 4-5). Significant differences between treatments were found only in Location 2 when pH was below 5.5 and when organic matter was below 34 g kg⁻¹ (Figure 4-3); micronutrient blend presented the highest values. The availability of manganese in solution is determined mainly by pH and redox potential (Leeper, 1947); pH < 5.5 tends to increase Mn's availability for plant uptake (Reid, 1976). Organic matter on Location 2 was the highest among locations; ranging from 29.5 to 39.1 g kg⁻¹ (Table 4-1). Manganese may complex with organic compounds, some form of organic matter may fix Mn and set it an un-available form for plant uptake (Moraghan et al. 1991).

Zinc

The locations 1 and 3 had certain plots with pH < 6.5, which are more likely to have reduced Zn availability. Zn levels were above 1 mg kg⁻¹ in Location 1 as opposed to Location 2 which had levels as low as 0.5 mg kg⁻¹. DTPA-extractable STZn levels below 0.9 mg kg⁻¹ are

considered marginal for optimum crop growth (Sawyer et. al, 2008). A poor relationship ($R^2:0.03$) was found between Zn tissue and soil in the strips that had no fertilizer applied (Figure 4-1). These results agreed with Pepper et al., (1983) on corn and Payne et al. (1986) on soybean, who reported the essentiality of including pH and organic matter to improve the relationship between STZn and Zn concentration in plants. Zinc concentration in soybean tissue was responsive to the broadcasted micronutrient blend compared to the control (Figure 4-4). Similar results were found by Mallarino (2013) with the broadcasted mixture which presented greater Zn concentration in plant tissue relative to the control. Significant differences were found for the three locations when pH was lower than 5.5 (for locations 1 and 2); this result concurs with Lohry (2007), who stated that Zn availability may start being compromised with a pH above 6.5 (Figure 4-4). Locations 1 and 2 had an adequate pH mean (5.5) for Zn availability, at this pH Zn^{2+} is the predominant form of Zn in soil, which favors plant uptake (Voss, 1998). Location 3 showed no difference among treatments when pH was above 6.9. On location 2, the micronutrient blend was significantly higher than the control regardless of pH and organic matter levels. When comparing conditions among the locations, Location 2 had an ideal pH range for Zn availability, organic matter was higher than the other locations and high amounts of Zn in soil (2.7 to 8.5 mg Zn kg⁻¹). Location 1 only presented significant differences when organic matter was higher than 29 g kg⁻¹.

CONCLUSION

Differences were found when dividing the data in levels of pH and organic matter. For Zn and B, soil pH and organic matter were the proper factors to consider in order explaining responses to micronutrient fertilization. Zinc fertilization was the only micronutrient with significant differences for all locations when pH was below the respective means of the three

locations (pH: 5.5 and 6.9). Copper showed no increase in response over the control at any location. Manganese content in tissue was significantly higher by the micronutrient blend over the control only in location 2, when pH ranged from 5.2 to 5.5 and organic matter ranged from 29 to 34 g kg⁻¹. Boron showed significant differences on two of three analyzed locations. Boron content in tissue was decreased by micronutrient fertilization when pH ranged from 5.5 to 7.6 in Location 1. High pH is a limiting soil condition for micronutrient availability for plant uptake. Tissue contents of Zn and B showed consistent responsiveness to the fertilization of Zn, B, Cu and Mn. Soil Cu presented the strongest relationship between soil test and tissue test and Zn in soil versus Zn in tissue presented the weakest relationship. Soil Zn is not a suitable indicator to understand Zn responses in tissue. Tissue testing can be a useful diagnostic test when accompanied by pH and organic matter.

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Table 4-1. Description of soil characteristics, precipitation, planting date and soybean varieties for the locations evaluated in 2014 and 2015

Location	County	Soil Series	Soil Subgroup	Precipitation [‡] (mm)		Planting Date	Soybean Variety
				30 yr. avg.	1 yr. avg.		
1	Nemaha	Chase SIL [†]	Aquertic Argiudolls	889	570	05/21/2014	§P 39T67R
2	Jefferson	Eudora SIL	Aquertic Argiudolls	939	672	05/23/2014	H 3811
3	Jefferson	Eudora SIL	Fluventic Hapludolls	939	672	06/11/2015	383-2R

[†] SIL, Silt Loam

[‡]Thirty year average from 1971 to 2000; annual average taken from data of January 1st until December 31st of each year; Location 3 received supplemental irrigation.

§ P,Pioneer; H, Hoegemeyer

Table 4-2. Initial soil chemical analysis and statistics (minimum, maximum and standard deviation) for the three experimental locations in 2014 and 2015.

Location	County	Statistic	pH	Organic Matter	P	K	B	Cu	Mn	Zn
				g kg ⁻¹	----- mg kg ⁻¹ -----					
1	Nemaha	Min.	4.6	19.2	23.8	120	0.35	0.79	10.2	1.02
		Max.	7.6	35.2	123	293	0.87	2.67	76.2	6.1
		SD	0.8	4.0	24.4	36.7	0.1	0.5	15.6	1.1
2	Jefferson	Min.	5.2	29.5	23.4	157.0	0.4	1.1	23.5	2.7
		Max.	6.0	39.1	69.3	256.0	0.7	2.0	45.1	8.5
		SD	0.2	2.4	11.5	24.2	0.1	0.2	5.2	1.6
3	Jefferson	Min.	6.5	15.6	22.6	198	0.30	0.54	16.4	0.49
		Max.	7.3	27.1	63.7	516	0.47	0.95	28.5	0.99
		SD	0.18	3.0	10.7	80	0.04	0.11	3.24	0.14

† Min., Minimum; Max., Maximum; SD, Standard deviation

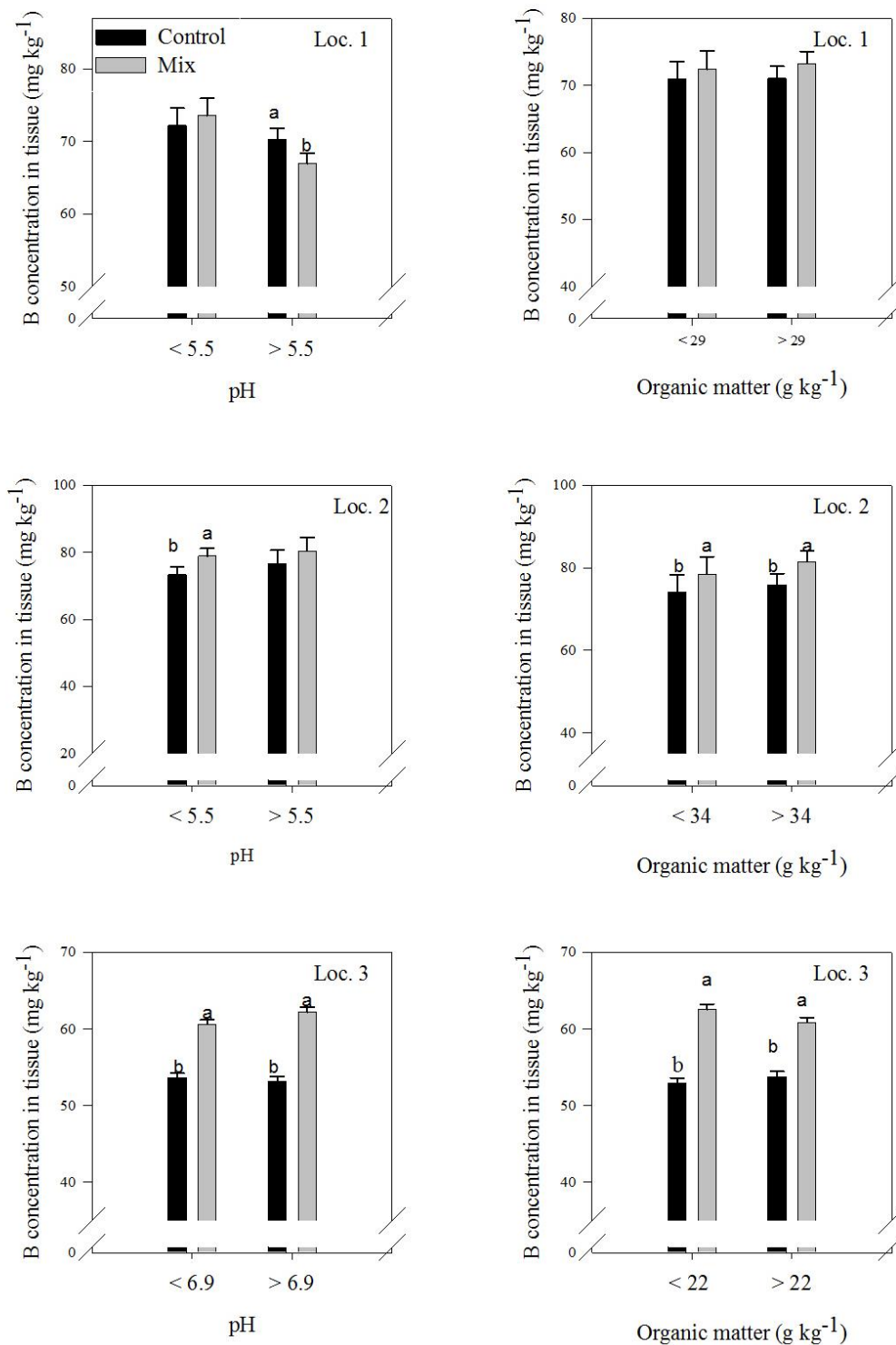


Figure 4-1. Boron concentration in tissue as affected by treatments and classified by soil pH and organic matter levels for the three locations in 2014 and 2015. Different letters between each bar indicate statistical difference at $P \leq 0.10$.

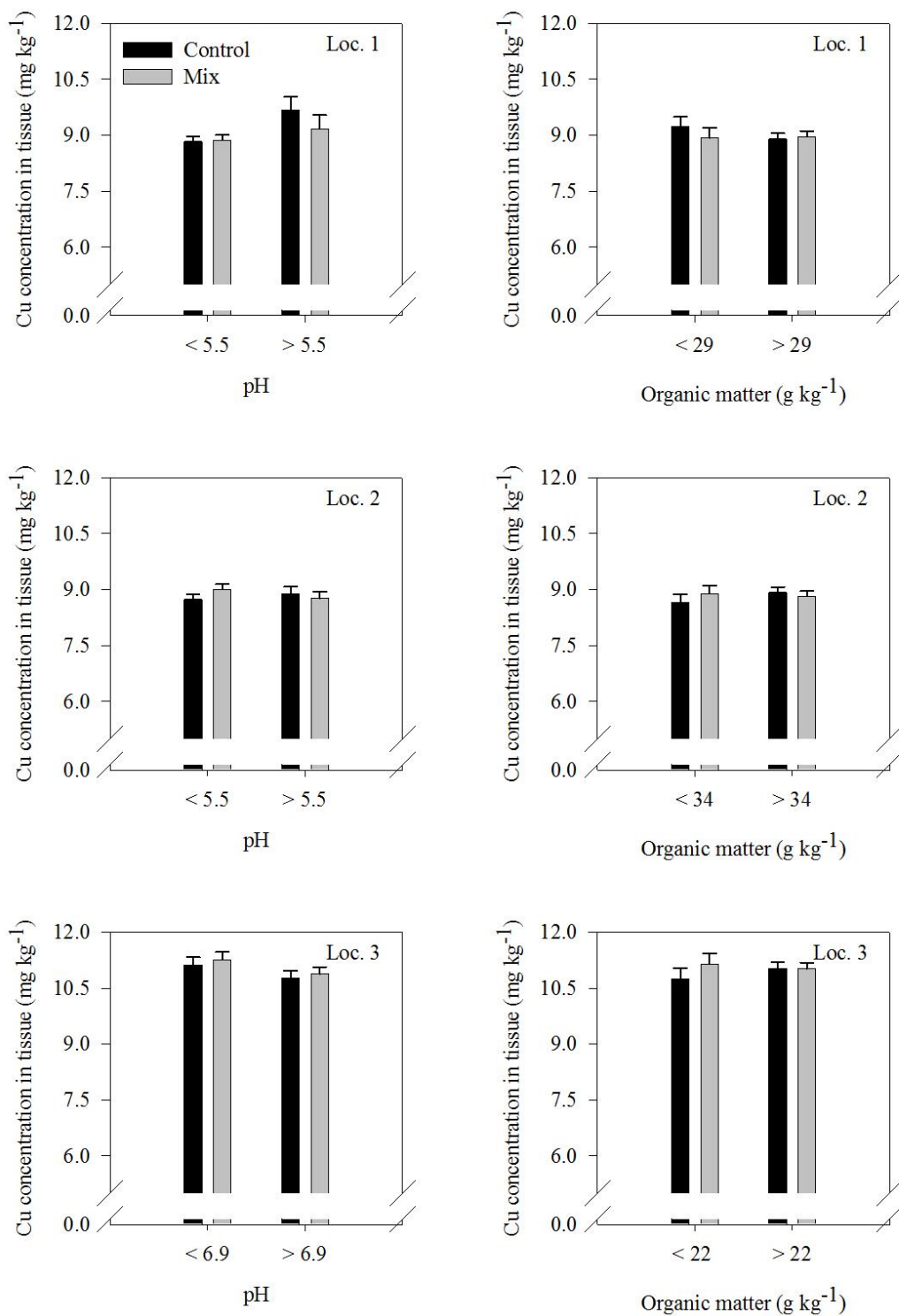


Figure 4-2. Copper concentration in tissue as affected by treatments and classified by soil pH and organic matter levels for the three locations in 2014 and 2015.

†Different letters between each bar indicate statistical difference at $P \leq 0.10$.

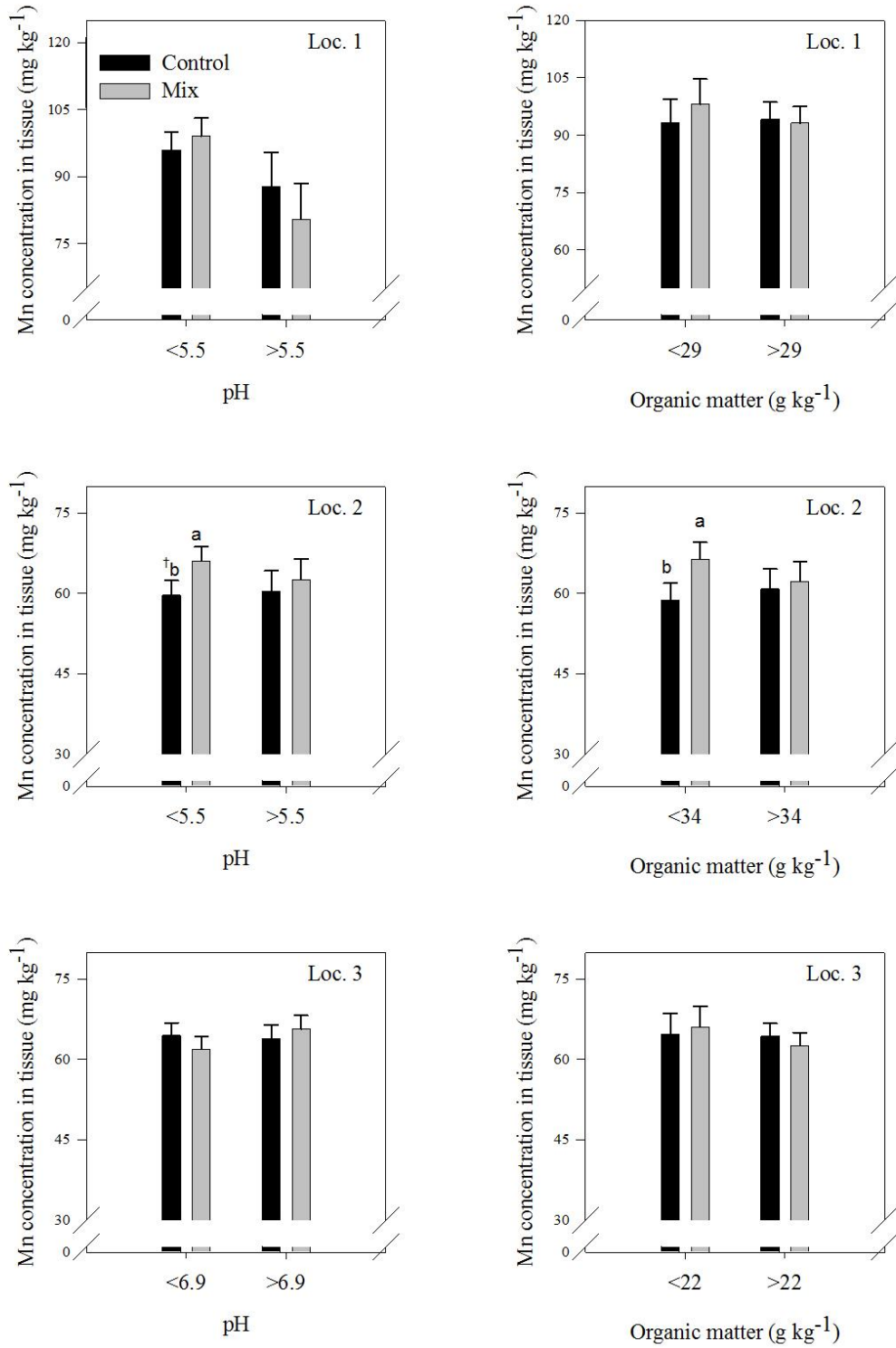


Figure 4-3. Manganese concentration in tissue as affected by treatments and classified by soil pH and organic matter levels for the three locations in 2014 and 2015.

† Different letters between each bar indicate statistical difference at $P \leq 0.10$

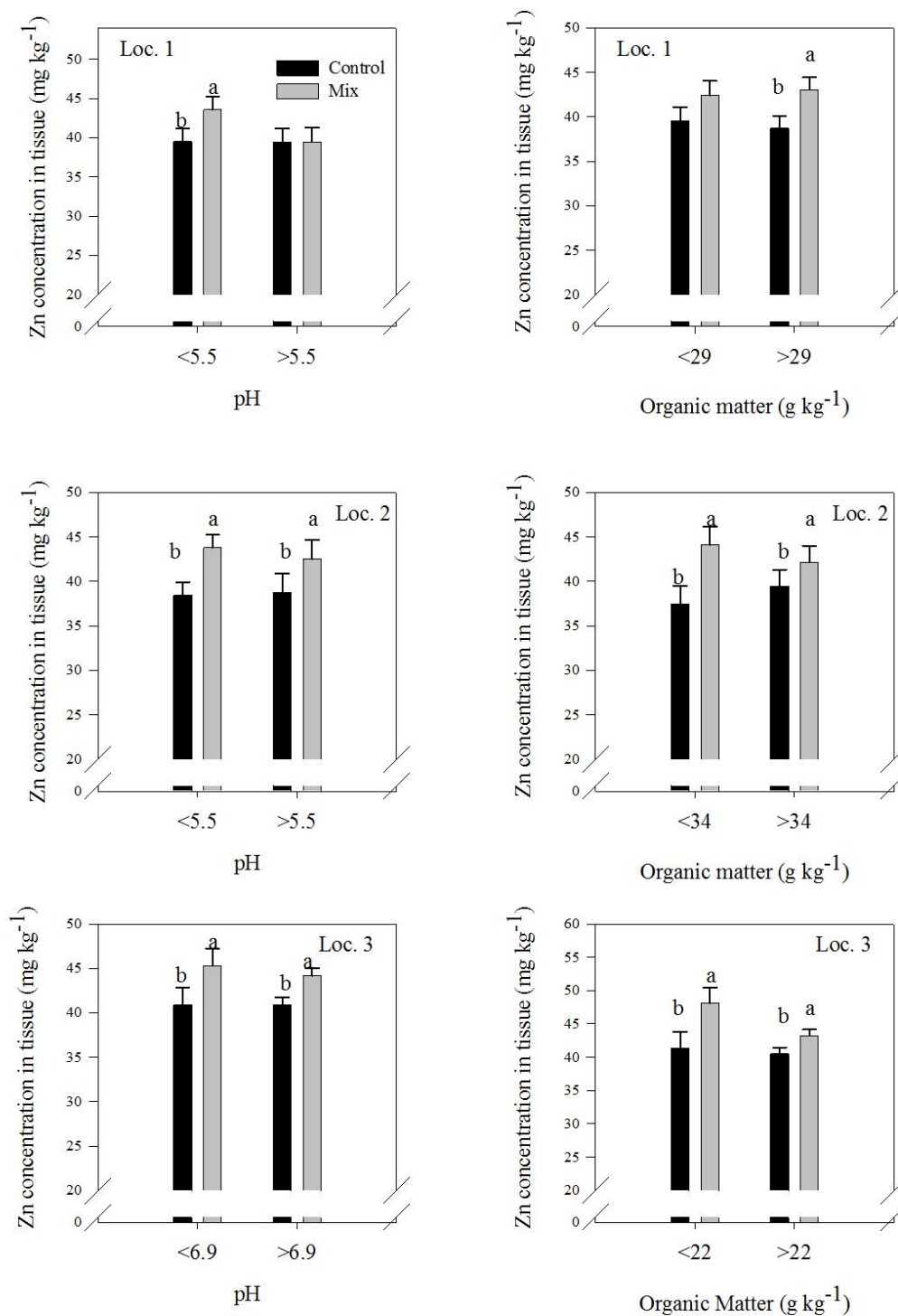


Figure 4-4. Zinc concentration in tissue as affected by treatments and classified by soil pH and organic matter levels for the three locations in 2014 and 2015.

† Different letters between each bar indicate statistical difference at $P \leq 0.10$

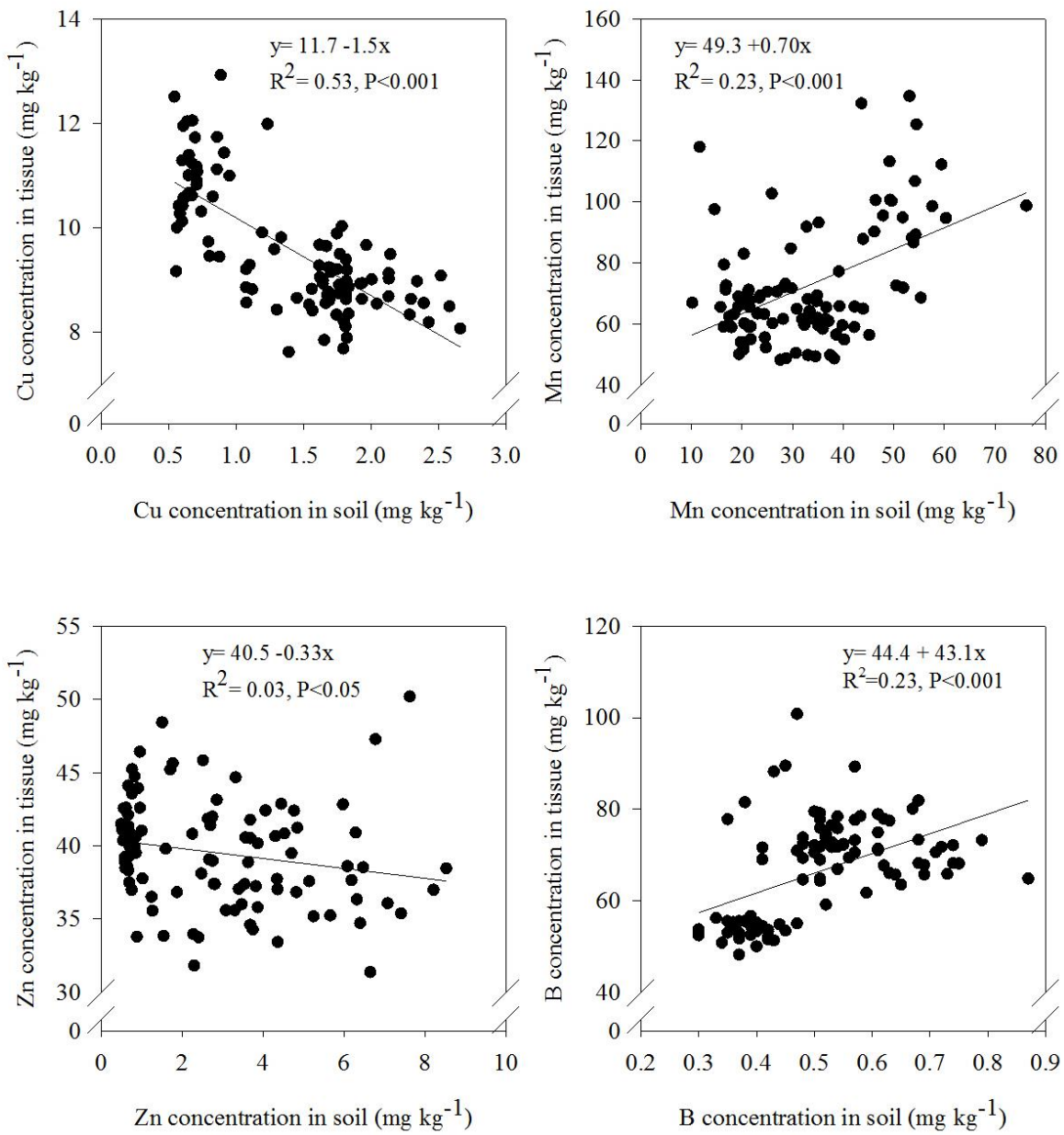


Figure 4-5. Relationship between Cu, Zn, Mn and B concentration in the leaf and their respective initial DTPA-extractable soil test for the unfertilized treatments.

Chapter 5 - General Conclusions

Within last decades, producers' uncertainty and questions about secondary and micronutrient fertilization in the Midwest have not grown parallel to appropriate answers and resourceful research; instead data has been limited. Research about secondary and micronutrient fertilization was found to be old, focused only on Zn and S disregarding the other micronutrients. Few studies about soybean were found. The review considering data since 1962 demonstrated the advantage in corn yield production when Zn is applied on soil, nevertheless soil Zn was not the best indicator of corn yield responsiveness to Zn fertilization. No yield increase were found by applying Zn to sorghum, wheat and soybean yet the limited amount of information deprived the analysis of other factors affecting the responses. Sulfur fertilization had no effect on corn and wheat yields.

The application of secondary and micronutrient fertilizers on soybeans after corn is not a common practice in Kansas, unless the area has had deficiencies or soil conditions prone to show deficiencies. The research shows that even when in low amounts of micronutrient level in soil and coarse textured soil, the fertilization of B, Cu, Mn, Zn and S (individually or applied together) had no effect on soybean yields. Zinc applied individually and in the broadcast blend increased Zn concentration in tissue and seed across locations. Across locations when applied in a band blend, Zn concentrations in seed had similar results as the control and significantly lower than Zn applied individually and broadcast blend. The seed quality is a subject that has been taking more importance. The results showing soybean with higher contents of Zn is of vast relevance for agronomic biofortification approaches. The fluid band blend could have had unfavorable complexations and interactions as opposed to the broadcast blend which was physically granulated and applied broadly not necessarily having all micronutrient in one same

spot. Micronutrient range between sufficiency and toxicity is narrow and special considerations must be taken prior the application of fertilizers. The soil collected post-harvest indicated that the broadcast blend increased significantly the amount of B, Cu, and Zn compared to the natural occurring, untreated soil. Future studies must evaluate and reveal the effects of the residual micronutrient fertilizers in soil, whether if complexations occurred, availability increased or toxicity could happen.

Due to the low reliability on micronutrient soil test, accounting soil variabilities such as pH and organic matter on large scale plots helped improve the proportion of variation explained about the effect of micronutrient effect on soybean tissue. Published nutrient sufficiency ranges (NSRs) are in discrepancy to results; no visual deficiency symptoms were encountered even when soil Cu content in soybean trifoliate was supposed to be below published sufficiency ranges. This published NSR may not be the best suited for the analysis of Cu in Kansas, therefore this ranges should be modified for local production. The application of the micronutrient fertilizer showed no increase on Cu concentration on soybean tissue. Tissue contents of Mn were higher with fertilization only when pH was below 5.5 and organic matter below 34 g kg^{-1} . Zinc in tissue was positively responsive over the untreated; B was not responsive on the site were pH averaged 5.5 and organic matter 29 g kg^{-1} .

In overall soybeans showed responsiveness mainly on Zn fertilization for seed, tissue and post-harvest analysis. Producers in Kansas will not benefit economically by fertilizing soybeans with B, Cu, Mn, S and Zn but major losses can happen if a deficiency of these nutrients appears. A proper nutrient management program is suggested to avoid unforeseen yield reductions due to S or micronutrients deficiencies.

These studies are a helpful approach to clear misleading information that circulates between commercial entities and local producers. Future studies should include: analyze micronutrients applied individually by band placement; try different soil test methods for all micronutrients; crop physiological aspects been studied such as seed per pod, pod per plant and seed weight; critical soil test values of micronutrients; nutrient sufficiency ranges for Kansas; and research analyzing the background of other crops to try to close gaps of missing information. Precautions must be taken if applying micronutrients as a blend, unfavorable interactions between Zn, Cu, and Mn may occur and high rates may lead to toxicities.

Appendix 1 – Description of the papers used for the summary in Chapter 2

Title	Author	Year	Source	Site
Zinc oxide and Zinc Sulfate as Zinc Sources for <i>Zea mays</i> and <i>Sorghum bicolor</i>	Patrick John Gallagher	1971	Thesis-KREX	Pottawatomie, Wallace, Pawnee, Sedgwick
Zinc Fertilizer Trials with Corn	-	1962	Kansas State Fertilizer Handbook 1962	Osborne, Smith
Trace Element Study at Three Central and Western Kansas Location	-	1964	Kansas State Fertilizer Handbook 1964	Scandia
Effect of Sulfur rate and Source on Early-Planted Short-seasoned Corn	G.M. Pierzynski, S. Glaze and R.E. Lamond	1994	Kansas Fertilizer Research 1994	Ashland
Sulfur on Early-Planted, Short season corn	R.E. Lamond	1991	Kansas Fertilizer Research 1991	Manhattan
Sulfur Fertilization of Corn	F.J. Wooding	1968	Kansas Fertilizer Handbook	Pottawatomie, Shawnee
Sulfur Fertilization effects on Irrigated Corn Yields and Leaf Composition	D. Buchholz, D.A. Whitney, R.E. Lamond, D. Leikam, K. Winter and M. Blocker	1978	Kansas Fertilizer Research Report of Progress 1978	Kiowa, Gray, Shawnee
Phosphorus-Zinc Fertilization of		1968	Kansas Fertilizer Handbook	Shawnee,

corn			1968	Pottawatomie
Sulfur Effects on Different Soybean Cultivars	G.Granade and D. Sweeney	1986	Kansas Fertilizer Progress 1986	Parsons
Soybean Fertilization Studies- Influence of Macronutrients and Micronutrient on Yields	L.W. Tobin	1970	Kansas Fertilizer Research Progress Report 1970	Cherokee
Zinc Experiment with Corn	-	1964	Kansas State Fertilizer Handbook 1964	Osborne, Shawnee and Pottawatomie
Effect of ACA, UAP, and Zinc Sulfate on Irrigated Corn	D. Whitney, L.Maddox and R.E. Lamond	1987	Kansas Fertilizer Research 1987	Rossville
Corn Hybrid Response to Starter Fertilizer combinations in a limited irrigation,ridge till production system	W.B. Gordon and G.M. Pierzynski	1996	Kansas Fertilizer Research 1996	Republic
Zinc Fertilization of Corn	-	1965	Kansas Fertilizer Handbook 1965	Shawnee, Pottawatomie and Osborne
Soybean Response to Application of Manganese and Zinc	N.Nelson, L.Maddox, M. David and A. Bontrager	2009	Kansas Fertilizer Research 2009	Ashland and Rossville
Phosphorus and sulfur nutrition on early corn/wheat rotation	M.Ashraf, G. Pierzynski and W. Gordon	1993	Kansas Fertilizer Research 1993	Norway and Rossville

Maximizing Irrigated Corn Yields in the Great Plains	W. Gordon	2005	Kansas Fertilizer Research 2005	Scandia
Responses of Corn Hybrid to Starter Fertilizer Combinations	W.B. Gordon and G.M. Pierzynski	1998	Kansas Fertilizer Research 1998	Scandia
Effects of Nitrogen, Phosphorus and Zinc on Yields of two Irrigated Corn Hybrids	B. Raney, G. TenEyck, D. Bonne and S. Clark	1969	Kansas Fertilizer Handbook 1969	Scandia
Evaluation of seven materials as sources for soybean (<i>Glycine Max L.</i>)	E. Salako	1975	Thesis-KREX	Pottawatomie, Pawnee
Varietal response and effects of different sources of zinc on soybean growth and yield	A. Bello	1977	Thesis-KREX	Greenhouse/Field
Plant responses to sulfur applications	J. Leiker	1970	Thesis-KREX	Field (Pottawotomie)
Effect of ACA, UAP and Zinc Sulfate on Grain sorghum	D. Whitney and R. Lamond	1987	Kansas Fertilizer Research 1987	Manhattan
Effect of Zinc, Manganese, and Copper on soil test Data, Grain Sorghum Yield and Leaf nutrient content	E. Beason	1970	Kansas Fertilizer Research Progress Report 1970	Southeast Kansas Branch Experiment Station

Influence of Macronutrients and Micronutrients on Yields of Dryland grain sorghum	L.W. Tobin	1970	Kansas Fertilizer Research Progress Report 1970	Sedgwick, Valley Center ,Andale and Elk
COOP Zn material Evaluations on Yield of Irrigated Corn and Grain Sorghum	Larry Murphy,Pat Gallagher and Jim Armbuster	1971	Kansas Fertilizer Research Report of Progress 1971	Geary and Sedgwick
Evaluation of Zn Materials for Irrigated Corn	Larry Murphy, Fred Meenen and Warren Miller	1971	Kansas Fertilizer Research Report of Progress 1971	Clay and St. Mary's
Effect of Macronutrients and Micronutrients on the Yield of Grain Sorghum in Osage and Washington Counties	L. Tobin	1971	Kansas Fertilizer Research Report of Progress 1971	Osage and Washington
Effect of Macronutrients and Micronutrients on the yield of Soybean in Johnson County	L.Tobin	1971	Kansas Fertilizer Research Report of Progress 1971	Johnson
Effect of application of sulfur micronutrient elements on yields	Roscoe Ellis,Jr.	1966	Kansas Fertilizer Handbook 1966	Butler
Effect of Zn when applied as a foliar fertilizer at Silk Stage to Corn Yield	J.D. Ball and G. TenEyck	1978	Kansas Fertilizer Research 1978	St. John
Effect of Zinc Rates and Sources on the Yield and composition of Irrigated Grain Sorghum	A.B. Bello, S. Schield,R. Lamond, P.J. Gallagher and L. Murphy	1976	Report of Progress Allied Chemical Corp	Sedgwick

Effect of Sulfur rate and sources on grain Sorghum	R. Lamond, G. TenEyck and R. Greenland	1989	Kansas Fertilizer Research	Stafford
Effects of Residual Sulfur on Double Crop Grain Sorghum	R. Lamond, D. Whitney,G. TenEyck and R. Greenland	1986	Kansas Fertilizer Research	Sandyland
Effects of Nitrogen,Phosphorus and Zinc on Irrigated Continous Grain Sorghum and Grain Sorghum Following Alfalfa	G. TenEyck and James Ball	1977	Kansas Fertilizer Research Report of Progress 1977	Stafford
Effects of Rates and Methods of Application of Zinc and Phosphorus on the Yield of Irrigated Grain Sorghum	Roscoe Ellis,Jr.	1968	Kansas Fertilizer Handbook	Sandyland
Effects of Potassium on Possible Phosphorus-Zinc Interactions in Irrigated Grain Sorghum	-	1968	Kansas Fertilizer Handbook	Sandyland
Effects of Zn Carriers and Phosphorus Carriers on Yield of Irrigated Grain Sorghum	Larry Murphy	1968	Kansas Fertilizer Handbook	Koehn Farm
Effects of Residual Sulfur on the Yield of Irrigated Grain Sorghum	Wallace Harris and Ronald Ibbetson	1968	Kansas Fertilizer Handbook	Colby
Zinc Fertilization of Grain Sorghum	B.G. Hopkins,D. Whitney, R. Lamond, V. Martin and	1994	Kansas Fertilizer Research	Ashland, Sandyland, Kansas River Valley

	L. Maddox			
Responses of Irrigated Grain Sorghum to Four Micronutrients	-	1965	Kansas Fertilizer Handbook	Roy Cudney
Zinc Fertilization on Wheat	R. Lamond and V. Martin	1994	Kansas Fertilizer Research 1994	Sandyland
Sulfur fertilization of Hard Red Winter Wheat	S.R. Duncan, R. Lamond, D. Whitney, G. McCirmack and J. Baker	1996	Kansas Fertilizer Research 1996	Barton, Cowley, Kingman and Pratt, Sandyland, Stafford
Effects of Sulphur Fertilization on Wheat Yields and Quality	R. Lamond, D. Whitney, L. Bonczkowski and J. Raney	1983	Kansas Fertilizer Research	Republic and Shawnee
Effects of Zinc on Wheat Yield and Grain Protein	L. Murphy, M. Claassen, R. Raney, R.E. Lamond and P. Gallagher	1977	Kansas Fertilizer Research Report of Progress	Belleville, Brown and Shawnee
Effect of Sulfur rates, sources and application times on wheat	R. Lamond, D. Whitney, L. Bonczkowski, R. Feyh, L. Maddox, G. TenEyck, G. Greenland, D. Mosieer and R. Wary, Jr	1988	Kansas Fertilizer Research	Cherokee, Morton, Shawnee, Stafford
Effects of Sulfur and Zinc Fertilization on Wheat	R. Lamond and D. Whitney	1986	Kansas Fertilizer Research	Gray and Shawnee