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MICRONUTRIENT FOLIAR ANALYSIS AND SUPPLEMENTATION IN NUTRIENT MANAGEMENT FOR HIGH YIELD MAIZE (ZEA MAYS L.)

by

Zachary P. Stewart

A DISSERTATION

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In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

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(Crop Physiology and Production / Soil Science)

Under the Supervision of Professors Charles A. Shapiro and Timothy M. Shaver

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MICRONUTRIENT FOLIAR ANALYSIS AND SUPPLEMENTATION IN NUTRIENT MANAGEMENT FOR HIGH YIELD MAIZE (ZEA MAYS L.)

Zachary P. Stewart, Ph.D.

University of Nebraska, 2016

Advisors: Charles A. Shapiro and Timothy M. Shaver

Micronutrient supplementation in maize production is of growing interest to producers and agronomists as means to further increase yield as other crop needs are increasingly met. Plant tissue and soil sampling for micronutrient concentrations have been used to determine likely responses to micronutrient supplementation. Nebraska soils are generally micronutrient sufficient and usually do not have soil or plant tissue micronutrient concentrations below critical levels, however, during precise periods where specific micronutrients are in greatest need due to physiological demands, there may be opportunity for micronutrient supplementation to increase grain yield. The compiled chapters indicate that in most scenarios in Nebraska, foliar micronutrient supplementation is not likely to result in increased maize yield, and yield reductions may occur with micronutrient supplementation. However, grain yield increases can occur with micronutrient supplementation even when soil or plant tissue micronutrient concentrations are above critical levels (Chapter 3 and 4). Models are needed that consider factors in addition to soil and plant micronutrient concentrations for improved prediction of maize yield responses to applied micronutrients (Chapter 2). Additionally, the following chapters provide recommendations on target growth stages for foliar-applied micronutrients (Chapter 4), opportunities for precision application technologies

with foliar-applied micronutrients in scenarios with confirmed micronutrient deficiency (Chapter 3, 4, and 6)), an assessment of soil and plant micronutrient correlations and their relationship with grain nutrient densities (i.e. biofortification) (Chapter 2 and 4), and opportunities for nanomaterials to improve the efficiency of foliar-applied micronutrients (Chapter 5).

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CHAPTER 1: A CONCEPTUAL FRAMEWORK OF PLANT TISSUE ANALYSIS FOR FOLIAR MICRONUTRIENT SUPPLEMENATION IN MAIZE PRODUCTION

1.1 Plant Tissue Analysis for Foliar Micronutrient Supplementation

Plant tissue analysis, shortened throughout to plant analysis, is used to diagnose plant nutrient status during the growing season, often complementing soil test results, to verify deficiency symptoms and to monitor nutrient levels during the growing season. This analysis provides the basis for determining if soil fertility levels and applied fertilizers are sufficient to meet crop nutritional needs (Mills et al., 1996).

The concept of plant analysis is built on Julius von Liebig and Carl Sprengel's "Law of the Minimum" in that plants grow to the limit imposed by the nutrient in least supply (van der Ploeg and Kirkham, 1999). Deficiency of any one of the essential plant nutrients can limit plant growth when other abiotic and biotic constraints are removed. Plant analysis makes use of this foundational concept by comparing the nutrient concentration of a particular plant part with established critical values or sufficiency ranges of the same plant species. This comparison of the nutrient content of the sampled plant and established critical values or sufficiency ranges is the basis for accessing the plant's nutrient status (Table 1-1). These values have been established for each crop based on the development of response curves, surveys, and experience. Critical values and sufficiency ranges have some overlap in interpretation but are different. Critical values are defined as the nutrient concentration at which there is a 5-10% yield reduction and is often associated with visual signs of deficiency, where as a sufficiency range is

defined as the range of nutrient concentration where there is no yield reduction due to either nutrient limitation or surplus. Often, there is no visual sign of deficiency at the lower value of the sufficiency range. By definition, the critical value is normally less than the lower level of the sufficiency range. This gap between the critical level and the lower value of the sufficiency range is where "hidden hunger" is proposed to occur (i.e. there is no visual sign of deficiency but still yield increase due to nutrient supplementation) (Marschner, 2012). However, other genetic, soil, cultural, and environmental factors have an influence on plant nutrient concentrations which may be considered, assuming adequate information, when interpreting plant analysis. Ratios of nutrient concentrations also have a significant effect on the nutritional status of plants. The most comprehensive application of ratios in the interpretation of plant analysis is the Diagnostic Recommendation Integration System (DRIS) (Walworth and Sumner, 1987). This system emphasizes nutrient concentration ratios rather than absolute concentrations alone.

Plant analysis involves sampling and sample preparation, laboratory analysis, and interpretation of results to provide a supplementation recommendation. An important aspect to interpreting plant analysis data is understanding how other factors influence on nutrient concentrations. Genetics, plant component and age, climate (light, temperature, rainfall, humidity), and soil properties (pH, soluble salts, moisture, temperature) have all been found to effect plant nutrient concentrations (Mills et al., 1996). Any one or combination of these factors may reduce the plant nutrient concentration even when there are adequate levels of that nutrient either available or unavailable in the soil. In practical terms, agronomic crops are most commonly constrained from reaching their genetic and environmental potential by the lack of nitrogen and water (Andrade et al., 2002).

However, as crops increasingly achieve sufficient levels of these and other agronomic inputs, micronutrients may become more likely to be the limiting growth factor and thus have attracted renewed interest.

1.2 Foliar Micronutrient Supplementation in Maize Production

There are 17 nutrients essential for plant growth and function. Eight are considered micronutrients i.e. boron, chlorine, copper, iron, manganese, molybdenum, nickel, and zinc. Boron, iron, manganese, and zinc are of most agronomic interest to maize production in Nebraska and were evaluated throughout the following studies (Chapter 2). Micronutrient foliar application is widely used in crop production, often to complement soil nutrient application (Fageria et al., 2009; Kannan, 2010). Plant leaves are specialized for capturing light and CO_2, but their ability to absorb certain nutrients has long been used in nutrient management (Fernández and Eichert, 2009; Gris, 1843). Foliar-applied micronutrients can penetrate leaves through the cuticle (solutes) or the stomata (gases and solutes) (Marschner, 2012). The effectiveness of foliar-applied micronutrient varies among plant species and also in relation to the chemical structure: salts, complexes, chelates, (Fernández and Ebert, 2005; Wojcik, 2004; Zhang and Brown, 1999a; Zhang and Brown, 1999b) or nanoparticles.

Soil properties can limit nutrient solubility and uptake by plant roots. For example, Fe, Mn, B, Cu, Ni, and Zn have relatively low availability in high pH, calcareous soils (Marschner, 2012). Thus, micronutrient foliar sprays are of general interest for use as tools to manage these nutrients and bypass soil limitations. Foliar

fertilization is frequently used because plant responses to foliar-applied micronutrients are normally more rapid than soil applications and generally have higher recovery rates compared to soil applications (Marschner, 2012). Therefore, foliar-applied micronutrients are of importance in nutrient correction within a given growing season. However, an unknown fraction of foliar-applied micronutrients are either sprayed directly onto the soil due to gaps in canopy cover or sprayed onto the foliage and subsequently washed off. In these circumstances, the foliar application can be effective, but soil-applied nutrients may also contribute to the micronutrient supplementation.

Recent research on foliar-applied micronutrients in maize production has reported mixed results (Heckman, 2002; Mueller and Diaz, 2011; Nelson and Meinhardt, 2011; Potarzycki and Grzebisz, 2009). Potarzycki and Grzebisz (2009) reported an increase in maize grain yield of nearly 18% (three-year average) with the application of 1.0 to 1.5 kg foliar Zn ha^{-1} in sandy, high P soils while many others report no yield increases in high-yielding situations. Responses have been most common in cases of confirmed micronutrient deficiency by soil or plant analysis prior to supplementation (i.e. deficiency correction theory). However, the effect of foliar micronutrients on maize grain yield in high yielding scenarios where there are sufficient soil concentrations and no confirmed micronutrient deficiency remains unclear and largely untested.

1.3 Research Justification

Bender et al. (2013) discussed the need to develop tools to better time nutrient applications to match each nutrient's uptake and mobilization characteristics especially

during periods of high vegetative uptake for high-yielding modern hybrids. For example, more than 70% of Zn uptake occurs during only one-third of the growing season, during late vegetative and early reproductive growth (Bender et al., 2013). About 65% of B uptake occurs during one-fifth of the growing season, during late vegetative and early reproductive growth (Bender et al., 2013). The increase in grain yield of modern hybrids has also been accompanied by an increase in total biomass yield (Hay, 1995; Lorenz et al., 2010.) This increase in biomass is the driving force for increased nutrient uptake and removal during harvest (Hanway, 1962a; Hanway, 1962b; Karlen et al., 1988; Karlen et al., 1987).

While Nebraska soils are generally fertile, maize has a high rate of nutrient uptake during the V4 to VT stage and demand may exceed supply. Demand exceeding supply may be especially important for less mobile micronutrients in that they may not be able to translocate in plant tissues to meet demands in other metabolically active plant tissues (Marschner, 2012). All these factors lead to our hypothesis that under high yield situations, even in field situations that do not indicate micronutrient deficiencies from soil or plant tissue sampling, there may be a yield response due to demand exceeding supply during key periods of high nutrient uptake as indicated by Bender et al. (2013).

As yield increases, producers are generally applying higher levels of macronutrients (N, P, K) which may increase the risk of a micronutrient being most limiting. Liebig's law of the minimum states that yield is proportional to the most limiting nutrient. For example, as sufficient levels of each of the macronutrients and all other constraints are being met, this increases the likelihood of a micronutrient deficiency being the yield limiting factor (Marschner, 2012). Additionally, increased plant growth

can induce low micronutrient concentrations due to dilution. As the plant increases in volume, the plant may have a lower concentration of the micronutrient even though the total micronutrient content has not changed or increased at a lower rate than the rate of volumetric increase (Jarrell and Beverly, 1981). Additionally, plants often respond to N and Zn together but not to Zn alone. The Zn deficiency is brought on by the increase in plant growth due to increased supplementation of N (Alloway, 2004).

Advances in maize yields have increased harvest removal of nutrients. Guidelines are needed to facilitate the efficient use of micronutrients. This issue is of general concern in the agricultural industry and has the support of agricultural laboratories, independent consultants, agronomists and extension educators. This research focuses on micronutrients and particularly locations that are high yielding and have a field history of soil tests and yield data that are likely to be responsive. On-farm strip trials complemented by small plot trials and greenhouse trials were designed and conducted to determine the effects of foliar application of nutrients and thereby obtain data for improving the interpretation of foliar results.

This project builds on past work conducted under different cropping systems and lower yield levels. The current NebGuide, Use and Management of Micronutrient Fertilizers in Nebraska, was originally written 30 years ago, and revised in 2013 (Wortmann et al., 2013). Research results from Nebraska during the past 30 years have largely confirmed these results. Current average irrigated yield is 100 bu ac^{-1} more than when previous work was conducted. In fact, previous determinations of critical levels (nutrient concentrations that indicate deficiency) in both plant and soil samples are also very old and may no longer be applicable. Also, growth stage, plant part, weather

conditions, and time of day of sampling affect foliar test results.

Interpretations of soil and foliar analysis differ between companies as they use very different criteria. Table 1-2 shows the challenge producers have in the interpretation of recommendations. The analysis data is from a V5 maize leaf sample collected in the spring of 2014 near Battle Creek, NE. Interpretations are listed from three anonymous laboratories, Company A, Company B, and Company C (Company C conducted the sampling). Note that the interpretation categories differ with Company A using deficient, responsive, adequate, and excessive; Company B using sufficient and deficient; and Company C using deficient, low-deficient, low, sufficient-low, sufficient, and high. Also, note the inconsistencies between companies in terms of whether a nutrient was sufficient or deficient. Companies B and C have no published recommendations for foliar nutrition but make recommendations on a case by case basis. The recommendations for Company A were specific products also sold by Company A (whose names would identify the company so were not included).

Testimonial yield increases from the application of micronutrients are usually associated with comparisons with multiple inputs versus a standard. For example, when fungicides, insecticides and high levels of macronutrients are applied with micronutrients, the specific source of any yield increase is difficult to determine as compared to applying the micronutrient treatment alone. Maize growers and their advisors need research-based information for better interpretation of foliar analyses to guide micronutrient application. At small yield increases, the value of maize and the cost of micronutrients make profitability variable, but if a high probability of success was documented, there would be wide spread adoption of the practice.

1.4 Research Goals

In order to address the concerns raised in the introduction, the following research plan was developed. Below articulates the research conducted to address these needs.

The primary focus of this research is to:

I. Literature Review → Develop a review of scientific research regarding the relationship between plant analysis and micronutrient supplementation in maize production and outline opportunities for future research (Chapter 1)

II. What is the micronutrient status in Nebraska and what are the key parameters involved in maize micronutrient status? → Survey sample maize production locations in the Nebraska for soil, plant tissue, and grain nutrient concentrations and yield to evaluate the micronutrient status of maize production locations relative to critical levels and explore relationships between nutrients in the soil, plant tissue, and maize grain with yield. These data will be valuable in determining micronutrients to target in subsequent yield response trials under similar field conditions and will provide insight into some of the key correlations between nutrient concentrations in soil, plant tissue, and grain samples which may aid in soil and plant tissue report interpretation (Chapter 2).

III. What is the effect of foliar micronutrient supplementation under current farmer-agronomist practices? → Evaluate the effect of foliar-applied

micronutrients on grain yield and plant tissue nutrient status under current farmer-agronomist practices using commercially available foliar micronutrient formulations (Chapter 3)

IV. What is the effect of foliar-applied micronutrient supplementation at different growth stages and different conditions? → Determine the effect of foliar-applied micronutrients on maize grain yield when applied at key growth stages (i.e. rapid nutrient uptake and demand) especially in circumstances where maize plants have low plant tissue or soil concentrations of the applied micronutrient but may not have a confirmed micronutrient deficiency (Chapter 3 and 4)

V. Does foliar micronutrient supplementation increase grain yield when there is no deficiency indicated by plant analysis? → Determine if there is a yield response during high uptake periods even without soil or plant samples indicating micronutrient deficiency based on established norms (Chapter 3 and 4)

VI. What is the fate of foliar-applied micronutrient supplementation? → Track the uptake, mobility, and partitioning of applied foliar micronutrients to determine the fate and recovery efficiency of the applied foliar micronutrients (Chapter 4)

VII. Do nanomaterials improve the effect of foliar micronutrient supplementation? → Compare the effect of foliar-applied Pheroid nanoparticles, chelate, and sulfate forms of Fe and Zn on biomass, nutrient uptake and mobilization in maize grown under Fe and Zn deficiency scenarios (Chapter 5)

VIII. Do foliar-applied micronutrient supplementations affect the nutrient composition of maize grain and do plant or soil micronutrient concentrations correlate with their concentration in grain? → Determine the effectiveness of foliar-applied micronutrients as a tool for the agronomic biofortification of maize which could have end use potential either for human or plant health goals (Chapter 2 and 4)

IX. What did this research teach us about foliar micronutrient supplementation and what are the opportunities for future research? → Develop objectives for future research where there is most promise for agronomic advances (Chapter 6)

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Table 1-1. Published critical nutrient concentrations and sufficiency ranges in maize (adapted from Escano et al. (1981)

Source	Growth Stage	N	P	K	Ca	Mg	S	Al	Mn	Fe	Cu	Zn	B	Mo
		----------%----------						----------mg kg^{-1}----------						
Mills and Jones (1996)	Plants <12" tall	3.50-5.00	0.30-0.50	2.50-4.00	0.30-0.70	0.15-0.45	0.15-0.50	-	20-300	50-250	5-20	20-60	5-25	0.10-10.00
Mills and Jones (1996)	Prior to tasseling	3.00-3.50	0.25-0.45	2.00-2.50	0.25-0.50	0.13-0.30	0.15-0.50	-	15-300	10-200	3-15	15-60	4-25	0.10-0.30
Mills and Jones (1996)	Initial Silk	2.70-4.00	0.25-0.50	1.70-3.00	0.21-1.00	0.20-1.00	0.21-0.50	-	20-200	20-250	6-20	25-100	5-25	0.10-0.20
Melsted et al (1969)	Initial Silk	3.0	0.25	1.9	0.40	0.25	-	-	15	15	5	15	-	-
Neubert et al. (1969)	Initial Silk	2.6-4.0	0.25-0.50	1.7-3.0	0.21-1.0	0.31-0.50	0.21-0.50	-	34-200	21-250	8-20	50-150	-	-
Jones (1967)	Initial Silk	2.8-3.5	0.25-0.40	1.7-2.5	0.21-1.0	0.21-0.60	-	200	20-150	21-250	6-20	20-70	-	-
Tyner (1946)	Initial Silk	2.9	0.30	1.3	-	-	-	-	-	-	-	-	-	-
Gallo et al (1968)	Initial Silk	2.9	0.23	1.7-2.7	-	-	-	-	-	-	-	-	-	-
Arnon (1975)	Initial Silk	3.1	0.33	1.7-2.0	-	-	-	-	-	-	-	-	-	-
Hanway and Dumenil (1965)	Initial Silk	3.2	0.34	1.7	-	-	-	-	-	-	-	-	-	-
Viets et al. (1954)	Initial Silk	2.8-2.9	-	-	-	-	-	-	-	-	-	-	-	-
Bennet et al. (1953)	Initial Silk	2.8-3.0	-	-	-	-	-	-	-	-	-	-	-	-
Loue (1963)	Initial Silk	-	-	1.7-2.0	-	-	-	-	-	-	-	-	-	-
Peaslee and Moss (1966)	Initial Silk	-	-	-	-	0.15	-	-	-	-	-	-	-	-
Grunes et al. (1963)	Initial Silk	-	-	-	-	-	-	-	-	-	-	15	-	-
Pumphrey et al. (1963)	Initial Silk	-	-	-	-	-	-	-	-	-	-	15	-	-
de L. Beyers (1969)	Initial Silk	-	-	-	-	-	-	-	-	-	-	17	-	-
Average Critical Value†	Initial Silk	3.02	0.29	1.63	0.00	0.20	-	200	15	15	5	15	-	-
Average Sufficeincy Range‡	Initial Silk	2.74-3.48	0.25-0.47	1.7-2.64	0.21-1.0	0.24-0.7	0.21-0.50	-	24.7-183	20.7-250	6.7-20	32-106	5-25	0.10-0.20

† The average maize nutrient concentration critical value at the initial silk growth stage.

‡ The average maize nutrient concentration sufficiency range at the initial silk growth stage.

Table 1-2. Example comparison of foliar analysis interpretation by three companies in Nebraska. 2015

Nutrient	Veg 5LS† Actual Analysis	Company A Interpretation	Company B Interpretation	Company C Interpretation
N	3.81%	Responsive	Sufficient	Sufficient-Low
P	0.25%	Deficient	Deficient	Deficient
K	3.83%	Adequate	Sufficient	High
Mg	0.16%	Deficient	Sufficient	Low
Ca	0.60%	Adequate	Sufficient	Sufficient
S	0.30%	Adequate	Sufficient	Sufficient
Fe	245 ppm	Excessive	Sufficient	High
Mn	64 ppm	Deficient	Sufficient	Low-Deficient
B	6 ppm	Deficient	Sufficient	Deficient
Cu	9 ppm	Adequate	Sufficient	Sufficient-Low
Zn	24 ppm	Deficient	Sufficient	Low-Deficient

† Vegetative 5 leaf stage

CHAPTER 2: A SURVEY OF SOIL, PLANT TISSUE, AND GRAIN NUTRIENT CONCENTRATIONS, AND MAIZE (ZEA MAYS L.) YIELD IN NEBRASKA AND IOWA

2.1 Abstract

In 2013 and 2014, 87 maize fields were sampled across eastern Nebraska and western Iowa to evaluate the micronutrient status of maize production locations relative to critical levels and explore relationships between nutrients in the soil, plant tissue, and maize grain with yield. Fields and sites within fields were randomly selected with no selection criteria except that the current crop was maize. All samples were taken within a 15 x 10 m area for soil, plant tissue, and grain nutrient concentrations and yield. There were 22, 30, 22, and 6% of locations with soil samples below critical levels of 20, 11, 0.5, and 0.75 mg kg^{-1} for P-Bray 1, S, B, and Zn, respectively. For plant tissue samples, 15, 2, 6, 27, 29, 11, 1, and 51% of locations were below critical levels of 27.0, 2.5, 17.0, 2.0, 2.1 g kg^{-1}, and 5, 6, 25 mg kg^{-1} for N, P, K, Mg, S, B, Cu, and Zn, respectively, at initial silk. Of these, S and Zn had 9 and 1%, respectively, of samples with both soil and plant concentrations below critical levels. Using Pearson's Method for correlation, soil K, Mg, Ca, S, and Mn each had significant positive correlations with their respective nutrient concentration in plant tissue samples. These data are suggestive that maize plants grown under similar conditions may increase uptake of each of these nutrients with increased soil supply. Soil NO_3, P, B, Cu, Fe, and Zn did not have significant correlations with their respective nutrient concentration in plant tissue. In respect to grain nutrient concentrations, all grain nutrient concentrations and grain protein concentration had negative correlations with grain yield, though there was a strong positive correlation of

nutrient uptake with grain yield. As yield increased, grain K, Mg, S, Fe, Mn, Zn and protein concentrations decreased while total nutrient uptake increased. Plant nutrient concentrations had little correlation with the corresponding nutrient concentrations in grain, however, soil extraction nutrient status of P, K, S, and Zn each had significant positive correlations with their corresponding nutrient concentration in grain. Stratification of soil samples from 0-10 cm and 10-20 cm revealed significant stratification of soil Zn concentration in the top 10 cm which had on average 1.9 mg kg^{-1} greater Zn concentration than the corresponding 10-20 cm soil depth.

Abbreviations: DRIS, Diagnosis and Recommendation Integration System; Buf, buffer index; OM, organic matter; CEC, cation exchange capacity; PB1, P Bray 1; PB2, P Bray 2; Pro, Crude Protein; VT, Vegetative Tassel; and R3: Reproductive Milk Stage

Keywords: Survey, Micronutrient, Plant Analysis, Soil Analysis, Interpretation, Grain Biofortification, Maize, Nebraska, Iowa

2.2 Introduction

Nebraska and Iowa soils are generally secondary and micronutrient sufficient with few deficiencies confirmed in maize production in the Western Corn Belt (Fixen et al., 2010; Wortmann et al., 2013). However, even in locations with sufficient levels of soil micronutrients, micronutrient concentrations in plant tissues may still have nutrient concentrations below critical levels which may limit grain yield (Mills et al., 1996). There are numerous soil-plant factors influencing nutrient movement and availability in agronomic systems and many remain unknown (Marschner, 2012). Developing a better understanding of these relationships under field conditions would be valuable to improve interpretations of soil and plant tissue laboratory reports to better predict yield response.

Many secondary nutrients and micronutrients are interrelated in their metabolic function and use similar rhizosphere transporters and therefore have known antagonistic relationships such as in the case of iron (Fe) and manganese (Mn) cations (Marschner, 2012). There is likely similar relationships with the movement of micronutrients from plant tissues to grains, but little is known about such mechanisms and their relationships with other plant tissue nutrients (Grusak et al., 1999; Pearson and Rengel, 1994). Grain Zn concentration can be low when the soil Zn availability is low (Cakmak, 2008; Yilmaz et al., 1998). These relationships would be valuable to understand since there is growing interest in grain biofortification both for improved seedling vigor and human health.

The objectives of this survey were to (i) evaluate maize production locations in the Western Corn Belt for soil, plant tissue, and grain nutrient concentrations and yield to assess the micronutrient status of maize production locations relative to critical levels, (ii) explore factors influencing micronutrient concentrations of soil, plant tissue, and grain

samples and their correlations with other soil, plant, and grain parameters and yield. These data will be valuable in determining secondary nutrients or micronutrients to target in subsequent yield response trials under similar field conditions. Additionally, these data will provide insight into some of the key correlations between nutrient concentrations in soil, plant tissue, and grain samples which may aid in soil and plant tissue report interpretation.

2.3 Materials and Methods

2.3.1 Study Sites

In the 2013-14 growing seasons, 87 maize producing field locations across central and eastern Nebraska and western Iowa were surveyed. Soil, plant tissue, grain samples and yield estimates were collected at each location from a 15 x 10 m area. Soil and plant tissue samples were collected during the field visit at the VT–R3 growth stage (Abendroth et al., 2011) and the grain and yield estimates were collected at physiological maturity from the same 15 x 10 m area. Sample locations were identified with the help of extension educators, local agronomists, and agricultural service laboratories. The maize fields were selected to avoid major constraints that would influence nutrient uptake such as excess or deficit soil water, insect or weed pressure, and wind or hail damage. Study sites had varying soil types, growing degree days, maize hybrids, topography, irrigation type and use, nutrient management, yield, crop rotation, tillage, and plant population but had no history of micronutrient or manure application in the previous 10 years. The sites

all had histories of high yields according to the producer. The locations were logged using GPS with the NutriSolution's mobile phone application (WinField Solutions®, Shoreview, MN) (https://play.google.com/store/apps/details?id=com.nutrisolutions.growthstages) and flagged in order to collect subsequent soil, leaf tissue, grain, and yield samples from the same location. The 87 samples locations were collected within the area no further North or South than 42°16'30"N to 40°12'30"N and no further East or West of 95°10'20"W to 99°05'00"W.

2.3.2 Plant Tissue Samples

Plant tissue samples were collected at the VT to R3 stages. The leaf collar method was used to stage the maize plants as described by Abendroth et al. (2011). These growth stages were of particular importance because they correspond with the period where maximum uptake of most nutrients has already occurred in plant tissues, and uptake has begun to plateau (Bender et al., 2013). Ten leaves were collected across the 15 x 10 m area and consolidated into one sample for the study site. The 15 leaves were collected below and opposite the ear node, and no more than one leaf sample was removed from a single plant (Westerman, 1990). At each stage, leaf samples were collected from plants that are representative of the surrounding crop and not lodged, damaged, or diseased. These tissue samples were put in paper bags and transported for laboratory analysis at Midwest Laboratories Omaha, NE within two days of collection where they were dried, ground, and analyzed. The laboratory analysis of leaf phosphorous (P), potassium (K),

sulfur (S), calcium (Ca), iron (Fe), manganese (Mn), zinc (Zn), and boron (B) were completed using microwave nitric acid digestion and concentrations were determined using inductively-coupled plasma emission spectroscopy (ICP-ES). The percent nitrogen (N) was determined using the Dumas Method with a Leco FP-428 (Horwitz and Latimer Jr, 1920).

Nutrient concentrations were compared to their corresponding critical levels as reported by Mills et al. (1996). It is important to note that for many nutrients, critical values that are published for other locations might be inappropriate for the location of interest, but there are few critical values published for most micronutrients in the target area beyond agricultural testing laboratory guidelines. In addition, critical levels can vary widely by source publication and should not be interpreted as absolutes but rather provide relative guidance (Chapter 1). For example, Neubert (1969) reports a Zn sufficiency range of 50-150 mg kg^{-1} and Mills et al. (1996) reports a Zn sufficiency range of 15-60 mg kg^{-1} for maize at initial silk.

2.3.3 Soil Samples

Soil samples were collected at the same time as the collection of the leaf samples (i.e. VT-R3) which was during August for most locations. Ten, 20 cm deep cores were collected using a hand probe (Oakfield Apparatus Company, 20 cm diameter) and composited into one sample from the 15 x 10 m area with the center of the soil sampling paired with the GPS coordinates. The 65 study locations were sampled from 0-10 cm and 10-20 cm depths, analyzed as stratified samples and were subsequently averaged and

combined for a 0-20 cm analysis so these location samples could be combined with 22 locations that were sampled from 0-20 cm depth. Samples were transported to Midwest Laboratories Omaha, NE within two days of collection for analysis. Laboratory procedures performed for each of the quantified nutrients and soil properties are listed in Table 2-1. Soil P was extracted using Bray 1 and 2 solutions. K, Mg, Ca, and S was extracted using (1N) ammonium acetate and detected with ICAP. Soil pH and buffer index was determined using a 1:1 soil: water mixture and a combination electrode. Cation exchange capacity (CEC) was calculated from the summation of cations (Ca, Mg, K, Na, and H) which were extracted with ammonium acetate saturation and displacement with NaCl and detection with distillation and titration. Organic matter was determined by loss of weight on ignition (LOI). Nitrate-N was extracted with a saturated CaO, cadmium reduction, and detected by segmental flow analysis (SFA). Copper, Fe, Mn, and Zn were extracted with DTPA and used ICAP detection. B was extracted using DTPA and detected by sorbitol ICAP.

2.3.4 Yield Samples

Yield samples were hand-harvested from 1/1000th of a hectare from each of the study sites as described by Lauer (2002). For 0.51 m row spacing, two rows of 9.8 m each were hand-harvested from within the 15 x 10 m collection area. For 0.76 m row spacing, one row of 13.1 m was hand harvested from within the 15 x 10 m collection site. Ears harvested from each of the study sites were shelled, weighed and grain moisture, and

test weights measured. Each yield sample was standardized to 155 g kg^{-1} water content and calculated for a kg ha^{-1} estimate.

2.3.5 Grain Samples

Grain was analyzed for mineral and crude protein (MWL FD PROC 70 which is based on AOAC 990.03) composition. For crude protein, samples were placed in a nitrogen combustion analyzer and the amount of nitrogen detected in a thermal conductivity cell which was quantified by comparison to a standard reference material of known nitrogen content. The N value was multiplied by a factor of 6.25 and that value reported as crude protein. For the quantification of P, K, S, Mg, Ca, sodium (Na), Fe, Mn, copper (Cu), and Zn the sample preparation followed MWL ME PROC 69 which was based on AOAC 935.13. The analysis of these data followed MWL ME PROC 29 which was based on AOAC 985.01. The sample was treated with a combination of heat and mineral acids to dissolve minerals and destroy organic materials. This extract was then introduced into the Inductively Coupled Argon Plasma (ICAP) emission spectrometer where energized plasma was produced. As the energized plasma cooled, specific wavelengths of light for each mineral were emitted. The intensity of the light was used to quantify each mineral concentration (Horwitz and Latimer Jr, 1920).

2.3.6 Data Analysis

Pearson correlation coefficients (r) and p-values for each pairwise correlation for plant x plant, soil x soil, soil x plant, grain x grain, soil x grain, plant x grain and yield x nutrient uptake parameters were computed using the sjt.corr function with R Statistical Software (R Development Core Team, 2015). The corrMethod=pearson (Appendix Code 2-1). The 65 of the 87 locations that had soil samples collected from 0-10 cm and 10-20 cm were averaged for a single 0-20 cm soil sample unit so the entire soil data set could be combined. Soil x plant correlations were plotted using the scatterplot function in Microsoft Excel (Microsoft Excel 2013, Microsoft Corp. Santa Rosa, CA). Critical plant tissue concentrations used in each graph were drawn from Mills et al. (1996) and critical soil concentrations were drawn from Nebraska or Iowa response functions where possible (Table 2-2) (Bordoli and Mallarino, 1998; Dodd and Mallarino, 2005; Mallarino and Blackmer, 1994; Shapiro et al., 2003; Ward, 2015; Wortmann et al., 2009).

2.4 Results and Discussion

2.4.1 Soil and Plant Tissue Nutrient Status

The mean yield was 14.4 Mg ha^{-1} (95% CI: 13.8-15.1 Mg ha^{-1}) expressed at 155 g kg^{-1} water content (Table 2-3). Of the 87 locations, 22, 30, 22, and 6% had soil samples below critical levels of 20, 11, 0.5, and 0.75 mg kg^{-1} for P-Bray 1, S, B, and Zn, respectively, (Figure 2-1; b, f, k, g) and 15, 2, 6, 27, 29, 11, 1, and 51% of locations with

plant tissue samples below critical levels of 27.0, 2.5, 17.0, 2.0, 2.1 g kg^{-1}, and 5, 6, 25 mg kg^{-1} for N, P, K, Mg, S, B, Cu, and Zn, respectively, at initial silk (Figure 2-1; a, b, c, d, f, k, j, g). Of these, S and Zn had 9 and 1%, respectively, of samples with both soil and plant concentrations below critical levels (Figure 2-1; f, g).

Soil K, Mg, and Cu availability was not low in all soil samples, but these nutrients were below their critical level in some foliar samples indicating possible extraneous factors limiting soil nutrient uptake into plant tissue or flaws in interpretation of foliar or soil test results (Figure 2-1; c, d, j). Plant tissue Zn concentrations were notably below critical levels with over 50% of the locations below a critical level of 25 mg kg^{-1} as reported by Mills et al. (1996) for maize at initial silk. These data are consistent with previous Nebraska field trials that has established a Zn critical level for maize (Shapiro et al., 2003; Wortmann et al., 2013).

Soil Zn was the only nutrient concentration that had clear stratification by soil depth with consistently higher Zn availability in upper 10 cm compared with the corresponding 10-20 cm sub-surface sample (Figure 2-2). The mean difference was 1.9 mg kg^{-1} (range: 0.2-8.8 mg kg^{-1} and standard deviation: 1.6 mg kg^{-1}). Our theory is that plant roots are likely drawing Zn throughout the 0-20 cm soil profile and after harvest, decomposing plant residue at the soil surface released Zn in the upper 0-10 cm of the soil profile. However, Zn is the most likely micronutrient applied to the 0-10 cm soil depth as a starter fertilizer which may have contributed to the stratification. All of the sampled locations practiced limited or no-till practices which likely further contributed to this stratification. Maize is commonly planted around 5 cm deep and thus, would likely be in soils during early rooting that have greater Zn concentration than what would be reported

in a 0-20 cm depth sample. Future research may be needed to correlate and calibrate Zn fertilizer recommendations with a 0-10 cm soil sample to evaluate more accurately soil available Zn concentration during early seedling development.

2.4.2 Soil, Plant, Grain, and Yield Nutrient Relationships

2.4.2.1 Soil, Plant, and Grain Nutrient Relationships with Yield

Numerous soil nutrient relationships have been reported to have significant effect on their nutrient status in plants, and in the case of plant nutrient ratios, have been widely evaluated with the Diagnosis and Recommendation Integration System (DRIS) (Mills et al., 1996; Walworth and Sumner, 1987). Our data support many of these already well-known relationships, is suggestive of others, and highlights those relationships that may influence grain yield.

Plant N, P, K, Na, and Cu concentrations each had significant positive correlations with grain yield (0.57, 0.55, 0.28, 0.53, 0.46, p= <0.001, <0.001, 0.01, <0.001, <0.001, respectively) whereas plant B and Fe had significant negative correlation with grain yield (-0.55, -0.25, p=<0.001, 0.03, respectively) (Table 2-4).

Soil Zn and B concentrations, pH, and buffer index each had significant positive correlations with grain yield (0.22, 0.28, 0.25, 0.26, p=0.05, 0.01, 0.02, 0.04, respectively) whereas soil organic matter (OM) (range: 22-42 g kg^{-1}, mean: 28 g kg^{-1}) and S concentration had significant negative correlations with grain yield (-0.51, -0.53, p<0.001, respectively) (Table 2-5). pH ranged from 4.5–8.2 with a mean pH of 6.0.

Under higher pH conditions, we would expect this relationship to switch to a negative correlation with grain yield. All grain nutrient concentrations and grain protein concentrations had negative correlations with grain yield. Grain K, Mg, S, Fe, Zn and protein concentrations each had significant negative correlations with grain yield (-0.43, -0.37, -0.44, -0.23, -0.22, -0.26, -0.34, p= <0.001, 0.001, <0.001, 0.04, 0.02, 0.002, respectively) (Table 2-6). As yield increases there is a decrease in the concentration of protein and mineral content. Total protein uptake in the grain also had a negative correlation with grain yield (-0.34, p=0.002). Inversely, all nutrient uptake except S had significant positive correlation with grain yield (P, K, Mg, S, Cu, Fe, Mn, Zn: 0.76, 0.65, 0.36, -0.08, 0.41, 0.53, 0.49, 0.23, p= <0.001, <0.001, 0.001, 0.47, <0.001, <0.001, <0.001, 0.04, respectively) as would be expected with increased grain biomass harvest (Table 2-7).

2.4.2.2 Plant Nutrient and Soil Property Relationships

There were several significant correlations identified between and among soil nutrient concentrations and plant nutrient concentrations which are reported in Table 2-4, Table 2-5, and Table 2-8. Of greatest interest was the correlations between soil nutrient concentrations and plant nutrient concentrations. Soil K, Mg, Ca, S, and Mn each had significant positive correlations with their respective nutrient concentration in plant tissue samples (0.28, 0.54, 0.52, 0.31, 0.63, p=0.01, <0.001, <0.001, 0.003, <0.001, respectively) (Table 2-8). These data are suggestive that maize plants grown under similar conditions may increase uptake of each of these nutrients with increased soil

supply. Soil NO_3, P, Ca, B, Cu, Fe, and Zn did not have significant correlations with their respective nutrient concentration in plant tissue at $p \leq 0.05$. Under similar conditions, these data suggest that it would be illogical to use only plant tissue analysis to predict soil availability of these nutrients and thus would not be advisable to view low plant tissue analysis as an indication of needed soil application of the corresponding nutrient.

As expected, soil pH had a significant negative correlation with plant Mn concentration (-0.59, $p<0.001$) and soil Mn concentration had a significant positive correlation with plant Mn concentration (0.63, $p<0.001$) (Table 2-8). Plant Mn also had a positive significant correlation with grain Mn (0.33, $p=0.002$) (Table 2-9), but soil Mn did not have a significant correlation with grain Mn at $p \leq 0.05$ (Table 2-10). These data suggest that Mn uptake from soil to plant tissue and from plant tissue to grain appears to have limited obstacles to overcome. Mn uptake does, however, have well-known antagonistic relationships with other nutrients and soil properties such as Fe and pH. An alternative explanation may be that maize plants and grains have greater demand for Mn and therefore readily increase Mn concentrations in plant tissues and grains when available.

2.4.2.3 Plant by Plant Nutrient Relationships

Plant N concentration had significant positive correlations with plant P, Mg, Ca, S, Na, Cu, and Zn concentration and yield (0.59, 0.23, 0.24, 0.32, 0.26, 0.72, 0.27, p= <0,001, 0.03, 0.03, 0.002, 0.02, <0.001, <0.001, respectively) (Table 2-4). Cu concentration in plant tissue had the strongest positive correlation (0.72, $p<0.001$) with N

concentration in plant tissue (Table 2-4). This strong positive correlation between plant Cu and plant N is consistent with past results indicating a catalyzing effect of Cu on plant metabolism thereby increasing nutrient uptake (Mills et al., 1996). However, soil Cu concentration had no significant correlation with plant N nor did soil nitrate concentration have any significant correlation with plant Cu (Table 2-8).

2.4.2.4 Soil by Soil Property Relationships

Several soil parameters had significant correlations with soil nutrient concentrations. Soil pH had highly significant negative correlations with soil Fe and Mn (-0.57, -0.79, $p<0.001$, respectively) which is well documented (Sarkar and Wynjones, 1982; SiMS, 1986). Inversely, soil pH had highly significant positive correlations with soil Mg, Ca, B and buffer index (0.37, 0.70, 0.71, 0.92, $p<0.001$, respectively). Buffer index is a measurement of a soil's ability to resist change in pH, therefore, this strong correlation was expected. Soil pH is well documented for its effect on the availability of soil nutrients. These data add to the body of literature by providing some indication of the magnitude and directionality of these correlations under field conditions which would aid in soil and plant analysis report interpretation. Soil cation exchange capacity (CEC) had significant positive correlations with soil OM, NO_3, P (Bray 2), Mg, Ca, S, B, Cu, Mn (0.32, 0.55, 0.38, 0.75, 0.66, 0.33, 0.24, 0.66, 0.22, $p= 0.003$, <0.001, <0.001, <0.001, <0.001, 0.002, 0.02, <0.001, 0.04, respectively) and had a significant negative correlation with buffer index (-0.45 $p<0.001$). CEC's positive correlation with cations was expected;

however, the strong correlations with anions NO_3^- and B, which is commonly in the soil profile as borate (BO_3^{3-}), was unexpected.

2.4.2.5 Grain by Grain and Plant by Grain Nutrient Relationships

Plant nutrient concentration had little correlation with the corresponding nutrient concentrations in grain. Only Mn had a positive correlation between plant Mn and grain Mn (0.33, p=0.002) (Table 2-9). Grain P concentration had significant positive correlation with grain K, Mg, Mn, and Zn concentrations (0.76, 0.76 0.40, 0.65, p<0.001, respectively) (Table 2-6). Of particular interest for grain biofortification, grain Zn concentration had significant positive correlations with grain P, K, Mg, S, Cu, Fe, Mn, and protein concentration (0.65, 0.60, 0.65, 0.33, 0.29, 0.49, 0.49, 0.23, p= <0.001, <0.001, <0.001, 0.002, 0.008, <0.001, <0.001, 0.03, respectively) (Table 2-6). These data suggest that altering the status of these nutrient concentrations in maize grain may also alter the status of Zn concentration in grain. Grain protein also had significant positive correlations with S, Fe, and Mn grain concentrations (0.45, 0.42, 0.58, p=<0.001, respectively) which also suggests altering the S, Fe, or Mn status of grain may alter the protein status of maize grain. There were few highly significant (p<0.001) plant nutrient concentrations correlations with grain nutrient concentrations. Plant P did have a highly significant negative correlation with grain K and S (-0.49, -0.40, p<0.001, respectively) (Table 2-9).

2.4.2.6 Soil by Grain Nutrient Relationships

Soil nutrient concentration of P (i.e. measured by Bray 1 and 2), K, S, and Zn each had positive correlations with their corresponding nutrient concentration in grain (0.55, 0.68, 0.24, 0.31, 0.33, $p<0.001$, <0.001, 0.03, 0.004, 0.002, respectively) (Table 2-10). Of particular significance, soil P, as measured by Bray 2, had highly significant correlations with grain P, K, Mg, and Zn (0.55, 0.56, 0.50, 0.38, $p<0.001$, respectively) (Table 2-10). Inversely, soil K had a highly significant correlation coefficient with grain P (0.38, $p<0.001$). Soil S also had highly significant correlations with grain P, K, Mg, S, and Zn (0.37, 0.44 0.55, 0.31, 0.41, $p=0.001$, <0.001, <0.001, 0.004, 0.004, respectively) (Table 2-10).

2.5 Conclusions

There were relatively few locations with soil and plant tissue samples below critical nutrient concentrations. However, P, S, and B each had greater than 10% of the sampled locations reporting soil concentrations below critical soil levels. N, Mg, S, Zn, and B each had greater than 10% of the sampled locations reporting plant tissue samples below critical levels (Figure 2-1; a, d, f, g, k). In particular, Zn had over 50% of the locations reporting plant tissue Zn concentrations below critical levels; however, Zn had only 6% of the sampled locations below critical soil concentrations (Figure 2-1; g). 5% of the locations were 10 mg kg^{-1} at or below the critical plant tissue Zn concentration of 25 mg kg^{-1} (Figure 2-1; g). S and Zn were the only nutrients with locations having both plant

tissue and soil samples below critical levels (i.e. 8 and 1%, respectively) (Figure 2-1; f, g). However, for most nutrients, low soil concentrations did not predict low plant concentrations. Each nutrient with samples below critical levels (i.e. P, S, Mg, Zn, B) is of particular interest for subsequent yield response trials under similar field conditions.

The survey protocol was not designed to determine if each site might be deficient. Locations with both soil and plant tissue nutrient concentrations below critical levels may be easiest to predict yield response; however, with timely in-season plant tissue testing, locations with only plant tissue samples below critical levels (i.e. Mg, S, Zn, B) may also be candidates for yield response to applications of the corresponding nutrient. This second scenario may be harder to predict yield response as there are likely numerous extraneous factors altering plant nutrient status throughout the growing season.

The significant ($p \leq 0.05$) correlation coefficient results have been summarized in Table 2-11 and have been organized by nutrient and parameter. These data provide insight into the numerous relationships between soil, plant, and grain nutrient concentrations and associated parameters and will be valuable for soil and plant tissue report interpretation. For an example of how these data are useful in soil and plant tissue laboratory report interpretation, instead of looking at a single plant or soil nutrient concentration status to determine if supplementation is needed, these data provide valuable information regarding other plant or soil parameters that should also be evaluated in addition to the nutrient of interest. If soil and plant tissue nutrient values do not correlate, then it would be inappropriate to assume that low plant nutrient concentrations indicate low soil concentrations and need for soil supplementation. Further, these data indicate which non-target nutrients or parameters may be influencing

the nutrient of interest. A well-known example of this occurs with Fe, Mn, and pH. Though there may be adequate soil Fe concentrations, high soil Mn or pH may be driving the low plant Fe concentrations. These correlations are not intended to be interpreted as causative but rather to aid in understanding nutrient relationships which may be driving nutrient concentrations in the plant and grain thereby influencing yield.

These data also highlight the challenge maize producers have in soil and plant tissue laboratory report interpretation. In many scenarios, soil nutrient concentrations do not correlate well with their nutrient concentration in plant tissues. Soil NO_3, P, Ca, B, Cu, Fe, and Zn did not have significant correlations with their respective nutrient concentration in plant tissue at $p \leq 0.05$. Under similar conditions, these data suggest that it would be illogical to use plant tissue analysis to predict soil availability of these nutrients and thus would not be advisable to view low testing plant tissue analysis as an indication of needed soil application of the corresponding nutrient. These data also add to the body of literature by providing some indication of the magnitude and directionality of these correlations under field conditions which would aid in soil and plant analysis report interpretation. As shown in this dataset, these nutrient relationships are driven by multiple factors, many of which were measured in this dataset but also many factors which were not included in this study (i.e. environmental, water, soil texture, plant genetics, etc.) that would have likely played a significant role and may have altered these relationships and is a significant limitation to this dataset. Further, higher order correlation tests, beyond first order, were not evaluated and thus, some relationships may have been overlooked.

There are many other significant correlations in this data set that were not discussed as the biology driving such relationships is not well known. However, these correlations may be beneficial for generating future research hypotheses.

Grain nutrient concentrations have been shown to be altered by soil and plant parameters (Cakmak, 2008; Chilimba et al., 2012; Rengel et al., 1999; White and Broadley, 2005; Yang et al., 2007). Of interest, our data showed a consistent negative relationship with grain yield and grain mineral and protein density which is consistent with the findings of Fan et al. (2008) in wheat grain (Table 2-6). Inversely, there was a consistent positive relationship with grain mineral uptake and grain yield (Table 2-7). As yield increased, total nutrient uptake increased (Chapter 4), however, these data show that the concentration of these nutrients in maize grain is negatively correlated with increasing grain yield (Table 2-6). This is suggestive that our breeding programs and nutrient management programs have been effective at increasing the total mass of maize grain production (i.e. likely due to increases in grain starches and saccharides) but have not been effective at maintaining mineral and protein concentrations.

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Table 2-1. Soil testing methodology as performed by Midwest Laboratories

Measured Nutrient/ Soil Property	Method	Source
Phosphorus (P1)	Extraction with dilute acid and ammonium fluoride (weak Bray)/colorimetric	NCR, p. 14-15
Phosphorous (P2)	Extraction with strong Bray solution (4 times the acid concentration of weak Bray)/colorimetric	NCR, p. 14-15
Potassium, Magnesium, Calcium, Sulfur	Neutral ammonium acetate (1 N) extraction/ Inductively Coupled Argon Plasma (ICAP) detection	RMST, p. 60-65 NCR, p.17-18
Soil pH, Buffer index	1:1 Soil:Water mixture/combination electrode	NCR, p. 5-8
Cation Exchange Capacity (CEC)	1. Summation of cations, Ca++, Mg++, K+, Na+, and H+ 2. Ammonium acetate saturation/displacement with NaCl/ distillation and titration	ASA, p. 149-151
Organic Matter	Loss of Weight on Ignition	NCR, p. 32
Nitrate-N	Saturated CaO Extraction/Cadmium Reduction/ Segmental Flow Analysis (SFA)	NCR, p. 11
Copper, Iron, Manganese, & Zinc	DPTA extraction/ICAP detection	NCR, p.18-19
Boron	DTPA/Sorbitol ICAP	NCR, p. 49-52

(Adapted from Midwest Laboratories) References: NCR: Recommended Chemical Soil Test Procedures for the North Central Region. No. 499, (Brown, 1998); ASA: Methods of Soil Analysis – Part 2: Chemical and Microbiological Properties, Second Edition, 1982. American Society of Agronomy, (Page, 1982); RMST: Handbook on Reference Methods for Soil Testing, 1974, Council on Soil Testing and Plant Analysis, (Kalra, 1997).

Table 2-2. Published critical plant tissue and soil nutrient concentrations and sufficiency ranges in maize

Source	Growth Stage	N	P	K	Ca	Mg	S	Mn	Fe	Cu	Zn	B	Mo
		g kg^{-1}						mg kg^{-1}					
Mills and Jones (1996)	Plants <0.3 m tall	35-50	3.0-5.0	25-40	3.0-7.0	1.5-4.5	1.5-5.0	20-300	50-250	5-20	20-60	5-25	0.10-10.00
Mills and Jones (1996)	Prior to tasseling	30-35	2.5-4.5	20-25	2.5-5.0	1.3-3.0	1.5-5.0	15-300	10-200	3-15	15-60	4-25	0.10-0.30
Mills and Jones (1996)	Initial Silk	27-40	2.5-5.0	17-30	2.1-10.0	2.0-10.0	2.1-5.0	20-200	20-250	6-20	25-100	5-25	0.10-0.20
		mg kg^{-1}											
			Bray 1	†	†	†	†	DTPA	0.1N HCl	0.1N HCl	DTPA	DTPA	
Shapiro (2003)	Soil	-	15	125	-	-	-	-	-	-	-	-	-
Wortman (2009)	Soil	-	15	125	-	-	8‡	-	-	-	-	-	-
Mallarino & Black (1992)	Soil	-	15-20	-	-	-	-	-	-	-	-	-	-
Bordi & Mallarino (1998)	Soil	-	12	112	-	-	-	-	-	-	-	-	-
Dodd & Mallarino (2005)	Soil		15-21	-	-	-	-	-	-	-	-	-	-
Ward (2015)	Soil	-	25	120	-	35	11	2.0	4.5	0.30	0.75	0.5	-
Voss (1998)	Soil	-	-	-	-	-	-	1.0	2.5	-	0.75	-	-

† Neutral ammonium acetate (1 N) extraction

‡ The critical sulfur level only applies to sandy soils with SOM less than 10 mg kg^{-1}. $Ca(H_2PO_4)_2$ extraction

Table 2-3. Descriptive statistics for all soil, plant (leaf tissue), and grain parameters for data collected from 87 locations in Nebraska and Iowa. Grain yield is expressed for 155 g kg^{-1} water content.

Parameter†	Mean	SD	Max.	Min.	Median	95% CI
Grain Yield, Mg ha^{-1}	14.4	3.0	20.8	6.0	16.15	(13.8-15.1)
Soil OM, g kg^{-1}	31.0	5.4	42.0	22.0	27.0	(29.8-32.1)
Soil NO^3, kg ha^{-1}	23.7	16.7	79.6	3.4	14.6	(20.2-27.2)
Soil P Bray 1, mg kg^{-1}	46.3	31.0	162.0	4.0	36.0	(39.8-52.8)
Soil P Bray 2, mg kg^{-1}	80.2	39.1	163.0	9.0	64.0	(72.0-88.4)
Soil K, mg kg^{-1}	325.2	109.4	784.0	174.0	278.0	(302.2-348.2)
Soil Mg, mg kg^{-1}	486.8	148.4	848.5	234.5	491.0	(455.7-518.0)
Soil Ca, mg kg^{-1}	2769	769	4635	1586	2667	(2607-2930)
Soil S, mg kg^{-1}	18.5	10.0	82.0	8.0	13.0	(16.4-20.6)
Soil B, mg kg^{-1}	0.6	0.2	1.1	0.3	0.6	(0.5-0.6)
Soil Cu, mg kg^{-1}	1.7	0.5	3.2	0.9	1.6	(1.6-1.9)
Soil Fe, mg kg^{-1}	67.5	24.1	187.0	18.0	63.5	(62.4-72.6)
Soil Mn, mg kg^{-1}	21.3	15.0	65.0	3.0	13.5	(18.2-24.5)
Soil Zn, mg kg^{-1}	2.2	1.6	9.8	0.8	2.0	(1.9-2.5)
Soil pH	5.9	0.7	8.2	4.5	6.1	(5.8-6.1)
Soil Buffer Index	6.4	0.3	6.8	5.7	6.6	(6.3-6.4)
Soil CEC, meq. 100 g^{-1}	23.7	4.6	33.9	14.7	22.9	(22.7-24.6)
Plant N, mg kg^{-1}	2.9	0.4	4.0	1.9	3.1	(2.9-3.0)
Plant P, mg kg^{-1}	0.3	0.1	0.5	0.2	0.3	(0.3-0.3)
Plant K, mg kg^{-1}	2.2	0.3	3.0	1.3	2.2	(2.2-2.3)
Plant Mg, mg kg^{-1}	0.2	0.1	0.5	0.1	0.2	(0.2-0.3)
Plant Ca, mg kg^{-1}	0.6	0.2	1.1	0.4	0.6	(0.6-0.6)
Plant S, mg kg^{-1}	0.2	0.0	0.3	0.2	0.2	(0.2-0.2)
Plant Na, mg kg^{-1}	0.007	0.007	0.023	0.001	0.006	(0.006-0.009)
Plant B, mg kg^{-1}	8.7	4.7	25.0	4.0	7.0	(7.7-9.7)
Plant Cu, mg kg^{-1}	10.0	2.1	17.0	4.0	10.0	(9.6-10.4)
Plant Fe, mg kg^{-1}	181.0	80.5	563.0	85.0	138.0	(164.1-198.0)
Plant Mn, mg kg^{-1}	80.6	26.4	163.0	47.0	71.0	(75.0-86.1)
Plant Zn,mg kg^{-1}	27.0	6.4	43.0	14.0	24.0	(25.7-28.4)
Grain P, g kg^{-1}	3.0	0.52	3.8	2.0	3.0	(2.9-3.1)
Grain K, g kg^{-1}	3.8	0.56	5.0	2.6	3.6	(3.7-3.9)
Grain Mg, g kg^{-1}	1.1	0.13	1.4	0.8	1.1	(1.1-1.1)
Grain S, g kg^{-1}	1.1	0.10	1.6	1.0	1.1	(1.1-1.2)
Grain Cu, mg kg^{-1}	2.8	0.8	6.0	1.6	2.8	(2.6-3.0)
Grain Fe, mg kg^{-1}	19.6	3.5	40.5	13.4	19.4	(18.8-20.3)
Grain Mn, mg kg^{-1}	5.2	1.1	9.0	3.2	5.3	(5.0-5.4)
Grain Zn, mg kg^{-1}	19.9	2.4	27.2	14.5	19.7	(19.3-20.4)
Grain Protein, g kg^{-1}	89.5	7.5	121.0	77.9	89.8	(87.9-91.1)
P Uptake‡, kg ha^{-1}	47.6	8.13	59.89	31.52	47.28	(45.8-49.3)
K Uptake, kg ha^{-1}	60.1	8.77	78.80	40.98	56.74	(58.2-61.9)
Mg Uptake, kg ha^{-1}	17.3	1.97	22.06	12.61	17.34	(16.9-17.7)
S Uptake, kg ha^{-1}	17.9	1.61	25.22	15.76	17.34	(17.5-18.2)
Cu Uptake, kg ha^{-1}	0.044	0.013	0.095	0.025	0.044	(0.041-0.047)
Fe Uptake, kg ha^{-1}	0.308	0.056	0.638	0.211	0.306	(0.296-0.320)
Mn Uptake, kg ha^{-1}	0.082	0.017	0.142	0.050	0.084	(0.078-0.085)
Zn Uptake, kg ha^{-1}	0.313	0.038	0.429	0.229	0.310	(0.305-0.321)
Protein Uptake, kg ha^{-1}	0.141	0.012	0.191	0.123	0.142	(0.139-0.144)

† OM, organic matter; CEC, cation exchange capacity

‡ Uptake calculated as elemental concentration multiplied by grain yield expressed as 0 g kg^{-1} water content

Table 2-4. Pearson correlation coefficients among plant nutrient concentrations and yield. Values directly below the correlation coefficients indicate p-values for each pairwise correlation. The horizontal and vertical labels indicate the nutrient chemical symbol of interest proceeded by a P indicating the source as plant leaf tissue. Non-chemical symbol key: YIELD=kg ha^{-1} at 155 g kg^{-1} water content.

	PN	PP	PK	PMg	PCa	PS	PNa	PB	PCu	PFe	PMn	PZn	YIELD
PN		0.59	0.03	0.23	0.24	0.32	0.26	-0.01	0.72	-0.18	0.04	0.27	0.57
		(<.001)	*0.80*	*0.03*	*0.03*	*0.002*	*0.02*	*0.95*	*(<.001)*	*0.09*	*0.75*	*0.01*	*(<.001)*
PP	0.59		0.13	0.26	0.32	0.02	0.31	-0.15	0.40	-0.12	-0.10	-0.02	0.55
	(<.001)		*0.24*	*0.02*	*0.003*	*0.84*	*0.004*	*0.18*	*(<.001)*	*0.29*	*0.38*	*0.85*	*(<.001)*
PK	0.03	0.13		-0.25	-0.40	-0.21	0.08	-0.21	0.14	-0.02	-0.19	0.24	0.28
	0.80	*0.24*		*0.02*	*(<.001)*	*0.06*	*0.48*	*0.05*	*0.19*	*0.83*	*0.09*	*0.02*	*0.01*
PMg	0.23	0.26	-0.25		0.51	-0.01	0.13	0.12	0.22	-0.10	-0.03	-0.15	0.02
	0.03	*0.02*	*0.02*		*(<.001)*	*0.95*	*0.23*	*0.28*	*0.04*	*0.35*	*0.82*	*0.17*	*0.85*
PCa	0.24	0.32	-0.40	0.51		0.43	0.33	-0.11	0.25	0.10	0.23	-0.22	0.12
	0.03	*0.003*	*(<.001)*	*(<.001)*		*(<.001)*	*0.002*	*0.30*	*0.02*	*0.37*	*0.03*	*0.04*	*0.27*
PS	0.32	0.02	-0.21	-0.01	0.43		0.08	0.10	0.21	0.004	0.02	0.02	0.09
	0.002	*0.84*	*0.06*	*0.95*	*(<.001)*		*0.49*	*0.36*	*0.05*	*0.97*	*0.87*	*0.88*	*0.44*
PNa	0.26	0.31	0.08	0.13	0.33	0.08		-0.56	0.20	-0.31	-0.09	-0.05	0.53
	0.02	*0.004*	*0.48*	*0.23*	*0.002*	*0.49*		*(<.001)*	*0.07*	*0.004*	*0.39*	*0.62*	*(<.001)*
PB	-0.01	-0.15	-0.21	0.12	-0.11	0.10	-0.56		-0.05	0.05	0.05	0.15	-0.55
	0.95	*0.18*	*0.05*	*0.28*	*0.30*	*0.36*	*(<.001)*		*0.65*	*0.67*	*0.66*	*0.17*	*(<.001)*
PCu	0.72	0.40	0.14	0.22	0.25	0.21	0.20	-0.05		0.002	0.25	0.45	0.46
	(<.001)	*(<.001)*	*0.19*	*0.04*	*0.02*	*0.05*	*0.07*	*0.65*		*0.99*	*0.02*	*(<.001)*	*(<.001)*
PFe	-0.18	-0.12	-0.02	-0.10	0.10	0.004	-0.31	0.05	0.002		0.40	0.23	-0.25
	0.09	*0.29*	*0.83*	*0.35*	*0.37*	*0.97*	*0.004*	*0.67*	*0.99*		*(<.001)*	*0.04*	*0.03*
PMn	0.04	-0.10	-0.19	-0.03	0.23	0.02	-0.09	0.05	0.25	0.40		0.09	0.21
	0.75	*0.38*	*0.09*	*0.82*	*0.03*	*0.87*	*0.39*	*0.66*	*0.02*	*(<.001)*		*0.41*	*0.06*
PZn	0.27	-0.02	0.24	-0.15	-0.22	0.02	-0.05	0.15	0.45	0.23	0.09		0.11
	0.010	*0.854*	*0.02*	*0.17*	*0.04*	*0.88*	*0.62*	*0.17*	*(<.001)*	*0.04*	*0.41*		*0.31*

Table 2-5. Pearson correlation coefficients among soil variables. Values directly below the correlation coefficients indicate p-values for each pairwise correlation. The horizontal and vertical labels indicate the nutrient chemical symbol or parameter of interest proceeded by an S indicating the source as soil. Non-chemical symbol key: Buf=buffer index, PB1=phosphorous Bray 1, OM=organic matter, PB2=phosphorous Bray 2, CEC=cation exchange capacity, YIELD=kg ha^{-1} at 155 g kg^{-1} water content.

	SOM	SNO_3	SPB1	SPB2	SK	SMg	SCa	SS	SB	SCu	SFe	SMn	SZn	SpH	SBuf	SCEC	YIELD
SOM		0.41	0.26	0.35	0.13	0.004	0.02	0.51	-0.19	0.24	0.25	0.32	0.03	-0.28	-0.43	0.32	-0.51
		(<.001)	*0.01*	*0.001*	*0.22*	*0.97*	*0.89*	*(<.001)*	*0.09*	*0.03*	*0.02*	*0.00*	*0.80*	*0.01*	*(<.001)*	*0.003*	*(<.001)*
SNO_3	0.41		0.23	0.40	-0.05	0.27	0.28	0.33	0.14	0.41	0.14	0.26	0.17	-0.10	-0.27	0.55	-0.17
	(<.001)		*0.04*	*(<.001)*	*0.65*	*0.01*	*0.01*	*0.002*	*0.19*	*(<.001)*	*0.21*	*0.01*	*0.12*	*0.36*	*0.03*	*(<.001)*	*0.13*
SPB1	0.26	0.23		0.82	0.50	-0.23	-0.22	0.12	-0.01	0.28	0.52	0.47	0.47	-0.33	-0.56	0.12	-0.14
	0.01	*0.04*		*(<.001)*	*(<.001)*	*0.03*	*0.04*	*0.29*	*0.96*	*0.01*	*(<.001)*	*(<.001)*	*(<.001)*	*0.002*	*(<.001)*	*0.27*	*0.20*
SPB2	0.35	0.40	0.82		0.33	0.11	0.16	0.39	0.17	0.53	0.29	0.34	0.34	-0.09	-0.44	0.38	-0.20
	0.00	*(<.001)*	*(<.001)*		*0.002*	*0.32*	*0.14*	*(<.001)*	*0.11*	*(<.001)*	*0.01*	*0.001*	*0.001*	*0.43*	*(<.001)*	*(<.001)*	*0.07*
SK	0.13	-0.05	0.50	0.33		-0.22	-0.19	0.07	0.14	0.04	0.35	-0.01	0.52	0.01	0.02	-0.20	0.01
	0.22	*0.65*	*(<.001)*	*0.002*		*0.04*	*0.08*	*0.51*	*0.19*	*0.69*	*0.001*	*0.92*	*(<.001)*	*0.95*	*0.89*	*0.06*	*0.94*
SMg	0.004	0.27	-0.23	0.11	-0.22		0.82	0.23	0.49	0.67	-0.48	-0.26	-0.11	0.37	0.24	0.75	-0.04
	0.97	*0.01*	*0.03*	*0.32*	*0.04*		*(<.001)*	*0.03*	*(<.001)*	*(<.001)*	*(<.001)*	*0.014*	*0.30*	*(<.001)*	*0.05*	*(<.001)*	*0.73*
SCa	0.02	0.28	-0.22	0.16	-0.19	0.82		0.23	0.69	0.68	-0.54	-0.48	0.04	0.70	0.46	0.66	0.05
	0.89	*0.01*	*0.04*	*0.14*	*0.08*	*(<.001)*		*0.03*	*(<.001)*	*(<.001)*	*(<.001)*	*(<.001)*	*0.72*	*(<.001)*	*(<.001)*	*(<.001)*	*0.65*
SS	0.51	0.33	0.12	0.39	0.07	0.23	0.23		-0.06	0.35	0.12	0.02	-0.05	-0.07	-0.06	0.33	-0.53
	(<.001)	*0.002*	*0.29*	*(<.001)*	*0.51*	*0.03*	*0.03*		*0.60*	*0.001*	*0.27*	*0.86*	*0.67*	*0.55*	*0.62*	*0.002*	*(<.001)*
SB	-0.19	0.14	-0.01	0.17	0.14	0.49	0.69	-0.06		0.56	-0.30	-0.44	0.42	0.71	0.55	0.24	0.28
	0.09	*0.19*	*0.96*	*0.11*	*0.19*	*(<.001)*	*(<.001)*	*0.60*		*(<.001)*	*0.004*	*(<.001)*	*(<.001)*	*(<.001)*	*(<.001)*	*0.02*	*0.01*
SCu	0.24	0.41	0.28	0.53	0.04	0.67	0.68	0.35	0.56		-0.07	-0.10	0.33	0.24	0.08	0.66	0.03
	0.03	*(<.001)*	*0.01*	*(<.001)*	*0.69*	*(<.001)*	*(<.001)*	*0.001*	*(<.001)*		*0.51*	*0.36*	*(<.001)*	*0.03*	*0.50*	*(<.001)*	*0.83*
SFe	0.25	0.14	0.52	0.29	0.35	-0.48	-0.54	0.12	-0.30	-0.07		0.44	0.15	-0.59	-0.32	-0.20	-0.11
	0.02	*0.21*	*(<.001)*	*0.01*	*0.001*	*(<.001)*	*(<.001)*	*0.27*	*0.004*	*0.51*		*(<.001)*	*0.16*	*(<.001)*	*0.01*	*0.06*	*0.34*
SMn	0.32	0.26	0.47	0.34	-0.01	-0.26	-0.48	0.02	-0.44	-0.10	0.44		-0.04	-0.79	-0.88	0.22	-0.14
	0.002	*0.01*	*(<.001)*	*0.001*	*0.92*	*0.01*	*(<.001)*	*0.86*	*(<.001)*	*0.36*	*(<.001)*		*0.72*	*(<.001)*	*(<.001)*	*0.04*	*0.21*
SZn	0.03	0.17	0.47	0.34	0.52	-0.11	0.04	-0.05	0.42	0.33	0.15	-0.04		0.17	0.20	-0.11	0.22
	0.80	*0.12*	*(<.001)*	*0.001*	*(<.001)*	*0.30*	*0.72*	*0.67*	*(<.001)*	*0.002*	*0.16*	*0.72*		*0.12*	*0.11*	*0.31*	*0.05*
SpH	-0.28	-0.10	-0.33	-0.09	0.01	0.37	0.70	-0.07	0.71	0.24	-0.59	-0.79	0.17		0.92	-0.02	0.25
	0.01	*0.36*	*0.002*	*0.43*	*0.95*	*(<.001)*	*(<.001)*	*0.55*	*(<.001)*	*0.03*	*(<.001)*	*(<.001)*	*0.12*		*(<.001)*	*0.84*	*0.02*
SBuf	-0.43	-0.27	-0.56	-0.44	0.02	0.24	0.46	-0.06	0.55	0.08	-0.32	-0.88	0.20	0.92		-0.45	0.26
	(<.001)	*0.03*	*(<.001)*	*(<.001)*	*0.89*	*0.05*	*(<.001)*	*0.62*	*(<.001)*	*0.50*	*0.01*	*(<.001)*	*0.11*	*(<.001)*		*(<.001)*	*0.04*
SCEC	0.32	0.55	0.12	0.38	-0.20	0.75	0.66	0.33	0.24	0.66	-0.20	0.22	-0.11	-0.02	-0.45		-0.20
	0.003	*(<.001)*	*0.27*	*(<.001)*	*0.06*	*(<.001)*	*(<.001)*	*0.00*	*0.02*	*(<.001)*	*0.06*	*0.04*	*0.31*	*0.84*	*(<.001)*		*0.07*

Table 2-6. Correlation coefficients among grain variables. Coefficients were computed using the Pearson Method. Values directly below the correlation coefficients indicate p-values for each pairwise correlation. The horizontal and vertical labels indicate the nutrient chemical symbol or parameter of interest proceeded by a G indicating the source as grain. Non-chemical symbol key: Pro=protein, YIELD=kg ha^{-1} at 155 g kg^{-1} water content.

	GP	GK	GMg	GS	GCu	GFe	GMn	GZn	GPro	YIELD
GP		0.76	0.76	0.17	0.21	0.21	0.40	0.65	0.18	-0.20
		(<.001)	*(<.001)*	*0.12*	*0.06*	*0.06*	*(<.001)*	*(<.001)*	*0.10*	*0.07*
GK	0.76		0.65	0.30	0.13	0.29	0.35	0.60	0.07	-0.43
	(<.001)		*(<.001)*	*0.01*	*0.24*	*0.01*	*0.001*	*(<.001)*	*0.55*	*(<.001)*
GMg	0.76	0.65		0.36	0.23	0.30	0.46	0.65	0.15	-0.37
	(<.001)	*(<.001)*		*0.001*	*0.04*	*0.01*	*(<.001)*	*(<.001)*	*0.19*	*0.001*
GS	0.17	0.30	0.36		-0.08	0.31	0.40	0.33	0.45	-0.44
	0.12	*0.01*	*0.001*		*0.47*	*0.004*	*(<.001)*	*0.002*	*(<.001)*	*(<.001)*
GCu	0.21	0.13	0.23	-0.08		0.41	0.43	0.29	0.14	-0.05
	0.06	*0.24*	*0.04*	*0.47*		*(<.001)*	*(<.001)*	*0.01*	*0.21*	*0.63*
GFe	0.21	0.29	0.30	0.31	0.41		0.53	0.49	0.42	-0.23
	0.06	*0.01*	*0.01*	*0.004*	*(<.001)*		*(<.001)*	*(<.001)*	*(<.001)*	*0.04*
GMn	0.40	0.35	0.46	0.40	0.43	0.53		0.49	0.58	0.22
	(<.001)	*0.001*	*(<.001)*	*(<.001)*	*(<.001)*	*(<.001)*		*(<.001)*	*(<.001)*	*0.05*
GZn	0.65	0.60	0.65	0.33	0.29	0.49	0.49		0.23	-0.26
	(<.001)	*(<.001)*	*(<.001)*	*0.002*	*0.01*	*(<.001)*	*(<.001)*		*0.03*	*0.02*
GPro	0.18	0.07	0.15	0.45	0.14	0.42	0.58	0.23		-0.34
	0.10	*0.55*	*0.19*	*(<.001)*	*0.21*	*(<.001)*	*(<.001)*	*0.03*		*0.002*

Table 2-7. Correlation coefficients among grain yield among grain nutrient uptake. Coefficients were computed using the Pearson Method. Values directly below the correlation coefficients indicate p-values for each pairwise correlation. The horizontal and vertical labels indicate the nutrient chemical symbol or parameter of interest. Parameters proceeded by a G indicating the source as grain. Non-chemical symbol key: Pro=protein, YIELD=kg ha^{-1} at 0 g kg^{-1} water content.

	GP Uptake	GK Uptake	GMg Uptake	GS Uptake	GCu Uptake	GFe Uptake	GMn Uptake	GZn Uptake	GPro Uptake
YIELD	0.76	0.65	0.36	-0.08	0.409	0.53	0.49	0.23	-0.34
	(<.001)	*(<.001)*	*0.001*	*0.47*	*(<.001)*	*(<.001)*	*(<.001)*	*0.04*	*0.002*

Table 2-8. Correlation coefficients among plant tissue leaf nutrients and soil variables. Coefficients were computed using the Pearson Method. Values directly below the correlation coefficients indicate p-values for each pairwise correlation. The horizontal and vertical labels indicate the nutrient chemical symbol or parameter of interest proceeded by an S or P indicating the source as soil or plant tissue, respectively. Non-chemical symbol key: Buf=buffer index, PB1=phosphorous Bray 1, OM=organic matter, PB2=phosphorous Bray 2, CEC=cation exchange capacity.

	SOM	SNO_3	SPB1	SPB2	SK	SMg	SCa	SS	SB	SCu	SFe	SMn	SZn	SpH	SBuf	SCEC
PN	-0.36	0.06	-0.18	-0.19	-0.08	0.09	0.16	-0.42	0.31	0.14	-0.12	-0.21	0.13	0.21	0.28	-0.01
	0.001	*0.57*	*0.10*	*0.08*	*0.44*	*0.39*	*0.13*	*(<.001)*	*0.004*	*0.21*	*0.28*	*0.05*	*0.23*	*0.05*	*0.02*	*0.94*
PP	-0.32	-0.07	0.06	-0.04	0.004	0.04	0.14	-0.36	0.38	0.21	0.001	-0.08	0.39	0.21	0.21	-0.04
	0.003	*0.54*	*0.55*	*0.72*	*0.97*	*0.69*	*0.18*	*0.001*	*(<.001)*	*0.05*	*0.99*	*0.45*	*(<.001)*	*0.05*	*0.10*	*0.71*
PK	-0.15	-0.20	0.13	-0.01	0.28	-0.37	-0.36	-0.30	-0.17	-0.31	0.28	0.10	0.03	-0.14	-0.10	-0.36
	0.17	*0.07*	*0.24*	*0.93*	*0.01*	*(<.001)*	*0.001*	*0.01*	*0.13*	*0.003*	*0.01*	*0.34*	*0.81*	*0.21*	*0.43*	*0.001*
PMg	-0.05	0.17	-0.20	-0.04	-0.48	0.54	0.51	-0.05	0.26	0.28	-0.28	-0.12	-0.24	0.23	0.09	0.44
	0.65	*0.11*	*0.07*	*0.72*	*(<.001)*	*(<.001)*	*(<.001)*	*0.64*	*0.01*	*0.01*	*0.01*	*0.28*	*0.02*	*0.03*	*0.49*	*(<.001)*
PCa	0.22	0.38	-0.03	0.21	-0.08	0.44	0.52	0.23	0.53	0.53	-0.20	-0.07	0.28	0.32	0.14	0.39
	0.04	*(<.001)*	*0.78*	*0.06*	*0.47*	*(<.001)*	*(<.001)*	*0.03*	*(<.001)*	*(<.001)*	*0.07*	*0.51*	*0.01*	*0.003*	*0.26*	*(<.001)*
PS	0.16	0.19	-0.24	0.05	-0.11	0.28	0.40	0.31	0.24	0.40	-0.28	-0.35	0.04	0.32	0.31	0.19
	0.15	*0.08*	*0.03*	*0.62*	*0.30*	*0.01*	*(<.001)*	*0.003*	*0.03*	*(<.001)*	*0.01*	*(<.001)*	*0.72*	*0.003*	*0.01*	*0.09*
PNa	-0.45	-0.004	-0.19	-0.16	0.05	0.17	0.11	-0.23	0.34	0.02	-0.06	-0.14	0.20	0.23	0.34	-0.10
	(<.001)	*0.97*	*0.08*	*0.13*	*0.62*	*0.12*	*0.32*	*0.03*	*0.001*	*0.87*	*0.57*	*0.21*	*0.06*	*0.03*	*0.01*	*0.34*
PB	0.25	0.10	0.12	0.15	-0.14	0.08	0.14	0.26	-0.11	0.16	0.02	-0.07	-0.12	-0.05	-0.06	0.21
	0.02	*0.35*	*0.28*	*0.17*	*0.20*	*0.47*	*0.18*	*0.02*	*0.32*	*0.15*	*0.86*	*0.50*	*0.26*	*0.67*	*0.65*	*0.06*
PCu	-0.19	0.03	-0.23	-0.27	-0.19	0.03	-0.03	-0.42	0.02	-0.08	-0.13	0.10	-0.05	-0.08	-0.08	0.05
	0.07	*0.75*	*0.03*	*0.01*	*0.08*	*0.82*	*0.76*	*(<.001)*	*0.86*	*0.49*	*0.22*	*0.36*	*0.63*	*0.49*	*0.54*	*0.63*
PFe	0.43	0.13	0.13	0.11	0.11	-0.27	-0.20	0.19	-0.31	-0.17	0.04	0.22	-0.001	-0.14	-0.23	-0.07
	(<.001)	*0.25*	*0.24*	*0.30*	*0.30*	*0.01*	*0.07*	*0.08*	*0.004*	*0.11*	*0.73*	*0.04*	*1.00*	*0.19*	*0.07*	*0.52*
PMn	0.31	0.25	0.21	0.13	-0.02	-0.25	-0.35	0.18	-0.36	-0.05	0.32	0.63	-0.01	-0.59	-0.56	0.16
	0.004	*0.02*	*0.05*	*0.23*	*0.88*	*0.02*	*0.001*	*0.09*	*0.001*	*0.67*	*0.003*	*(<.001)*	*0.90*	*(<.001)*	*(<.001)*	*0.13*
PZn	0.02	0.05	-0.06	-0.14	-0.03	-0.22	-0.19	-0.22	-0.27	-0.26	-0.07	0.13	-0.13	-0.12	-0.24	-0.07
	0.87	*0.63*	*0.58*	*0.19*	*0.81*	*0.04*	*0.08*	*0.04*	*0.01*	*0.01*	*0.50*	*0.24*	*0.23*	*0.26*	*0.05*	*0.53*

Table 2-9. Correlation coefficients among plant and grain variables. Coefficients were computed using the Pearson Method. Values directly below the correlation coefficients indicate p-values for each pairwise correlation. The horizontal and vertical labels indicate the nutrient chemical symbol or parameter of interest proceeded by a P or G indicating the source as plant tissue or grain, respectively. Non-chemical symbol key: Pro=protein.

	GP	GK	GMg	GS	GCu	GFe	GMn	GZn	GPro
PN	-0.27	-0.37	-0.25	-0.22	0.02	0.13	-0.01	-0.12	0.01
	0.01	*0.001*	*0.02*	*0.05*	*0.85*	*0.26*	*0.96*	*0.29*	*0.96*
PP	-0.13	-0.49	-0.28	-0.40	0.12	-0.06	-0.23	-0.16	-0.01
	0.23	*(<.001)*	*0.01*	*(<.001)*	*0.27*	*0.57*	*0.04*	*0.14*	*0.94*
PK	0.08	0.03	-0.20	-0.31	-0.01	-0.08	0.001	-0.01	-0.04
	0.45	*0.78*	*0.08*	*0.004*	*0.91*	*0.46*	*0.99*	*0.93*	*0.74*
PMg	-0.26	-0.15	-0.19	-0.05	0.003	0.03	-0.31	-0.29	-0.01
	0.02	*0.19*	*0.08*	*0.65*	*0.98*	*0.81*	*0.004*	*0.01*	*0.95*
PCa	0.18	0.09	0.26	0.07	-0.02	0.08	-0.16	0.14	-0.12
	0.10	*0.40*	*0.02*	*0.53*	*0.87*	*0.49*	*0.14*	*0.22*	*0.30*
PS	0.05	0.004	0.30	0.19	-0.07	0.18	0.03	0.26	-0.14
	0.64	*0.97*	*0.01*	*0.08*	*0.50*	*0.10*	*0.78*	*0.02*	*0.21*
PNa	-0.17	-0.21	-0.32	-0.16	-0.19	-0.17	-0.17	-0.07	-0.22
	0.13	*0.06*	*0.003*	*0.16*	*0.09*	*0.12*	*0.14*	*0.54*	*0.05*
PB	0.04	0.20	0.28	0.20	0.06	0.31	0.22	0.17	0.27
	0.73	*0.07*	*0.01*	*0.07*	*0.60*	*0.01*	*0.05*	*0.13*	*0.02*
PCu	-0.21	-0.24	-0.25	-0.35	-0.01	-0.09	-0.11	-0.20	-0.18
	0.05	*0.03*	*0.02*	*0.001*	*0.96*	*0.42*	*0.33*	*0.07*	*0.11*
PFe	0.30	0.25	0.29	-0.08	0.30	0.05	0.11	0.16	0.10
	0.01	*0.02*	*0.01*	*0.49*	*0.01*	*0.67*	*0.35*	*0.15*	*0.35*
PMn	0.21	0.18	0.22	-0.08	0.20	0.04	0.33	0.07	0.10
	0.06	*0.11*	*0.04*	*0.46*	*0.07*	*0.74*	*0.002*	*0.55*	*0.35*
PZn	-0.10	0.01	-0.03	-0.23	0.02	-0.04	0.04	-0.07	-0.19
	0.38	*0.94*	*0.82*	*0.04*	*0.84*	*0.69*	*0.75*	*0.51*	*0.09*

Table 2-10. Correlation coefficients among soil and grain variables. Coefficients were computed using the Pearson Method. Values directly below the correlation coefficients indicate p-values for each pairwise correlation. The horizontal and vertical labels indicate the nutrient chemical symbol or parameter of interest proceeded by an S or G indicating the source as soil or grain, respectively. Non-chemical symbol key: Buf=buffer index, PB1=phosphorous Bray 1, OM=organic matter, PB2=phosphorous Bray 2, CEC=cation exchange capacity, Pro=protein.

	GP	GK	GMg	GS	GCu	GFe	GMn	GZn	GPro
SOM	0.33	0.48	0.50	0.14	0.19	0.19	0.07	0.24	-0.04
	0.002	*(<.001)*	*(<.001)*	*0.21*	*0.08*	*0.09*	*0.52*	*0.03*	*0.70*
SNO_3	0.16	0.26	0.23	0.12	0.16	0.23	0.01	0.23	0.04
	0.15	*0.02*	*0.04*	*0.28*	*0.15*	*0.04*	*0.94*	*0.04*	*0.75*
SPB1	0.55	0.36	0.29	-0.13	0.09	0.05	0.18	0.23	0.23
	(<.001)	*0.001*	*0.01*	*0.25*	*0.40*	*0.67*	*0.11*	*0.04*	*0.04*
SPB2	0.68	0.56	0.50	0.06	0.12	0.10	0.13	0.38	0.12
	(<.001)	*(<.001)*	*(<.001)*	*0.62*	*0.27*	*0.39*	*0.23*	*(<.001)*	*0.29*
SK	0.38	0.24	0.17	-0.01	0.04	0.17	0.27	0.23	0.34
	(<.001)	*0.03*	*0.12*	*0.91*	*0.70*	*0.12*	*0.01*	*0.04*	*0.002*
SMg	-0.12	0.02	0.03	0.25	-0.05	0.05	-0.23	-0.01	-0.06
	0.27	*0.89*	*0.78*	*0.02*	*0.68*	*0.63*	*0.04*	*0.91*	*0.60*
SCa	-0.02	0.05	0.14	0.34	-0.07	0.16	-0.18	0.12	-0.04
	0.86	*0.68*	*0.22*	*0.002*	*0.56*	*0.14*	*0.11*	*0.28*	*0.69*
SS	0.37	0.44	0.55	0.31	0.15	0.22	0.12	0.41	-0.02
	0.001	*(<.001)*	*(<.001)*	*0.004*	*0.17*	*0.05*	*0.27*	*(<.001)*	*0.86*
SB	0.01	-0.10	-0.01	0.25	-0.21	0.09	-0.13	0.12	0.11
	0.94	*0.38*	*0.92*	*0.03*	*0.06*	*0.43*	*0.23*	*0.30*	*0.33*
SCu	0.19	0.15	0.30	0.21	0.04	0.16	-0.05	0.22	-0.02
	0.09	*0.18*	*0.01*	*0.05*	*0.69*	*0.14*	*0.63*	*0.05*	*0.85*
SFe	0.23	0.20	0.06	-0.11	0.20	0.10	0.27	0.21	0.14
	0.04	*0.07*	*0.58*	*0.31*	*0.06*	*0.38*	*0.01*	*0.05*	*0.20*
SMn	0.27	0.23	0.11	-0.30	0.13	-0.23	0.02	-0.05	-0.08
	0.01	*0.04*	*0.32*	*0.01*	*0.24*	*0.04*	*0.86*	*0.68*	*0.48*
SZn	0.32	0.06	0.09	-0.14	0.07	0.19	0.08	0.33	0.15
	0.003	*0.58*	*0.42*	*0.22*	*0.51*	*0.08*	*0.46*	*0.002*	*0.17*
SpH	-0.06	-0.11	-0.01	0.27	-0.16	0.14	-0.14	0.06	0.03
	0.60	*0.32*	*0.93*	*0.01*	*0.14*	*0.22*	*0.21*	*0.61*	*0.83*
SBuf	-0.27	-0.25	-0.21	0.20	0.04	0.28	0.04	0.05	0.04
	0.03	*0.04*	*0.09*	*0.12*	*0.78*	*0.02*	*0.77*	*0.68*	*0.74*
SCEC	0.07	0.19	0.21	0.18	0.04	0.06	-0.11	0.06	-0.04
	0.55	*0.09*	*0.06*	*0.11*	*0.69*	*0.60*	*0.32*	*0.59*	*0.75*

Table 2-11. Summary of significant ($p<0.05$) correlation coefficients calculated using the Pearson Method organized by parameter

Parameter	Plant† x Plant‡	Plant† x Soil‡	Soil† x Soil‡	Grain† x Grain‡	Plant† x Grain‡	Soil† x Grain‡
N†	P‡, Mg, Ca, S, Na, Cu, Zn, Yield	-OM, -S, B, -Mn, pH, Buf	-	-	-P, -K, -Mg, -S,	-
P†	N, Mg, Ca, Na, Cu, Yield	-OM, -S, B, Zn, pH	-	K, Mg, Mn, Zn	-K, -Mg, -S, -Mn	-
K†	-Mg§, -Ca, Zn, Yield	K, -Mg, -Ca, -S, -Cu, Fe, -CEC	PB1, PB2, -Mg, Fe, Zn	P, Mg, S, Fe, Mn, Zn, -Yield	-S	P, K, Mn, Zn, Pro
Mg†	N, P, -K, Ca, Cu	-K, Mg, Ca, B, Cu, -Fe, -Zn, pH, CEC	NO_3, -PB1, -K, Ca, S, B, Cu, -Fe, -Mn, pH, CEC	P, K, S, Cu, Fe, Mn, Zn, -Yield	-P, -Mn, -Zn	S, -Mn
Ca†	N, P, -K, Mg, S, Na, Cu, Mn, -Zn	OM, NO_3, Mg, Ca, S, B, Cu, Zn, pH, CEC	NO_3, -PB1, Mg, S, B, Cu, -Fe, -Mn, pH, Buf, CEC	-	Mg	S
S†	N, Ca	-PB1, Mg, Ca, S, B, Cu, -Fe, -Mn, pH, Buf	OM, NO_3, PB2, Mg, Ca, Cu, CEC, -Yield	K, Mg, Fe, Mn, Zn, Pro, -Yield	Mg, Zn	P, K, Mg, S, Zn
Na†	N, P, Ca, -B, -Fe, Yield	-OM, -S, B, pH, Buf	-	-	-Mg, -Pro	-
B†	-Na, -Yield	OM, S	Mg, Ca, Cu, -Fe, -Mn, Zn, pH, Buf, CEC, Yield	-	Mg, Fe, Mn, Pro	S
Cu†	N, P, Mg, Ca, Mn, Zn, Yield	-PB1, -PB2, -S	OM, NO_3, PB1, PB2, Mg, Ca, S, B, Zn, pH, CEC	Mg, Fe, Mn, Zn	-K, -Mg, -S	Mg, Zn
Fe†	-Na, Mn, Zn, -Yield	OM, -Mg, -B, Mn	OM, PB1, PB2, K, -Mg, -Ca, -B, Mn, -pH, -Buf	K, Mg, S, Cu, Mn, Zn, Pro, -Yield	P, K, Mg, Cu,	P, Mn
Mn†	Ca, Cu, Fe	OM, NO_3, PB1, -Mg, -Ca, -B, Fe, Mn, -pH, -Buf	OM, NO_3, PB1, PB2, -Mg, -Ca, -B, Fe, -pH, -Buf, CEC	P, K, Mg, S, Cu, Fe, Zn, Pro, Yield	Mg, Mn	P, K, -S, -Fe
Zn†	N, K, -Ca, Cu, Fe	-Mg, -S, -B, -Cu	PB1, PB2, K, B, Cu, Yield	P, K, Mg, S, Cu, Fe, Mn, Pro, -Yield	-S	P, Zn
NO_3†	-	-	OM, PB1, PB2, Mg, Ca, S, Cu, Mn, -Buf, CEC	-	-	K, Mg, Fe, Zn
P-Bray 1†	-	-	OM, NO_3, PB2, K, -Mg, -Ca, Cu, Fe, Mn, Zn, -pH, -Buf	-	-	P, K, Mg, Zn, Pro
P-Bray 2†	-	-	OM, NO_3, PB1, K, S, Cu, Fe, Mn, Zn, -Buf, CEC	-	-	P, K, Mg, Zn
Protein†	-	-	-	S, Fe, Mn, Zn, -Yield	-	
SOM†	-	-	NO_3, PB1, PB2, S, Cu, Fe, Mn, -pH, -Buf, CEC, -Yield	-	-	P, K, Mg, Zn
pH†	-	-	-OM, -PB1, Mg, Ca, B, Cu, -Fe, -Mn, Buf, Yield	-	-	S
CEC†	-	-	OM, NO_3, PB2, Mg, Ca, S, B, Cu, Mn, -Buf	-	-	none
Buffer Index†	-	-	-OM, -NO_3, -PB1, -PB2, Ca, B, -Fe, -Mn, pH, CEC, Yield	-	-	-P, -K, Fe

† Parameters in the first column corresponds with the first value in each of the subsequent column headings

‡ Parameters in the table indicate significant correlation at $p \leq 0.05$ with the associated parameter in the first column. Yield, grain yield expressed for 155 g kg^{-1} water content; OM, organic matter; Buf, Buffer Index; PB1, P Bray 1; PB2, P Bray 2; Pro, Crude Protein

§ Parameter preceeded by (-) indicates a significant negative correlation coefficient

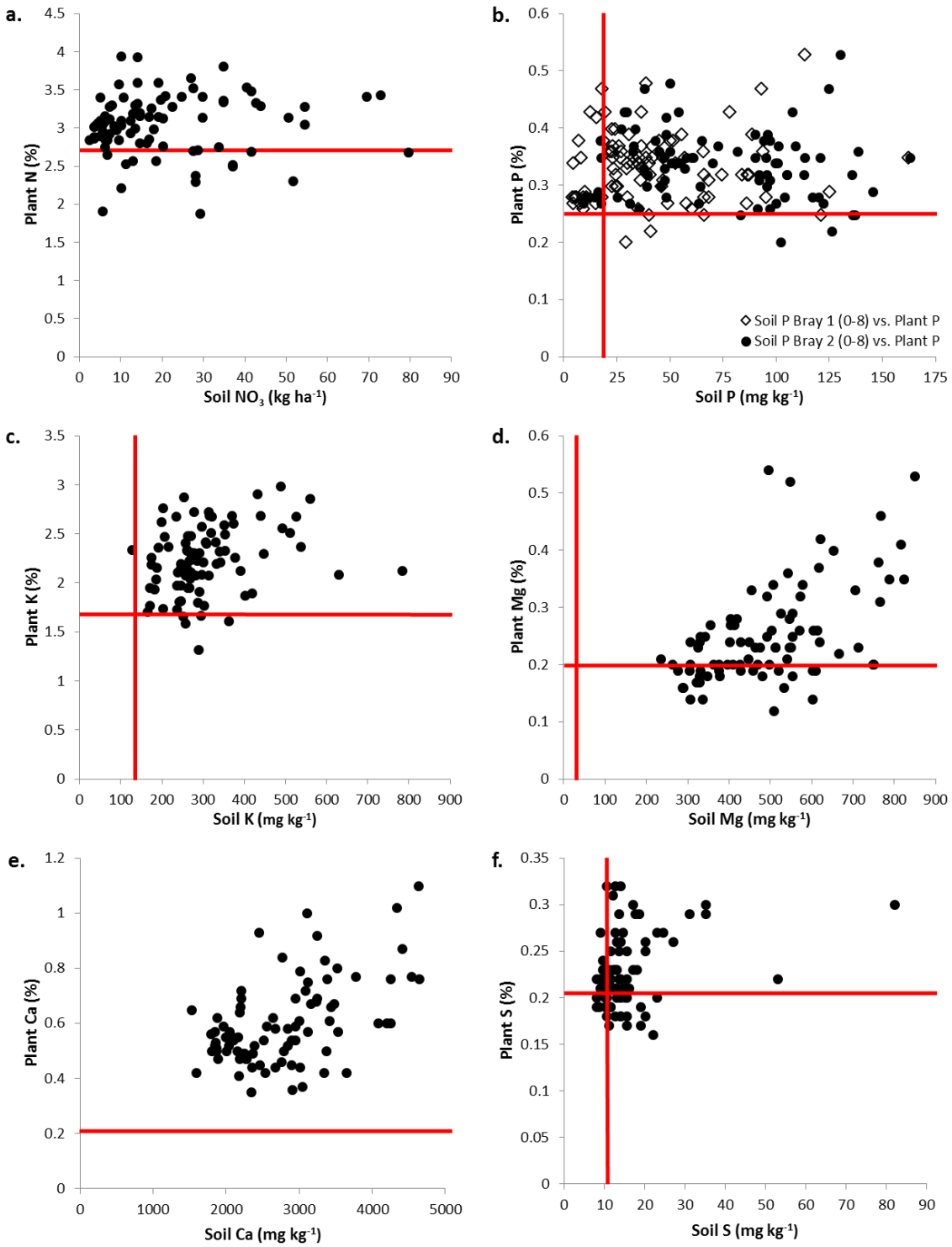

Figure 2-1. Soil and maize leaf nutrient relationships. Maize critical values are presented as horizontal lines for leaf samples and as vertical lines for soil samples collected at VT-R3 (Mills et al., 1996; Voss, 1998; Ward, 2015; Wortmann et al., 2009). In many cases,

these critical values varied by source publication and should not be interpreted as absolutes but are provided for relative guidance. Soil test critical levels were not intended for mid-season sampling. Where there was no published critical soil level, a vertical line was not presented. There was no soil nitrate critical level included as soil nitrate sufficiency late in the growing season (VT-R3) is considered highly variable and location specific. For the soil phosphorous critical level, Bray 1 is presented. Soil and plant samples were collected at the same 15 x 10 m location and time.

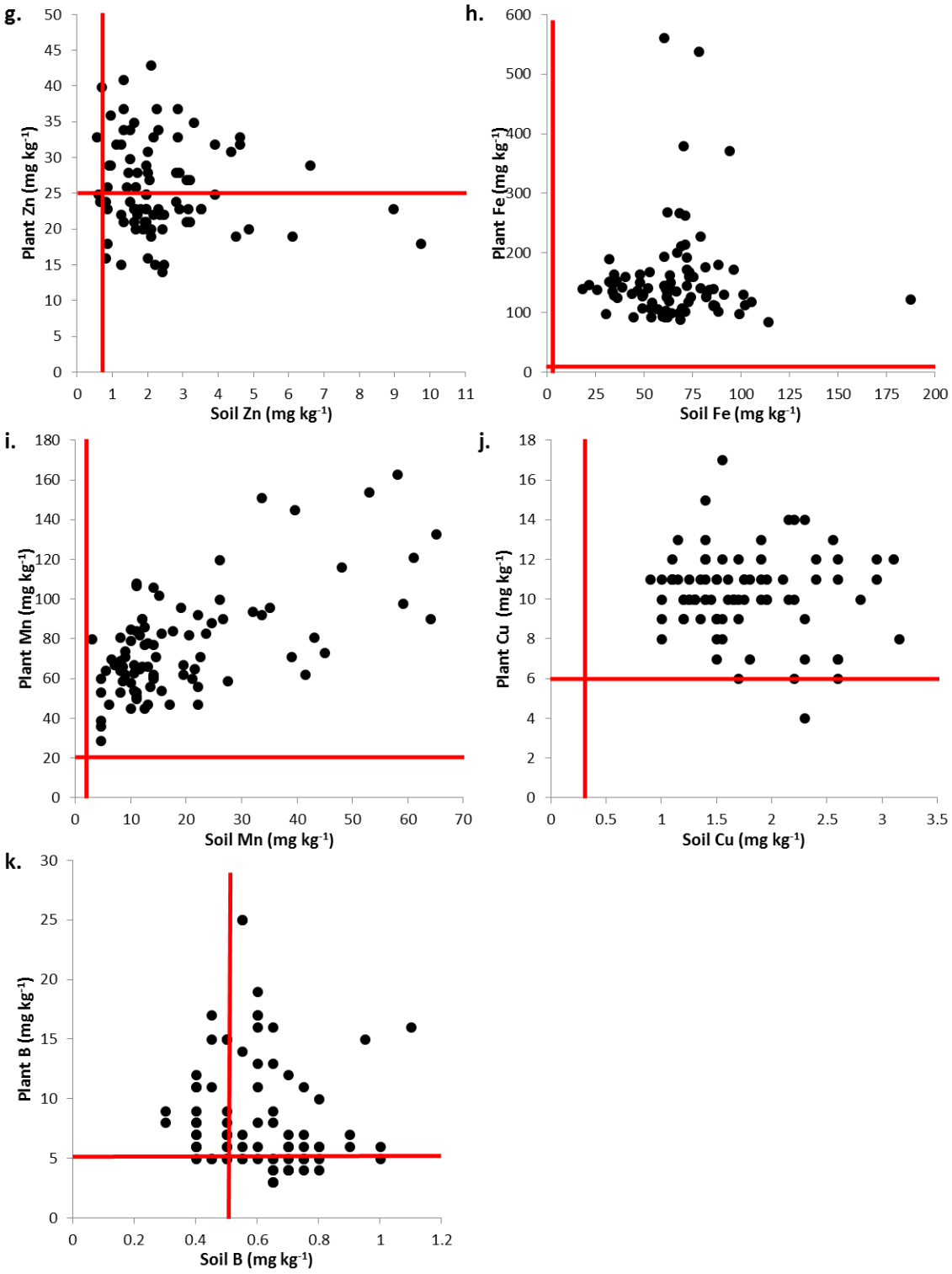

Figure 2-1. Continued.

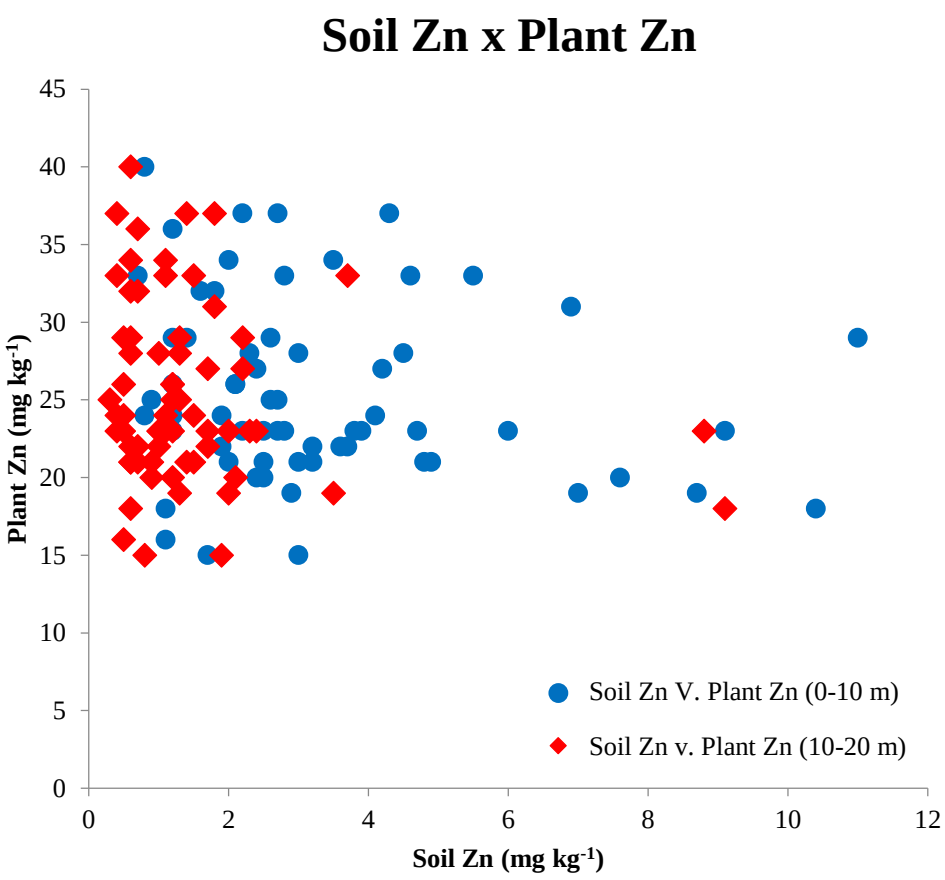

Figure 2-2. Stratified soil Zn concentration 0-10 cm and 10-20 cm sampling depths and plant tissue leaf Zn concentrations for 65 locations. No other nutrient concentrations were stratified at the sampled locations.

CHAPTER 3: FOLIAR MICRONUTRIENT APPLICATION IN PRODUCTION FIELDS EFFECTS ON MAIZE IN NEBRASKA

3.1 Abstract

In the growing seasons of 2013-15, 26 on-farm paired comparison strip trials were conducted across the state of Nebraska testing the effect of foliar-applied micronutrients on maize (*Zea mays* L.) yield and plant tissue nutrient concentrations under current farmer-agronomist practices using commercially available foliar-applied micronutrients. Treatments were applied from V6 to V14 at relatively high yielding locations (10.9 to 16.4 Mg ha^{-1}). Soils ranged in texture from silty clays and silty loams to fine sands. No soil micronutrient levels were below critical levels and no plant tissue micronutrient concentrations taken after micronutrient application were below reported critical values for maize prior to tassel. For a three-year average, locations receiving a foliar application of Zn, Mn, or B ranged in non-significant grain yield differences due to treatment from -0.38 to 0.75 Mg ha^{-1}. Foliar applications of Zn (87 to 119 g Zn ha^{-1}) at 5 of 17 locations, increased Zn concentration in plant tissue by 4 to 9 mg kg^{-1}. However, this increase was not consistently associated with an increase in grain yield. Foliar applications of 87 to 89 g Mn ha^{-1} at 2 of 17 locations significantly increased leaf Mn concentrations by 12 to 16 mg kg^{-1} and the change in leaf Mn had a positive correlation with increasing grain yield r=0.54. Foliar applications of B at a rate of 3 to 235 g B ha^{-1} did not increase foliar B concentration at any of the 15 locations receiving foliar B and there was no combined effect on grain yield. There were 10 locations with visual signs of Fe deficiency where 123 g foliar Fe ha^{-1} increased plant tissue Fe concentration an average of 8.1 mg kg^{-1}

(p=0.1), and was the only plant nutrient concentration increase associated with a highly significant increase in grain yield by an average of 0.4 Mg ha^{-1}. Neither the soil nor plant tissue concentrations were below reported critical values and were not predictive of the associated increase in grain yield. These data are supportive of foliar-applied micronutrient where there are visual symptoms of deficiency and do not support the application of foliar-micronutrients to high yielding/ high demand maize without deficiency.

Abbreviations: V(1-T): vegetative growth stages; and ANOVA: analysis of variance

Keywords: On-farm, Strip-trials, Micronutrients, Foliar, Maize, Boron, Manganese, Iron, Zinc

3.2 Introduction

Nebraska soils are generally micronutrient sufficient with few locations reporting soil and/or plant tissue samples below critical values for maize production (Chapter 2). However, maize has a high rate of micronutrient uptake during critical growth stages and demand may exceed supply. Further, advances in maize yield have increased total micronutrient uptake and removal during harvest adding further demand for micronutrients by modern maize hybrids. In order to produce 12.0 Mg ha^{-1} of grain, maize requires 1.4 kg Fe, 0.5 kg Mn, 0.5 kg Zn, and 0.08 kg B ha^{-1} (Bender et al., 2013). For most Nebraska soils, agronomic crops are most likely constrained from reaching their genetic and environmental potential by the lack of nitrogen and water (Dobermann and Shapiro, 2004). However, as crops increasingly achieve sufficient levels of these and other agronomic inputs, micronutrients may become more likely to be the limiting growth factor.

Micronutrient foliar sprays are of widespread use in agricultural production and are commonly used as a complementary strategy to soil nutrient amendments. Although plant leaves are specialized in capturing light and CO_2, their ability to absorb certain nutrients has long been recognized and used in nutrient management (Fernández and Eichert, 2009). Foliar-applied micronutrients have been found to penetrate the leaf surface through the cuticle, cuticular cracks and imperfections, and stomata trichomes, and lenticels (Marschner, 2012).

Micronutrients such as boron (B), manganese (Mn), iron (Fe), and zinc (Zn), are essential to plant physiological function and are needed in relatively small but critical

amounts by maize (Marschner, 2012). Each of these micronutrients are of general interest to Nebraska producers and agronomists and were selected for inclusion in this trial based on discussions with Nebraska agronomists and agronomic laboratory personnel, in-season plant tissue analysis, and a soil and plant tissue survey study (Chapter 2).

Advances in maize yields have increased the removal of nutrients harvested and even in scenarios where plant or soil analysis do not indicate concentrations below critical levels it may be theorized that plant demand may exceed soil supply during periods of rapid uptake. While Nebraska soils are generally fertile, maize has a high rate of nutrient uptake during the V4 to VT stage and demand may exceed supply. The application of foliar micronutrients to correct or avoid micronutrient deficiencies under conditions where soils provide limited availability of such micronutrients is one of the most commonly practiced uses of foliar fertilization worldwide (Fageria et al., 2009; Kannan, 2010). Numerous soil properties can limit micronutrient solubility and uptake by plant roots. For example, micronutrients (e.g. Fe, Mn, copper (Cu), and Zn) have limited availability in high pH, calcareous soils or excessively low pH in acidic soils (Wortmann et al., 2013). Thus, micronutrient foliar sprays are of general interest as tools to manage these nutrients and subsequently bypassing these soil limitations. Further, plant responses to foliar micronutrients are normally more rapid than soil applications and for most nutrients have higher recovery rates applied to the foliage as compared to soil applications (Marschner, 2012). Therefore, foliar-applied micronutrients are of importance for nutrient correction within a given growing season.

As yield increases, producers are applying higher levels of macronutrients which may increase the risk of micronutrient deficiencies. Liebig's law of the minimum states

that yield is proportional to the most limiting nutrient. For example, as sufficient levels of each of the macronutrients are being met, this increases the likelihood of a micronutrient deficiency being the yield limiting factor (Marschner, 2012). In the pursuit of ever high yields, maize producers see micronutrient supplementation as a suitable addition to their fertilizer program without clear evidence of its actual benefit under their specific field conditions. Further, most foliar micronutrient supplements can be tank mixed with herbicides and pesticides which adds to their convenience and appeal.

The effectiveness of foliar micronutrient treatments varies significantly in relation to their ingredients such as salts, surfactants, complexes, or chelates and as such, when evaluating micronutrient effects it is likely not adequate to evaluate a singular micronutrient formulation (Fernández and Ebert, 2005; Wojcik, 2004; Zhang and Brown, 1999) (chapter 5). In an attempt to avoid this pitfall, it was the goal of this study to evaluate commercial formulations which included a variety of micronutrient mixes with a variety of additives. Recent foliar trials of micronutrient foliar treatments on maize have seen mixed results with one trial reporting an increase in maize grain yield of nearly 18% for a three-year average with the application of 1.0 to 1.5 kg foliar Zn ha^{-1} (Potarzycki and Grzebisz, 2009), while many others report no significant increase in yield (Heckman, 2002; Mueller and Diaz, 2011; Nelson and Meinhardt, 2011).

Nutrient concentrations in plant tissue is widely used for determining the nutritional status of maize throughout the growing season. This concept is built on Julius von Liebig and Carl Sprengel's "Law of the Minimum" in that plants grow to the limit imposed by the nutrient in least supply (van der Ploeg and Kirkham, 1999). Deficiency of any one of the essential plant nutrients can limit plant growth. Plant analysis makes use of

this foundational concept by comparing the nutrient concentration of a particular plant part with established critical values or sufficiency ranges of the same plant species. This comparison of the nutrient concentration of the sampled plant and established critical values or sufficiency ranges is the basis for accessing the plant's nutrient status. In simplistic terms, a plant analysis with a nutrient concentration below the sufficiency range or critical value would imply a deficiency of the nutrient in question and would imply that the nutrient is likely limiting, or the nutrient is in least supply. Plant nutrient concentrations are not static throughout the growing season. Thus, plant tissue samples that are near critical levels but not below may also be candidates for micronutrient application (Mundorf et al., 2015). We also theorize that plants that have received micronutrient application will have greater nutrient concentrations of the applied micronutrient in new growth tissues.

The objective of this study was to evaluate the effect of foliar-applied micronutrients on grain yield and plant tissue nutrient status under current farmer-agronomist practices using commercially available foliar micronutrients. Paired comparison strip trials were the experimental designs of choice as this allowed maize producers to use conventional practices to apply (i.e. high clearance and aerial applicators) and select their foliar micronutrient treatments based on their relationship with their local agronomist, the availability of foliar micronutrient formulations, and their knowledge of which micronutrient formulation best fits their field location's demand. These data could also be valuable as an extension education tool.

3.3 Materials and Methods

During the growing seasons of 2013, 2014 and 2015, 26 paired comparison strip trials were performed across the state of Nebraska (Figure 3-1) testing the effect of various commercial foliar micronutrient products. Locations had between 3 and 20 replications depending on farmer equipment and willingness (Table 3-1). Two of the 26 trials were on popcorn (Table 3-2). Each of the foliar micronutrient treatments were selected by the producer and their local agronomist as to best fit the micronutrient needs of each field as based on prior soil and plant tissue recommendations from commercial laboratories. No soil micronutrient levels were below critical levels as reported by Ward (2015) and no plant tissue micronutrient concentrations were below reported critical values for maize prior to tasseling as reported by Mills et al. (1996). Only locations 20-24 had visual signs of Fe deficiency (i.e. interveinal chlorosis).

Sites were selected to be representative of Nebraska soils and encompassed 23 soil series (Table 3-1). The selected locations had no history of micronutrient or manure applications in the previous 10 years. Nebraska soils are generally micronutrient fertile so finding micronutrient deficient sites proved to be relatively unobtainable (Wortmann et al., 2013) as confirmed by a Nebraska wide survey of soil and plant tissue samples and data obtained from Nebraska agronomic testing laboratories (Chapter 2). Pre-season nitrogen applications varied by source, application time, and rate but all locations applied nitrogen at a rate sufficient for at least 12.0 Mg ha^{-1} maize grain production (Shapiro et al., 2003).

Products ranged in micronutrient, rate, adjuvant formulation, application method, application date and application growth stage (Table 3-1). The most relevant site information including locations, soils, rainfall, tillage system, row spacing, previous crop, irrigation, hybrid, planting date and harvest date can be found in Table 3-2, and background soil analysis and texture can be found in Table 3-3. Treatments were applied aerially or with a high-clearance applicator in strips with and without the foliar treatments and split into adjacent pairs for analysis. Strips ranged in size from approximately 27-34 m wide x 715 m long. The high-clearance applicator did not go through control strips.

Plant tissue samples were collected three to five vegetative growth stages (Abendroth et al., 2011) after application from new growth / unsprayed leaves (i.e. a composite sample of 10 upper most fully collared leaves), a soil sample (0-20 cm) was collected (i.e. a composite sample of 14 subsamples collected across the treatment strips), and grain yield was collected by hand (i.e. 1/1000 ha^{-1} estimate), weigh wagon, or yield monitor from each strip. Grain samples were adjusted to 155 g kg^{-1} water content. Plant tissue and soil samples were sent to Midwest Laboratories (Midwest Laboratories, Omaha, NE) for nutrient concentration analysis.

Laboratory analysis of plant tissue phosphorous (P), potassium (K), sulfur (S), Fe, Mn, Zn, and B were completed using microwave nitric acid digestion and concentrations determined using inductively-coupled plasma emission spectroscopy (ICP-ES). Percent nitrogen (N) was determined using the Dumas Method with a Leco FP-428 (Horwitz and Latimer Jr, 1920). Soil samples were dried at 40°C and ground to pass through a 2-mm sieve prior to analysis. Boron, Mn, and Zn used DPTA (diethylenetriaminepentaacetic

acid) extraction, Fe and Cu used 0.1 N HCl extraction, and was measured with ICAP detection (Brown, 1998). B used sorbitol ICAP detection.

3.3.1 Statistical Design and Analysis

An analysis of variance (ANOVA) for treatment effects was conducted on yield and plant tissue nutrient concentrations for 26 paired comparison strip trials. Each location had 3 to 20 replications. Location results were analyzed separately, and results from sites with identical treatments and crop were combined and analyzed across locations and years. Yield and plant tissue nutrient concentrations were analyzed as a paired comparison design using Statistix 10.0 Analytical Software (Analytical Software Tallahassee, Florida, USA) assuming fixed treatment effects and random site effects. A mean comparison test using Tukey's HSD was used to compare treatment effects (Appendix Code 3-1). Data were managed using Microsoft Excel (Microsoft Excel 2013, Microsoft Corp. Santa Rosa, CA) and plotted to test correlations of plant tissue and soil parameters with grain yield response using the scatterplot and correlation coefficient (r) function.

3.4 Results and Discussion

Soil micronutrient concentrations were above published critical levels at all locations as reported by Ward (2015) (Table 3-3). The soil B, Fe, Mn, and Zn concentrations ranged from 0.3 to 1.0, 4.0 to 71.7, 2.0 to 23.2, and 1.0 to 6.7 mg kg^{-1}, respectively. Soil organic matter ranged from 10 to 33 g kg^{-1} and pH ranged from 5.6 to

8.2. Locations varied widely in soil texture and included clays, loams, and sands (Table 3-3). Plant tissue micronutrient concentrations were also above published critical levels at all locations for values cited in Mills et al. (1996). Maize tissue B, Fe, Mn, and Zn concentrations ranged from 3.7 to 16, 59 to 213, 38 to 109, 18 to 57 mg kg^{-1}, respectively, in untreated plots prior to tassel. Though not below critical levels, only locations 20-24 had visual signs of micronutrient deficiency (i.e. Fe deficiency: interveinal chlorosis in upper new growth leaves) (Figure 3-2).

Under conditions without visual signs of deficiency, responses of leaf micronutrient concentrations and grain yield to foliar micronutrient treatments were limited and inconsistent. Locations receiving foliar Zn, Mn, or B supplementation ranged in grain yield differences from -0.38 to 0.75 Mg ha^{-1}. Of the foliar treatments containing Zn, Mn, and/or B, there was no significant yield increase ($p<0.05$) for a three-year average for any product formulation (Table 3-5). In conditions with visual signs of deficiency, foliar Fe supplementation was effective at increasing grain yield by an average of 0.4 Mg ha^{-1} ($p=0.008$) (Figure 3-6).

In conditions without confirmed micronutrient deficiency, yield responses were unpredictable and included both significant yield increases and decreases. Overall, the average yield difference across all locations was an increase of 0.08 Mg ha^{-1} for micronutrient treatments, though not significant. Three of the 26 locations had significant yield increases (locations 1, 14, 23) and two had yield decreases (locations 10, 13) (Table 3-4). These locations had some combination of foliar B, Mn, Zn, and +/-Fe applied. Location 23 received foliar Fe only (Table 3-1). Of the locations that had significant yield increases, the average yield increase was 0.69 Mg ha^{-1}. Two of these yield increasing

locations had relatively high or low pH (i.e. 5.6 and 7.8) which may have contributed to reduced micronutrient availability and increased the likelihood of response. Of the locations that had significant yield decreases, the average yield decrease was -0.38 Mg ha^{-1}. These significant negative effects on grain yield only occurred in locations that received their foliar micronutrient supplementation by a high-clearance applicator at V8 and V11 and not by aerial application which might suggest the yield reduction may be associated with damage incurred during treatment application. The high-clearance applicator did not make a pass through the control strips.

Inversely, the combined analysis of variance for identical treatments at differing locations indicates that a foliar application of 123 g Fe ha^{-1} increased grain yield ($p=0.008$) by an average of 0.4 Mg ha^{-1} under conditions of high pH (i.e. 7.2 to 8.0), sufficient but low soil Fe concentrations (i.e. 8.0 to 13.0 mg kg^{-1}), and sufficient but low plant tissue Fe concentration prior to tassel (i.e. 68 to 86 mg kg^{-1}), and visual signs of Fe deficiency (Figure 3-2) (Table 3-5). Additionally, visual observations of treated strips showed small re-greening patterns where foliar Fe droplets contacted the leaf surface on the V5-6 leaf (Figure 3-2). No re-greening appeared in upper untreated leaves as shown in image a) of Figure 3-2. Fe and micronutrients are considered relatively immobile within plant tissue. Thus, no re-greening in upper untreated new growth leaves was expected. These data are consistent with the previously described deficiency correction theory. However, plant and soil Fe concentrations alone were not predictive indicators of grain yield response to foliar Fe. There were several significant ($p \leq 0.05$) treatment*site interactions of the treatments on grain yield which were likely due to unique site

conditions such as soil parameters or various hybrids varying in their micronutrient deficiency susceptibility.

Foliar micronutrient treatments were more consistent in increasing their respective micronutrient concentrations in leaf tissue than increasing grain yield, especially in the case of Zn (Figures 3-3, 4, 5, 6) (Table 3-4). Of the locations that received foliar Zn, 47% had a significant increase ($p \leq 0.10$) of an average of 4 mg Zn kg^{-1} in the leaf tissue for all locations receiving Zn supplementation (Figure 3-3) (Table 3-4). Foliar treatments also effected plant N and P concentrations. The combined analysis of locations 1, 2, and 3 which received 116, 87, 87, 87, and 7 g of N, S, Mn, Zn, and B ha^{-1}, respectively increased plant N by 1.3 g kg^{-1} and plant P by 0.2 g kg^{-1} ($p \leq 0.05$) (Table 3-5). The increase in N would be expected with the addition of foliar N. However, the increase in P was not as clear. Warnock (1970) previously showed that P and Fe, Mn, and Zn have significant interaction with their respective plant tissue concentrations which is likely contributing to this treatment effect.

For the combined year and location analysis, foliar applications of Zn at 17 locations, ranging in application rate from 87 to 119 g Zn ha^{-1} and regardless of product formulation, significantly increased Zn concentration ($p \leq 0.05$) in plant tissue by 2.9 to 6.1 mg kg^{-1}, however, this increase was not consistently associated with an increase in grain yield under these conditions (Table 3-5). Foliar applications of 87 to 89 g Mn ha^{-1} at 17 locations also had significant increase in plant tissue Mn concentrations by 9.3 ($p \leq 0.05$) to 4.2 ($p \leq 0.10$) mg kg^{-1}, and had a positive correlation with increasing grain yield $r=0.54$ but for a three-year average did not significantly increase grain yield in any product formulation. (Figure 3-4) (Table 3-5). Foliar applications of B at a rate of 3 to

235 g B ha^{-1} did not have significant ($p \leq 0.05$) effect on plant tissue concentrations of B nor grain yield at any of the 15 locations receiving foliar B (Table 3-5) (Figure 3-5).

Inversely, 123 g foliar Fe ha^{-1}, applied at 10 locations, increased plant tissue Fe concentration ($p=0.1$), and was the only plant nutrient concentration increase associated with a highly significant increase in grain yield by an average of 0.4 Mg ha^{-1} ($p=0.008$) (Table 3-5). This response of plant tissue concentrations and grain yield to the foliar Fe treatment was consistent with the deficiency correction theory in that there were visual signs of Fe deficiency identified at locations 20-23 prior to foliar Fe treatment and thus, there was an increase in grain yield due to the application of the deficient nutrient. However, neither the soil or plant tissue concentrations were below reported critical values for Fe and thus were not predictive of the associated increase in grain yield. Additionally, locations receiving foliar B, Mn, and Zn treatments, under conditions with no respective deficiency identified by plant tissue samples, soil samples, or visual symptoms, did not have a yield increase for a three-year average.

Due to increased micronutrient demand of higher yielding locations, we also theorized that locations with higher yields may be more likely to have increased yield response due to foliar micronutrient supplementation. The combined analysis for maize production locations showed no relationship ($r=0.03$) between higher yielding locations and yield response to foliar micronutrient supplementation (Figure 3-7). Additionally, locations with micronutrient concentrations in soil or plant tissue near critical levels but not below were not consistently associated with an increased likelihood of increased grain yield ($r<0.1$), except in the case of Fe, which had visual signs of deficiency. Neither SOM, pH nor soil/plant P was correlated ($r<0.01$) with a positive increase in grain yield.

3.5 Conclusions

The objective of these on-farm strip trials was to evaluate the effect of foliar-applied micronutrients on grain yield and plant tissue nutrient status under current farmer-agronomist practices using commercially available foliar micronutrients. This research was conducted across a wide range of maize production conditions, and the results are widely applicable to high yield maize production with similar soil and plant micronutrient status. These data are largely supportive of the deficiency correction theory and refute the indiscriminate use of foliar micronutrient applications. The most noteworthy result of these trials was the unpredictable response on grain yield, both positive and negative in cases without visual signs of micronutrient deficiency. Nebraska soils are generally fertile and in most cases micronutrient treatments are likely not necessary. These data are consistent with the results of comparable micronutrient foliar treatments on soybeans under similar field conditions in Iowa (Enderson et al., 2015). Additionally, soil samples and plant tissue nutrient concentrations were not predictive of response under Nebraska conditions. Further, no locations reported soil or plant tissue micronutrient concentrations below critical levels as reported by Mills et al. (1996). Nonetheless, there were some locations that did have significant yield increases indicating that there is need for predictive tools to aid producers to better forecast yield response.

These data are not sufficient to either confirm or disprove previously established critical values but do suggest that locations with a plant tissue or soil sample reporting Fe

concentrations near but not below critical values may still be candidates for foliar Fe treatments, especially in scenarios with visual signs of deficiency and alkaline pH. Without visual signs of deficiency or other confirmation of a micronutrient deficiency, predicting yield response to foliar applications of micronutrients will be illusive. These data also highlight an opportunity to fine tune predictive tools for yield response to micronutrient treatments where maize plants may have low levels of a micronutrient but may not be below reported critical values. As shown in this data set, there can still be positive yield increases to foliar micronutrient treatments when soil and plant tissue micronutrient concentrations are low but still above reported critical levels, however, in order for this supplementation to be economically feasible, there is need for predictive tools in addition to the current practice of assessing soil and plant tissue micronutrient concentrations such as those relationships discussed in Chapter 2. Without a confirmed micronutrient deficiency, there is an equivalent chance for grain yield reductions with the application of foliar micronutrient treatments.

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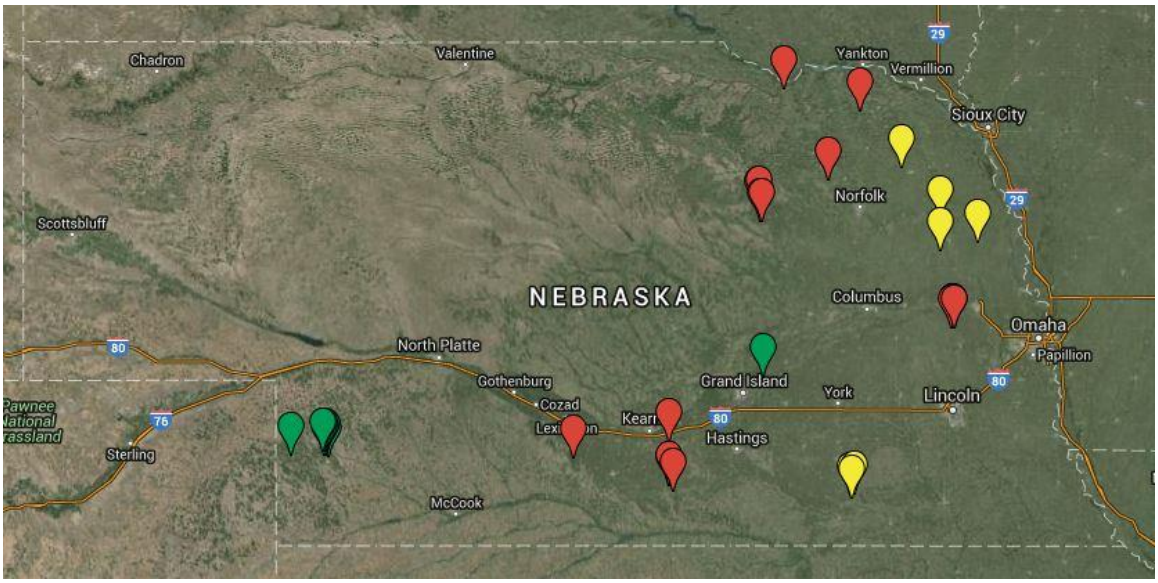

Figure 3-2. Locations of the 26 on-farm strip trials color coded by year. (2013: yellow, 2014: red, 2015: green)

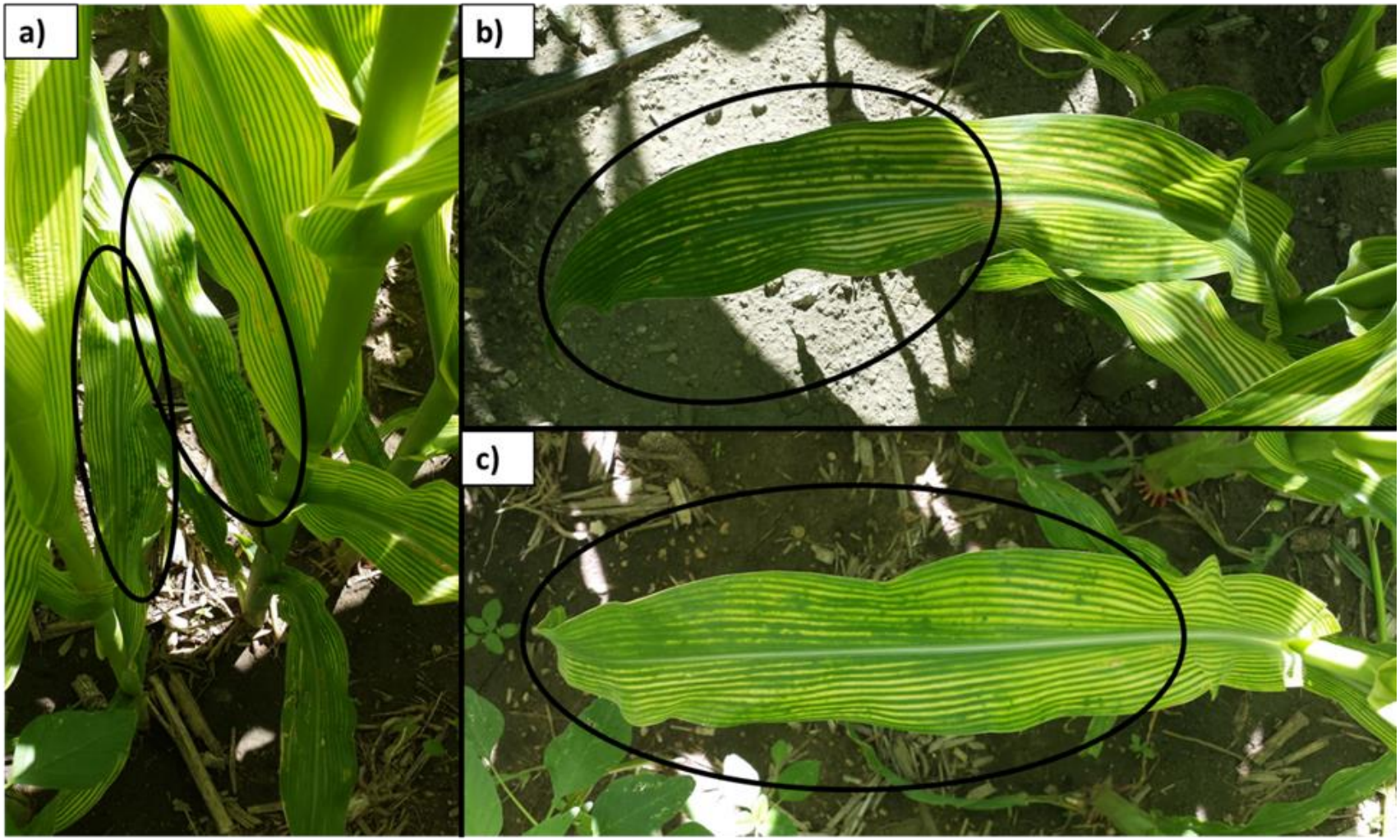

Figure 3-2. Images from foliar Fe treated maize plants in strip trials showing re-greening patterns where foliar Fe droplets contacted the leaf surface on the V5 leaf. No re-greening appears in upper untreated leaves as shown in image a).

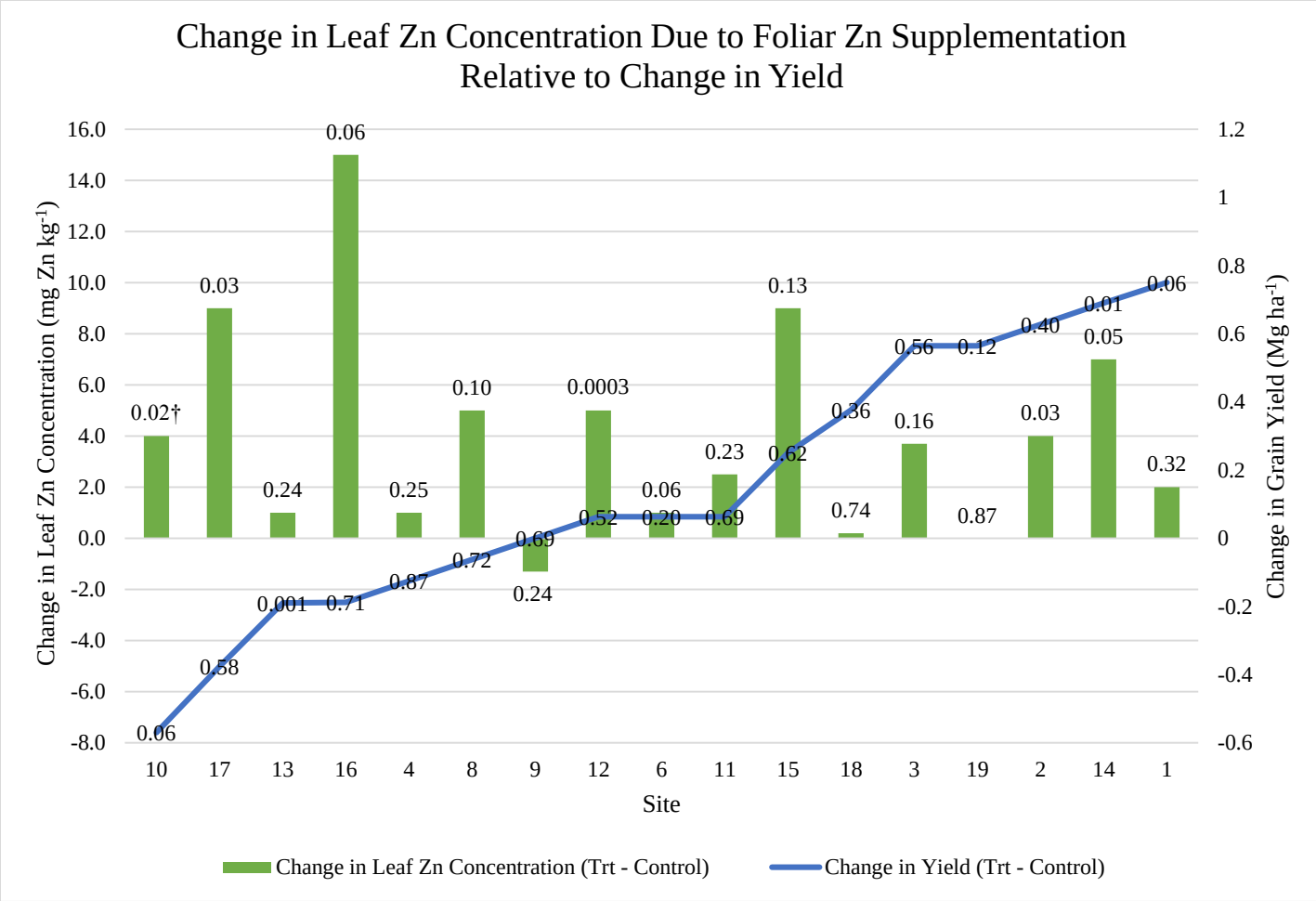

Figure 3-3. Change in leaf Zn concentration denoted as bars (Foliar-treated plot leaf Zn concentration - control plot leaf Zn concentration) due to foliar Zn supplementation correlating with the change in maize grain yield denoted as a line (treated plot yield – control plot yield) at all sites that received Zn containing foliar supplementation. Data labels on both the line and bar graphs indicate p-values† for mean comparison differences. The correlation coefficient (r) for these data is 0.2.

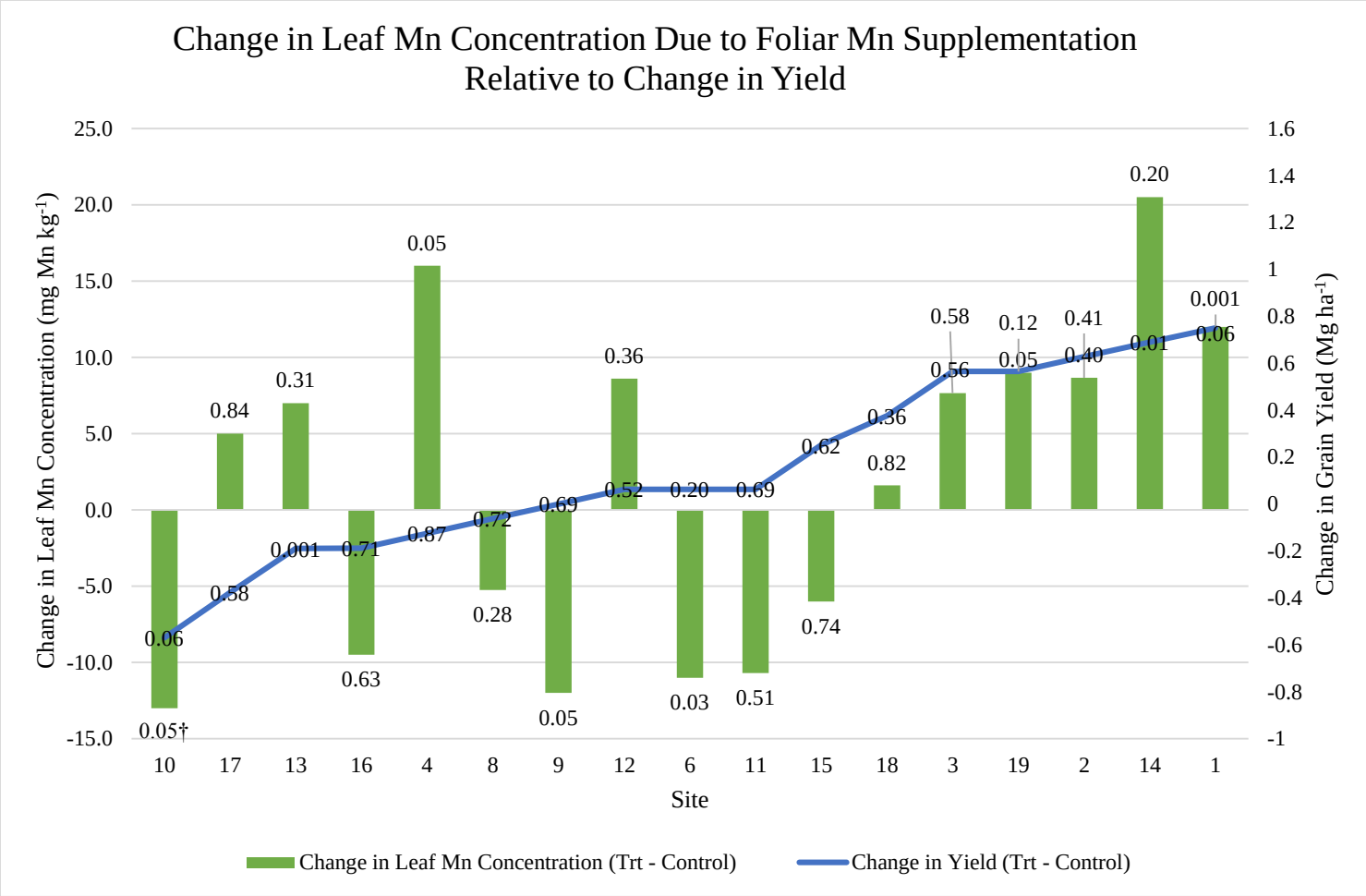

Figure 3-4. Change in leaf Mn concentration denoted as bars (Foliar-treated plot leaf Mn concentration - control plot leaf Mn concentration) due to foliar Mn supplementation correlating with the change in maize grain yield denoted as a line (treated plot yield – control plot yield) at all sites that received Mn containing foliar supplementation. Data labels on both the line and bar graphs indicate p-values† for mean comparison differences. The correlation coefficient (r) for these data is 0.54.

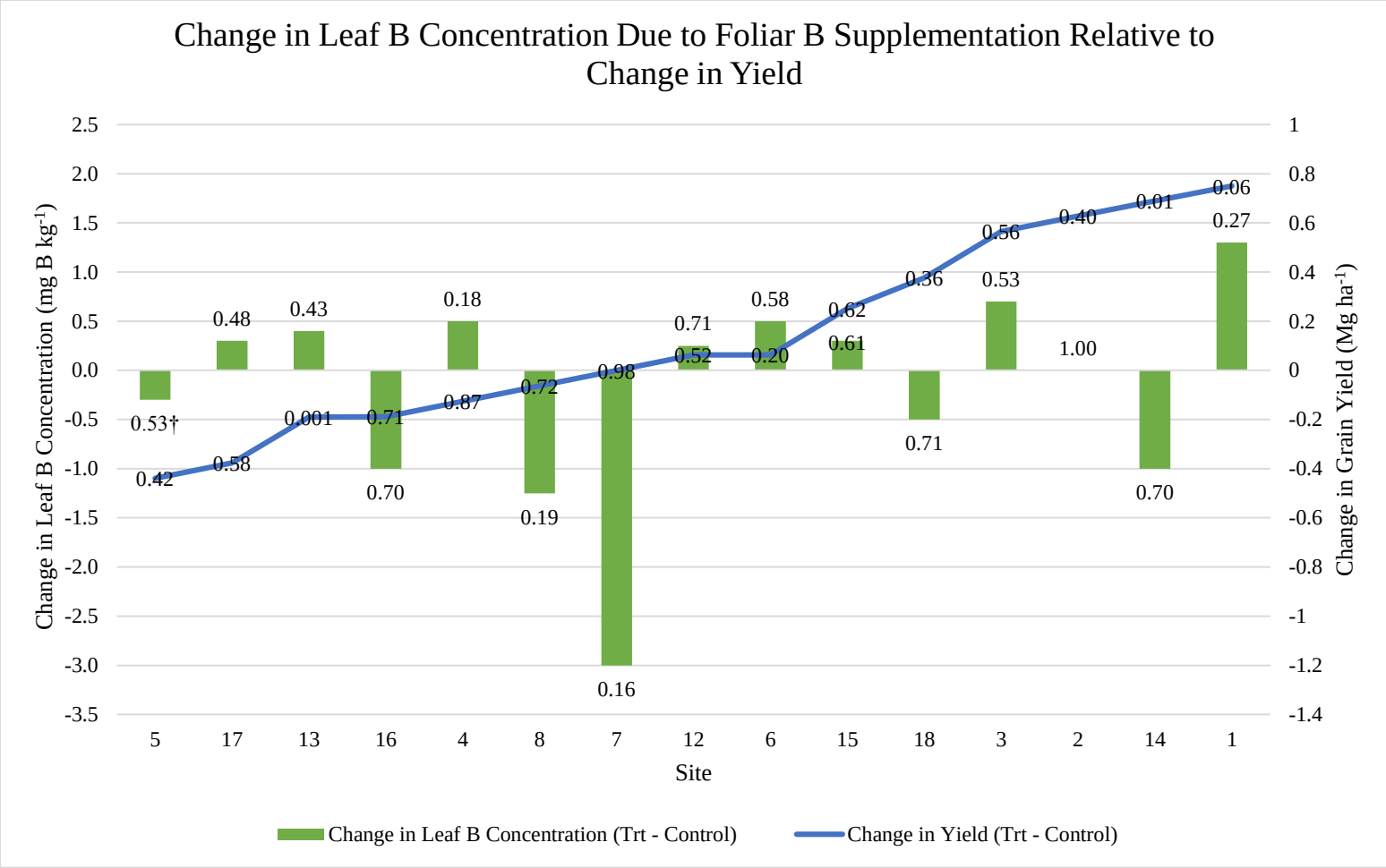

Figure 3-5. Change in leaf B concentration denoted as bars (Foliar-treated plot leaf B concentration - control plot leaf B concentration) due to foliar B supplementation correlating with the change in maize grain yield denoted as a line (treated plot yield – control plot yield) at all sites that received B containing foliar supplementation. Data labels on both the line and bar graphs indicate p-values† for mean comparison differences. The correlation coefficient (r) for these data is 0.2.

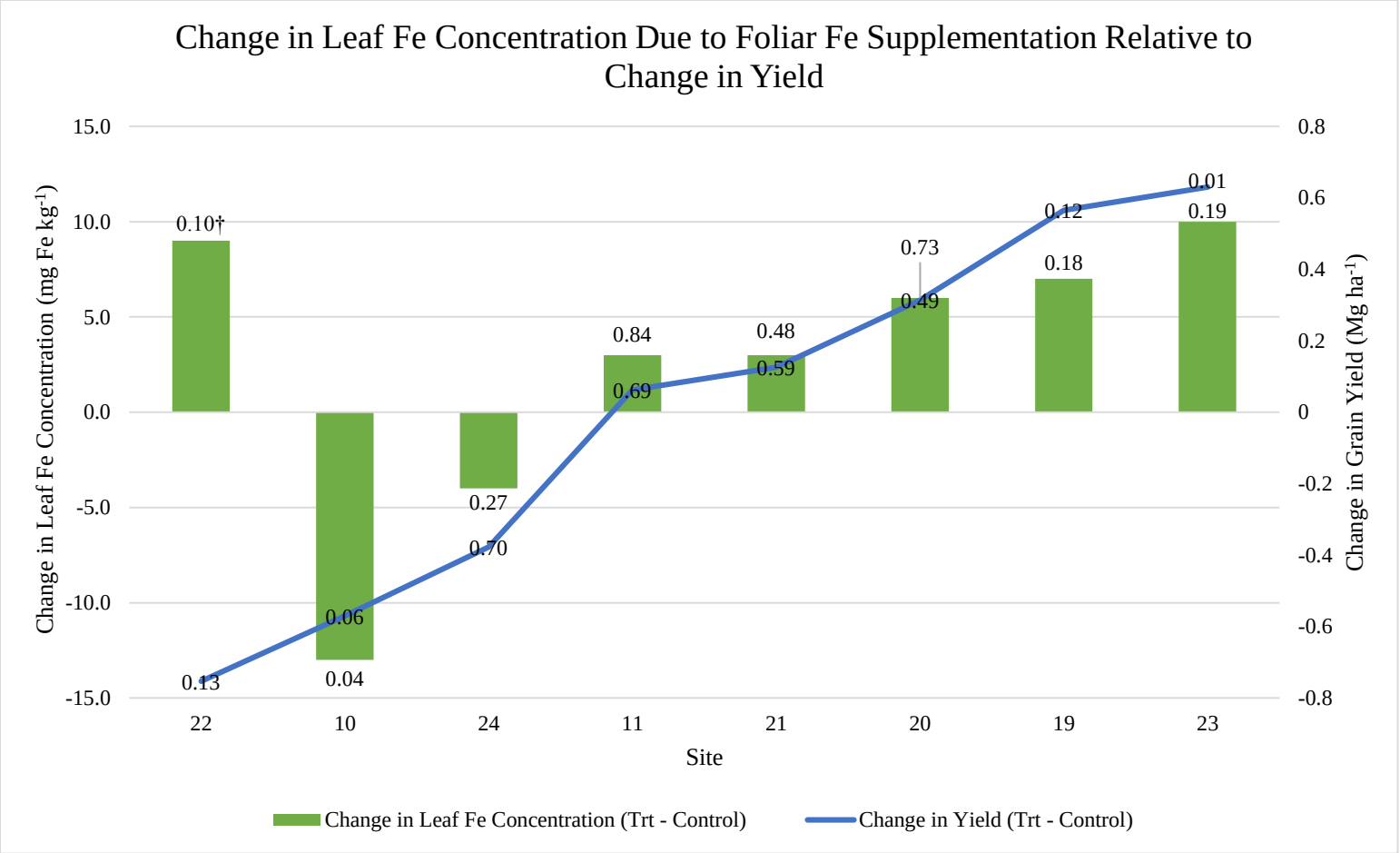

Figure 3-6. Change in leaf Fe concentration denoted as bars (Foliar-treated plot leaf Fe concentration - control plot leaf Fe concentration) due to foliar Fe supplementation correlating with the change in maize grain yield denoted as a line (treated plot yield – control plot yield) at all sites that received Fe containing foliar supplementation. Data labels on both the line and bar graphs indicate p-values† for mean comparison differences. The correlation coefficient (r) for these data is 0.54. These locations were the only locations with visual signs of micronutrient deficiency.

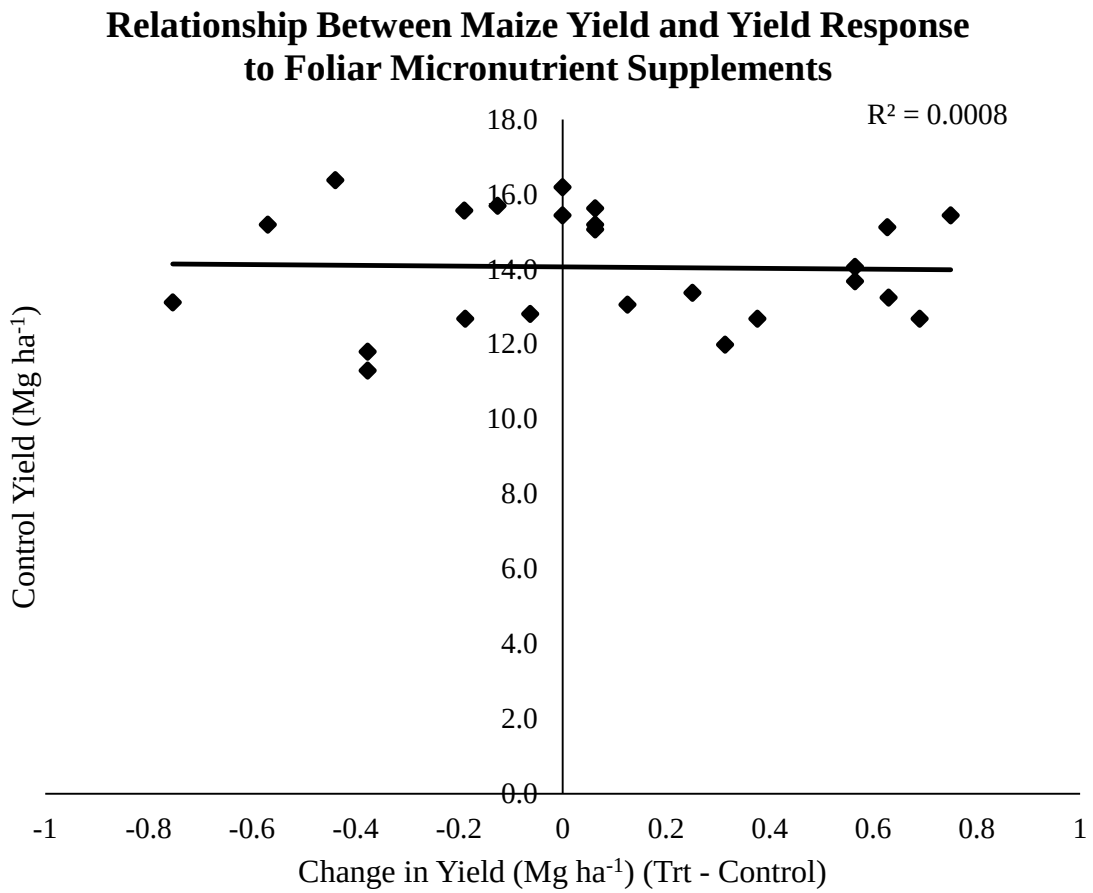

Figure 3-7. Relationship between control grain yield and the change in grain yield (treatment – control yield) due to foliar micronutrient treatments for all maize locations receiving foliar micronutrient supplementation. All grain yields are expressed in Mg ha^{-1} adjusted to 155 g kg^{-1} water content. Our hypothesis was that higher yielding locations would have a greater probability of increased yield response. This figure suggests that there is no relationship (r=0.03) between higher yielding locations and an increased likelihood of yield response to foliar micronutrient supplementations.

Table 3-1. Treatment micronutrient formulation and application background information for each of the 26 sites

Site	Product	Applied Nutrients	Rate	Elemental Rate	Application Method	Application Date	Application Stage	Reps
		g kg^{-1}	L ha^{-1}	g element ha^{-1}				
1	Brandt Smart Trio (Zn, Mn, B)	40 N, 30 S, 30 Mn, 30 Zn, 2.5 B	2.3	116 N, 87 S, 87 Mn, 87 Zn, 7 B	High Clearance Applicator	2-July	V10	3
2	Brandt Smart Trio (Zn, Mn, B)	40 N, 30 S, 30 Mn, 30 Zn, 2.5 B	2.3	116 N, 87 S, 87 Mn, 87 Zn, 7 B	High Clearance Applicator	2-July	V12	3
3	Brandt Smart Trio (Zn, Mn, B)	40 N, 30 S, 30 Mn, 30 Zn, 2.5 B	2.3	116 N, 87 S, 87 Mn, 87 Zn, 7 B	High Clearance Applicator	2-July	V12	3
4	MAX-IN Ultra ZMB	36 S, 10 B, 30 Mn, 40 Zn	2.3	107 S, 89 Mn, 119 Zn, 3 B	High Clearance Applicator	18-June	V6	4
5	MAX-IN Boron	80 B	2.3	235 B	High Clearance Applicator	17-June	V6	6
6	MAX-IN Ultra ZMB	36 S, 10 B, 30 Mn, 40 Zn	2.3	107 S, 89 Mn, 119 Zn, 3 B	High Clearance Applicator	18-June	V5	6
7	MAX-IN Boron	80 B	1.2	123 B	High Clearance Applicator	13-June	V5	6
8	MAX-IN Ultra ZMB + MAX-IN Boron	36 S, 10 B, 30 Mn, 40 Zn + 80 B	2.3	107 S, 89 Mn, 119 Zn, 3 B + 235 B	High Clearance Applicator	26-June	V8	4
9	FullTec Zn	300 P_2O_5, 80 Mn, 30 Zn	0.4	206 P_2O_5, 55 Mn, 21 Zn	High Clearance Applicator	12-June	V5	4
10	Attain and MAX-IN Boron	80 N, 30 S, 20 Mn, 30 Zn, 10 Fe + 80 B	2.3	238 N, 89 S, 56 Mn, 89 Zn, 30 Fe	High Clearance Applicator	26-June	V8	8
11	Attain and MAX-IN Boron	80 N, 30 S, 20 Mn, 30 Zn, 10 Fe + 80 B	2.3	238 N, 89 S, 56 Mn, 89 Zn, 30 Fe	High Clearance Applicator	26-June	V9	8
12	MAX-IN Ultra ZMB + MAX-IN Boron	36 S, 10 B, 30 Mn, 40 Zn + 80 B	2.3	107 S, 89 Mn, 119 Zn, 3 B + 235 B	High Clearance Applicator	3-July	V11	20
13	MAX-IN Ultra ZMB + MAX-IN Boron	36 S, 10 B, 30 Mn, 40 Zn + 80 B	2.3	107 S, 89 Mn, 119 Zn, 3 B + 235 B	High Clearance Applicator	3-July	V11	20
14	MAX-IN Ultra ZMB	36 S, 10 B, 30 Mn, 40 Zn	2.3	107 S, 89 Mn, 119 Zn, 3 B	Aerial	10-July	V13	4
15	MAX-IN Ultra ZMB	36 S, 10 B, 30 Mn, 40 Zn	2.3	107 S, 89 Mn, 119 Zn, 3 B	Aerial	10-July	V14	4
16	MAX-IN Ultra ZMB	36 S, 10 B, 30 Mn, 40 Zn	4.7	218 S, 182 Mn, 242 Zn, 6 B	Aerial	10-July	V13	4
17	MAX-IN Ultra ZMB	36 S, 10 B, 30 Mn, 40 Zn	2.3	107 S, 89 Mn, 119 Zn, 3 B	Aerial	10-July	V13	2
18	MAX-IN Ultra ZMB + MAX-IN Boron	36 S, 10 B, 30 Mn, 40 Zn + 80 B	2.3	107 S, 89 Mn, 119 Zn, 3 B + 235 B	High Clearance Applicator	3-July	V11	10
19	Attain + N-Cline	80 N, 30 S, 30 Zn, 20 Mn, 10 Fe + 280 N	2.3 and 9.4	238 N, 89 S, 56 Mn, 89 Zn, 30 Fe + 3343 N	High Clearance Applicator	23-June	V7	6
20	Versa Fe liquid Fe + Lockdown Surfactant	180 Fe-EDDHA	5.8 L ha^{-1} and 0.3 kg ha^{-1}	123 Fe	Aerial	26-June	V6	4
21	Versa Fe liquid Fe + Lockdown Surfactant	180 Fe-EDDHA	5.8 L ha^{-1} and 0.3 kg ha^{-1}	123 Fe	Aerial	26-June	V6	4
22	Versa Fe liquid Fe + Lockdown Surfactant	180 Fe-EDDHA	5.8 L ha^{-1} and 0.3 kg ha^{-1}	123 Fe	Aerial	26-June	V6	4
23	Versa Fe liquid Fe + Lockdown Surfactant	180 Fe-EDDHA	5.8 L ha^{-1} and 0.3 kg ha^{-1}	123 Fe	Aerial	26-June	V6	4
24	Pro Iron 5	60 N, 30 S, 50 Fe-EDTA	2.3	175 N, 88 S 146 Fe	High Clearance Applicator	25-June	V6	6
25	Versa Fe liquid Fe + Lockdown Surfactant	180 Fe-EDDHA	5.8 L ha^{-1} and 0.3 kg ha^{-1}	123 Fe	Aerial	26-June	V6	4
26	Versa Fe liquid Fe + Lockdown Surfactant	180 Fe-EDDHA	5.8 L ha^{-1} and 0.3 kg ha^{-1}	123 Fe	Aerial	26-June	V6	4

Table 3-2. Location background information and cultural practices for 26 field strip trials testing foliar micronutrients.

Site	Year	Crop	County	Soil Classification	Rainfall†	Till‡	Row Spacing	Previous Crop	Irrigation	Hybrid	Plant Date	Harvest Date
				Soil Series	Season							
					mm		cm		Y/N			
1	2013	Maize	Thayer	Crete	724	NT	76.2	Soybean	Y	Pioneer P1690HR	13-May	26-Oct
2	2013	Maize	Thayer	Crete	724	NT	76.2	Soybean	Y	Pioneer 33D47	6-May	26-Oct
3	2013	Maize	Thayer	Crete	724	NT	76.2	Soybean	Y	Pioneer P1690HR	7-May	26-Oct
4	2013	Maize	Wayne	Nora	902	NT	76.2	Soybean	Y	Pioneer 1625 HR	5-May	26-Oct
5	2013	Maize	Cuming	Belfore	864	CT	50.8	Maize	Y	Dekalb DKC61-06RIB	5-May	26-Oct
6	2013	Maize	Cuming	Belfore	953	NT	76.2	Soybean	Y	Dekalb DK62 98 VT pro	13-May	26-Oct
7	2013	Maize	Burt	Zook	889	CT	76.2	Maize	Y	Golden Harvest GH14R38	15-April	27-Oct
8	2014	Maize	Thurman	Thurman	673	NT	76.2	Soybean	Y	Pioneer 1266	29-April	21-Oct
9	2014	Maize	Kearney	Boel and Valentine	711	CT	76.2	Maize	Y	Pioneer 33D47	10-May	18-Oct
10	2014	Maize	Kearney	Holdrege and Detroit	762	CT	76.2	Maize	Y	Pioneer 33D53 AMI	9-May	18-Oct
11	2014	Maize	Kearney	Holdrege and Detroit	762	CT	76.2	Maize	Y	Pioneer 1469 AMI	9-May	13-Nov
12	2014	Maize	Saunders	Yutan	1016	NT	76.2	Soybean	Y	Pioneer 1690 HR	16-May	13-Nov
13	2014	Maize	Saunders	Yutan	1016	NT	76.2	Soybean	Y	Pioneer 1690 HR	27-April	3-Nov
14	2014	Maize	Antelope	Thurman and Nora	597	CT	76.2	Maize	Y	Pioneer 1625 HR	15-May	19-Nov
15	2014	Maize	Antelope	Thurman and Doger	648	CT	76.2	Maize	Y	Channel 209-53 STX RIB	27-April	26-Oct
16	2014	Maize	Antelope	Thurman and Nora	584	CT	76.2	Maize	Y	Channel 213-40 VT3	15-May	24-Oct
17	2014	Maize	Antelope	Thurman and Doger	648	CT	76.2	Maize	Y	Channel 213-40 VT3	25-May	26-Oct
18	2014	Maize	Cedar	Crofton and Nora	914	NT	50.8	Maize	Y	Pioneer 1197-AM	27-Apr	5-Nov
19	2015	Maize	Merrick	Cozad and Alda	940	CT	91.4	Maize	Y	Pioneer 1311-AM	25-April	9-Nov
20	2015	Maize	Chase	Blanche and Tassel-Duda	554	NT	76.2	Maize	Y	Channel 209-69 VT3PRIB	28-April	28-Oct
21	2015	Maize	Chase	Blanche and Tassel-Duda	554	NT	76.2	Soybean	Y	Channel 209-69 VT3PRIB	8-May	28-Oct
22	2015	Maize	Chase	Rosebud-Canyon, Blanche, Duda-Tassel	526	CT	76.2	Maize	Y	Pioneer 1151	25-April	21-Nov
23	2015	Maize	Chase	Rosebud-Canyon	526	CT	76.2	Maize	Y	Pioneer 1151	25-April	21-Nov
24	2015	Maize	Chase	Rosebud, Rosebud-Canyon, and Kuma	516	CT	76.2	Maize	Y	Prairie Brand 5825	18-May	9-Nov
25	2015	Popcorn	Chase	Valent, Duda-Tassel, and Jayem	572	NT	76.2	Maize	Y	R-98114	28-April	9-Nov
26	2015	Popcorn	Chase	Jayem, Ascalon, and Valent	561	NT	38.1	Wheat	Y	R-427	2-May	20-Oct

† Observed rainfall during growing season

‡ Till = tillage system including conventional tillage consisting of disk or chisel plow tillage (CT) or no-till (NT)

Table 3-3. Soil background information for each location. No soil micronutrient levels were below critical levels ¶ as reported by Ward (2015).

Site	Soil Analysis (0-20 cm)															
	Texture†	SOM‡	CEC§	pH	NO_3–N	P Bray 1	P Bray 2	K	Mg	S	Ca	Mn¶	B¶	Zn¶	Fe¶	Cu
		g kg^{-1}	*meq./100g*	*-log(H+)*	*kg ha^{-1}*	*(mg kg^{-1})*										
1	siL	23.9	18.7	5.6	7.6	7.1	12.3	285	374	10.4	2102	15.3	0.4	2.4	66.6	1.0
2	siL	23.2	17.8	6.2	6.6	20.8	33.0	304	407	11.9	2364	9.5	0.5	1.1	63.3	1.1
3	siL	26.6	18.7	6.0	6.3	5.7	13.0	344	476	9.6	2177	11.0	0.5	1.0	64.2	1.3
4	siL	28.7	26.0	7.1	27.6	29.4	70.5	302	601	10.6	3821	8.3	0.9	3.6	26.6	0.8
5	siCL	26.1	23.9	6.2	17.7	68.3	95.4	354	558	11.9	3015	23.2	0.8	5.9	71.7	1.2
6	siCL	24.2	19.7	6.4	11.9	14.3	23.3	266	468	10.6	2662	11.8	0.7	1.9	51.7	1.5
7	siC	29.8	32.2	6.5	48.9	35.2	107.3	273	793	18.3	4402	15.1	0.8	1.9	53.5	2.8
8	lS	26.0	12.6	6.3	17.9	28.0	53.0	301	206	15.0	1765	7.0	0.4	2.1	37.0	0.9
9	saL and lfS	14.0	12.1	7.6	12.3	74.0	175.0	220	151	13.0	2063	3.0	0.6	5.2	20.0	0.9
10	siL	22.0	22.4	5.6	87.4	68.0	136.0	446	415	17.0	2498	17.0	0.6	1.9	42.0	1.5
11	siL	27.0	20.7	6.0	20.2	110.0	135.0	591	377	14.0	2591	18.0	0.7	3.3	60.0	1.7
12	siCL	21.0	16.1	6.1	9.5	26.0	48.0	224	331	11.0	2116	21.0	0.5	2.1	51.0	1.2
13	siCL	23.0	16.2	6.0	9.0	24.0	43.0	219	336	12.0	2082	22.0	0.5	1.9	49.0	1.1
14	lS	14.0	5.6	6.2	9.0	18.0	22.0	83	96	10.0	771	6.0	0.3	4.6	21.0	0.6
15	lS	13.0	5.6	6.3	8.1	17.0	21.0	81.0	101	11.0	786	6.0	0.3	4.7	23.0	0.5
16	lS	13.0	5.8	6.1	7.8	19.0	26.0	74	98	10.0	799	5.0	0.3	5.9	25.0	0.5
17	lS	12.0	5.9	6.0	7.9	18.0	24.0	76.0	97.0	10.0	773	6.0	0.4	4.9	22.0	0.6
18	siCL	33.0	24.6	7.9	13.5	26.0	84.0	274	342	13.0	4206	5.0	0.9	2.0	18.0	1.9
19	L	27.4	14.1	7.1	53.1	45.0	99.4	595	301	36.6	1983	6.8	0.9	4.1	14.3	0.4
20	lS	17.0	16.9	7.2	78.5	44.0	140.0	332	148	13.0	2956	2.0	0.6	5.6	13.0	0.6
21	vfSL and lS	18.0	17.7	7.5	94.2	13.0	130.0	390	146	13.0	3087	2.0	0.6	5.9	8.0	0.7
22	vfSL and lS	25.0	21.4	8.0	46.0	39.0	118.0	528	192	15.0	3695	2.0	1.0	5.2	10.0	0.7
23	L	23.0	18.8	7.8	59.4	51.0	144.0	495	175	17.0	3216	2.0	1.0	6.7	10.0	0.6
24	L and siL	17.0	22.5	8.2	78.5	4.0	99.0	612	236	26.0	3799	3.0	0.9	3.3	4.0	0.4
25	lS and fS	18.0	13.9	6.3	38.1	66.0	120.0	502	241	14.0	1815	6.0	0.5	2.8	26.0	1.2
26	lS and saL	10.0	7.8	6.1	22.4	43.0	52.0	210	170	10.0	957	6.0	0.4	2.7	48.0	1.3

† Soil texture classes: silt loam (siL), silty clay loam (siCL), silty clay (siC), loamy sand (lS), sandy loam (saL), loamy fine sand (lfS), loam (L), very fine sandy loam (vfSL), and fine sand (fS)

‡ SOM = soil organic matter

§ CEC = cation exchange capacity

¶ Soil critical levels for B, Mn, Fe, and Zn: 0.1, 0.5, 1.0, 0.25 mg kg^{-1}, respectively

Table 3-4. Analysis of variance results for yield and plant nutrient concentration data

	Yield			Plant Tissue Concentration											
Site	Trt	Control	Statistic	N	Control N	Statistic	P	Control P	Statistic	K	Control K	Statistic	S	Control S	Statistic
	Mg ha^{-1}		P>F	g kg^{-1}		P>F	g kg^{-1}		P>F	g kg^{-1}		P>F	g kg^{-1}		P>F
1	16.19+†	15.44+	0.06	31.0	30.4	0.27§	3.0	2.8	0.12	22.8	22.8	1.00	1.8	1.8	0.59§
2	15.75	15.13	0.40	31.2	30.3	0.61§	3.1+	2.9+	0.07	24.7	25.3	0.67	2.0	2.1	0.21§
3	14.62	14.06	0.56	31.0	29.1‡	0.22§	2.9	2.7	0.27	22.6	23.6	0.21	1.7	1.7	0.68§
4	15.57	15.69	0.87	35.1	34.8	0.81	3.7+	3.6+	0.09	20.5	21.0	0.43	1.9	1.9	0.73§
5	15.94	16.38	0.42	32.5	33.7	0.46	4.3*	4.6*	0.03	19.3‡	20.7	0.19	2.3	2.1	0.19
6	15.25	15.19	0.20	28.2‡	30.2	0.41	3.7+	4.4+	0.08	22.4	21.9	0.30	2.7	2.6	0.13§
7	15.44	15.44	0.98	32.5	34.2	0.13	3.6	3.6	0.92	24.6	23.1	0.30	2.5	2.5	0.35
8	12.74	12.80	0.72	28.6‡	27.3‡	0.17	3.4	3.4	0.94	25.3	25.8	0.60	1.6	1.7	0.32§
9	16.19	16.19	0.69	33.7	33.4	0.28	2.9	2.7	0.50§	22.1	22.7	0.54	1.9	1.9	0.64
10	14.62+	15.19+	0.06	28.5‡	28.4‡	0.23§	2.8	2.8	1.00	29.4	29.3	0.92	2.2***	2.3***	0.001§
11	15.13	15.06	0.69	29.7‡	29.5‡	0.93§	3.1	3.0	0.43	30.0	30.0	0.89	2.3	2.3	0.81§
12	15.69	15.63	0.52	24.5‡	24.9‡	0.58	2.3‡	2.3	0.65	22.3	21.0	0.15	1.8	1.8	0.17§
13	15.38***	15.57***	0.001	25.9‡	26.1‡	0.71	2.6	2.7	0.43	22.3	22.7	0.28	1.9	1.9	0.62§
14	13.37**	12.68**	0.01	20.0‡	22.0‡	0.48	2.3‡	2.6	0.13	19.0‡	21.0	0.54	1.6	1.6	0.52§
15	13.62	13.37	0.62	22.5‡	22.7‡	0.67	3.1	3.0	0.59	20.7	20.9	0.73	1.9	1.8	0.33§
16	12.49	12.68	0.71	26.3*‡	23.5*‡	0.05	3.2	3.1	0.83	15.9‡	14.7‡	0.13	2.1	1.8	0.13§
17	11.42	11.80	0.58	31.9	30.9	0.34	3.2	3.4	0.35	23.9	24.2	0.42	2.1	2.1	0.47§
18	13.06	12.68	0.36	31.2	30.4	0.25	3.7	3.7	0.83	25.8	26.2	0.83	1.9	1.9	0.79§
19	14.25	13.68	0.12	31.4	29.7‡	0.21§	3.1+	2.9+	0.08	26.8	26.3	0.74	1.8	1.9	0.84§
20	12.30	11.99	0.49	34.5	32.7	0.27	3.9	3.6	0.17	30.5*	26.3*	0.04	2.3	2.2	0.44
21	13.18	13.06	0.59	33.1	31.1	0.25	4.0	3.9	0.62	32.3	33.3	0.70	2.3	2.2	0.14
22	12.37	13.12	0.13	33.8	31.2	0.27	5.3	4.7	0.13	34.7	34.9	0.91	2.2+	2.1+	0.06
23	13.87**	13.24**	0.01	30.3	31.7	0.35	4.5*	5.0*	0.02	33.3	32.3	0.72	2.0	2.1	0.41
24	10.92	11.30	0.70	32.7	32.7	0.29§	3.9	3.6	0.41	31.2	29.4	0.81	2.9	3.0	0.36
25	4.90	4.90	0.97	32.0	31.5	0.84	3.9*	3.6*	0.05	36.2	34.5	0.46	2.1	2.0	0.39
26	7.59	7.47	0.28	37.5	38.1	0.71	4.2	4.4	0.44	29.8	28.9	0.46	2.6	2.8	0.25

† Means followed by mean comparison significant F test: Not Significant >0.10; + >0.05; *>0.01; **>0.001; ***≤0.001

‡ Plant tissue concentration is below reported critical value for maize prior to tasseling as reported by Mills and Jones 1996. Critical values used are as follows: 3.00 g kg^{-1} N, 0.25 g kg^{-1} P, 2.00 g kg^{-1} K, 0.15 g kg^{-1} S, 15 mg kg^{-1} Zn, 15 mg kg^{-1} Mn, 4.0 mg kg^{-1} B, and 10 mg kg^{-1} Fe.

§ Plant parameter received a foliar application of the corresponding analyzed elemental concentration

Table 3-4. Continued

Plant Tissue Concentration											
Zn	Control Zn	Statistic	Mn	Control Mn	Statistic	B	Control B	Statistic	Fe	Control Fe	Statistic
------mg kg^{-1}------		P>F	------mg kg^{-1}------		P>F	-----mg kg^{-1}-----		P>F	------mg kg^{-1}------		P>F
32	30	0.32§	87***	75***	0.001§	6.3	5.0	0.27§	189	181	0.47
35*	31*	0.03§	80	71	0.41§	5.0	5.0	1.00§	124	122	0.75
31	27	0.16§	84	77	0.58§	7.0	6.3	0.53§	158	153	0.63
34	33	0.25§	73*	57*	0.05§	5.5	5.0	0.18§	197	213	0.14
20	21	0.87	80	98	0.32	5.5	5.8	0.53§	137	136	0.91
23+	22+	0.06§	46*	57*	0.03§	4.2	3.7	0.58§	138	140	0.87
29	27	0.13	67	64	0.76	5.0	8.0	0.16§	176	171	0.19
23	18	0.10§	54	59	0.28§	6.5	7.8	0.19§	118	124	0.27
17	18	0.24§	50*	62*	0.05§	6.4	6.7	0.15	103	107	0.22
27*	23*	0.02§	96*	109*	0.05§	8.5+	9.3+	0.08	128*	141*	0.04§
22	19	0.23§	67	78	0.51§	9.2	9.7	0.66	125	122	0.84§
23**	18**	0.0003§	90	82	0.36§	4.9	4.6	0.71§	131	134	0.32
19	18	0.24§	48	41	0.31§	4.7	4.3	0.43§	164	161	0.83
26*	19*	0.05§	59	38	0.20§	5.5	6.5	0.70§	117	112	0.25
29	20	0.13§	57	63	0.74§	6.4	6.1	0.61§	113	109	0.64
41+	26+	0.06§	48	58	0.63§	5.5	6.5	0.70§	152	146	0.45
31*	22*	0.03§	59	54	0.84§	6.2	5.9	0.48§	150	146	0.33
18	18	0.74§	68	67	0.82§	5.5	6.0	0.71§	163	154	0.32
21	21	0.87§	69*	60*	0.05§	7.0	6.0	0.50	79	72	0.18§
33	27	0.13	66	48	0.35	17.0	16.0	0.96	86	80	0.73§
30	30	0.87	57	58	0.59	16.0	12.0	0.36	72	69	0.48§
41	42	0.91	71	72	0.90	12.0	11.0	0.52	68+	59+	0.10§
40	43	0.54	67	69	0.75	11.0	12.0	0.60	70	60	0.19§
54	57	0.19	98	97	0.41	11.0	12.0	0.72	68	72	0.27§
32	30	0.46	48	51	0.51	8.0	8.0	1.00	94	92	0.73§
45	47	0.42	67	71	0.40	21.0	21.0	0.87	116	124	0.33§

Table 3-5. Three-year combined analysis of variance testing treatment effects of identical treatments on grain yield and leaf tissue nutrient concentrations

Product	Combined Studies	Total Reps	Yield			N			P		
			Trt†	Site‡	Trt*Site‡	Trt†	Site‡	Trt*Site‡	Trt†	Site‡	Trt*Site‡
			-Mg ha^{-1}-	--P>F--	--P>F--	g kg^{-1}	--P>F--	--P>F--	g kg^{-1}	--P>F--	--P>F--
Brandt Smart Trio (Zn, Mn, B)	1, 2, 3	9	0.7+§	0.03*	0.97	01.3*¶	0.59¶	0.62¶	0.2**	0.13	0.68
MAX-IN Boron	5, 7	12	-0.2	0.06+	0.56	-1.5+	0.77	0.74	-0.1	<0.001***	0.13
MAX-IN Ultra ZMB (Zn, Mn, B)	4, 6, 14, 15, 16, 17	25	0.1	<0.001***	0.63	-0.1	<0.001***	0.29	-0.2	<0.001***	0.17
MAX-IN Ultra ZMB and MAX-IN Boron	8, 12, 13, 18	54	0.1	<0.001***	0.17	0.60	<0.001***	0.22	0.010	<0.001***	0.94
Attain (Zn, Mn, Fe, B) and MAX-IN Boron	10, 11	16	-0.3	0.35	0.05*	-0.4¶	0.08+¶	0.55¶	0.07	0.10+	0.38
Versa Fe liquid Fe + Lockdown surfactant (corn)	20, 21, 22, 23	16	0.4**	0.04*	0.03*	1.20	0.44	0.28	0.20	<0.001***	0.02*
Versa Fe liquid Fe + Lockdown surfactant (popcorn)	25, 26	8	0.1	<0.001***	0.56	-0.06	0.002**	0.63	0.06	0.02*	0.07+

† Mean difference between control and treatment. Negative values indicate the control is greater than the treatment mean.

‡ P statistic for site effect and trt*site interactions.

§ Significant F test: Not Significant >0.10; + >0.05; *>0.01; **>0.001; ***≤0.001

¶ Plant parameter received a foliar application of the corresponding analyzed elemental concentration

Table 3-5. Continued

K			S			Zn			Mn			B			Fe		
Trt†	Site‡	Trt*Site‡	Trt†	Site‡	Trt*Site‡	Trt†	Site‡	Trt*Site‡	Trt†	Site‡	Trt*Site‡	Trt†	Site‡	Trt*Site‡	Trt†	Site‡	Trt*Site‡
g kg^{-1}	--P>F--	--P>F--	g kg^{-1}	--P>F--	--P>F--	-mg kg^{-1}-	--P>F--	--P>F--	-mg kg^{-1}-	--P>F--	--P>F--	-mg kg^{-1}-	--P>F--	--P>F--	-mg kg^{-1}-	--P>F--	--P>F--
-0.5	0.01**	0.68	-0.03¶	0.03*¶	0.24¶	3.4**¶	0.34¶	0.45	9.3*¶	0.44¶	0.91¶	0.7¶	0.05*¶	0.47¶	5.0	0.17	0.25
0.04	0.004**	0.10+	0.10	0.14	0.43	1.2	<0.001***¶	0.14	-7.2	0.03*	0.28	-1.7+¶	0.35¶	0.15¶	3.0	0.09+	0.16
-0.2	<0.001***	0.26	0.02¶	0.006**¶	0.01*¶	6.1***¶	<0.001***¶	<0.001***¶	4.2+¶	0.10+¶	0.005**¶	-0.3¶	0.004**¶	0.34¶	-2.0	0.34	0.13
0.10	<0.001***	0.45	-0.003¶	0.02*¶	0.48¶	3.3***¶	0.30¶	0.01**¶	1.7¶	0.04*¶	0.53¶	-0.5¶	0.01**¶	0.53¶	-2.3	0.01**	0.33
-0.02	0.45	0.87	-0.07¶	0.36¶	0.58¶	2.9**¶	0.009**¶	0.68¶	-11.5¶	0.01**¶	0.91¶	-0.6¶	0.60¶	0.81¶	-5.0¶	0.10+¶	0.22¶
1.00	0.02*	0.33	0.040	0.21	0.22	0.8	0.007**	0.50	3.4	0.06+	0.35	1.1	0.29	0.91	8.1+¶	0.04*¶	0.91¶
1.30	<0.001***	0.72	-0.04	<0.0001***	0.11	0.3	0.02*	0.26	-3.8	0.02*	0.88	0.1	<0.001***	0.89	-3.6¶	0.005**¶	0.27¶

CHAPTER 4: EFFECT OF FOLIAR MICRONUTRIENTS (B, MN, FE, ZN), APPLIED AT DIFFERENT RATES AND TIMES, ON MAIZE (ZEA MAYS L.) GRAIN YIELD, AND MICRONUTRIENT RECOVERY, UPTAKE, AND PARTITIONING

4.1 Abstract

Timing of micronutrient demand and acquisition by maize is nutrient specific and associated with key vegetative and reproductive growth stages. The objective of this study was to determine the fate of foliar-applied B, Fe, Mn, Zn, and Fe/Zn together, evaluate the effect of foliar micronutrients applied at multiple rates and growth stages on maize grain yield, and determine their apparent nutrient recovery efficiency (ANR). Five RCBD experiments were conducted in 2014 and 2015 at five locations across Nebraska. Total dry matter was collected at 5-6 stages, and separated into leaves, stalk, and reproductive tissue as appropriate to determine micronutrient uptake, partitioning, and translocation. Foliar B, Mn, Zn, and Fe/Zn had no effect on grain yield for most application time by rate levels, though, at the foliar Mn site, there was a 19% yield increase due to a V18 application of 0.73 kg Mn ha^{-1} which corresponded with reduced Mn uptake in maize grown in control plots. At the foliar Zn site, there was a yield decrease of 4.5% due to a split application of foliar 0.84 kg Zn ha^{-1} total, applied at V11 and V15 which increased leaf Zn concentrations greater than the established toxic level. Only the Fe site had consistent grain yield response and was the only experiment that had visual signs of micronutrient deficiency. Regardless of application time from V6 to R2, there was a 13.5-14.6% increase in grain yield due to a foliar application of 0.22 kg Fe ha^{-1}. Foliar applications of B, Mn, and Zn increased their respective micronutrient

concentration and uptake in leaf, stalk, and reproductive organs. Applications of Mn and Zn also affected grain Mn and Zn concentration and uptake, respectively. Most micronutrients had limited or no translocation, however, early season applications of B, prior to V10, had significant mobilization to reproductive tissues at or after VT. Foliar Mn, Zn, and B application had ANR LSmeans of 9.5, 16.9, and 2.5%, respectively whereas the Fe/Zn mix had negative ANR LSmeans of -9.1% Fe and -1.3% Zn indicating suppression. These data highlight the importance of confirming a micronutrient deficiency prior to foliar application, guide specific growth stages to target with specific micronutrients, track the fate of foliar-applied micronutrients, and describe the variable effect of foliar-applied micronutrients on grain yield.

Abbreviations: V(1-18): vegetative growth stage; R(1-6): reproductive growth stage; RCBD: randomized complete block design; ANOVA: analysis of variance; ANR: apparent nutrient recovery; T: Time of foliar application (1 : early (V6-11), 2: middle (V15-18), 3: 4: late (R1-4)); and R: Treatment rate (rate 1: lower level of industry recommendation & rate 2: upper level of industry recommendation)

Keywords: Foliar, Maize, Boron, Iron, Manganese, Zinc, Uptake, Partitioning, Translocation

4.2 Introduction

Best nutrient management practices require matching nutrient application and availability with plant demand and nutrient uptake. In order to maximize fertilizer uptake and utilization, it is essential to apply or have nutrients available at the time of greatest demand (Roberts, 2007). Timing of nutrient demand and acquisition by maize is nutrient specific and associated with key vegetative or reproductive growth stages (Bender et al., 2013). Bender et al. (2013) discuss the need to develop recommendations to better time nutrient applications to match each nutrient's uptake and mobilization characteristics especially during periods of high vegetative uptake for high yielding modern maize hybrids. This is especially critical for micronutrient applications, as micronutrients are needed in relatively small but critical amounts by maize at specific growth stages during the growing season (Marschner, 2012).

For most nutrients, seasonal uptake in maize is sigmoidal with the maximum rates of nutrient uptake occurring between V10 and V14 and plateauing at VT/R1. As much as two-thirds of boron (B), manganese (Mn), and iron (Fe) uptake occurs before reproductive growth stages compared to only one-half of zinc (Zn) uptake. For Zn, more than 70% of Zn uptake occurs slightly later during one-third of the growing season in late vegetative and early reproductive growth. B uptake follows a similar trend with 65% of B uptake occurring during one-fifth of the growing season during late vegetative growth. Fe uptake has two periods of critical accumulation; between V10 and V14, and after R4, whereas Mn uptake is more gradual with a majority of Mn uptake occurring from V10 to

R4 (Bender et al., 2013). Each of these periods of high micronutrient uptake and demand should be targeted for micronutrient specific application.

Micronutrient foliar sprays are of widespread use in agricultural production. The application of foliar micronutrients to correct or avoid micronutrient deficiencies under conditions where soils provide limited availability is a common practice worldwide (Fageria et al., 2009; Kannan, 2010). Further, while Nebraska soils are generally fertile, maize has a high rate of nutrient uptake during specific growth stages and demand may exceed supply (Bender et al., 2013). This is especially true for less mobile nutrients, such as Ca and Mn, in that they are not able to translocate in plant tissues to meet demands in other plant tissues when soils temporally have limited micronutrient supply (Marschner, 2012).

Foliar applications have several benefits which often makes this method an ideal choice for application of micronutrients over soil applications. These benefits include: (1) the avoidance of interaction with soil properties that may inhibit the solubility and availability of the applied micronutrient to the plant such as excessively acidic or basic soil pH, limited soil water, herbicidal tie-up, sorption of metallic micronutrients (Cu, Fe, Mn, and Zn) by soil clays, etc.; (2) foliar applications of micronutrients can be applied in-season at precise times during crop utilization and often combined with other agrochemical applications; (3) plants often display a more rapid response to foliar-applied micronutrients, and (4) a greater uptake often occurs due to foliar-applied micronutrients as compared to soil applications (Fernández et al., 2013). Thus, micronutrient foliar sprays are of general interest for use as a tool to manage micronutrients. However, unlike soil applications which can be available for plant uptake

over a period of time, foliar applications of micronutrients are usually only cost effectively delivered at a one-time application time. Therefore, the application time of foliar micronutrients is critical to achieving the greatest likelihood of response.

Most research on foliar-applied B, Mn, Fe, and Zn on maize have focused on single application times, both in deficient and sufficient field locations and have reported inconsistent and mixed results (Arif et al., 2007; Bukvić et al., 2003; Godsey et al., 2003; Heckman, 2002; Mascagni Jr and Cox, 1984; Mueller and Diaz, 2011; Nelson and Meinhardt, 2011; Potarzycki and Grzebisz, 2009; Ziaeyan and Rajaie, 2009) (Chapter 3 and 5). Potarzycki and Grzebisz (2009) reported an increase in maize grain yield of nearly 18% for a three-year average with the application of 1.0 to 1.5 kg foliar Zn ha^{-1} and Nelson and Meinhardt (2011) reported an increase in maize grain yield of 6% for a three-year average with the application of 0.56 kg foliar B ha^{-1} while many others show no significant yield increase. Differential yield responses for each of these studies were not associated with a consistent predictor such as low soil or plant nutrient concentration, soil organic matter, pH, or texture.

Within the plant, essential nutrients have been classified as highly mobile (N, P, K, Mg, S, Cl), conditionally mobile (Fe, Zn, Cu, B, Mo), and immobile (Ca, Mn) but this is species dependent, and little is known about the specific mobility of each in maize (Marschner, 2012). Foliar applications of more mobile nutrients are likely to translocate and induce more systematic response; in contrast to less mobile nutrients which are likely to have only a localized effect. For example, foliar applications of Zn, Mn, Ca, and Fe have largely local effect with only limited transport out of the sprayed leaf tissue

(Fernández et al., 2013; Zhang and Brown, 1999). Nevertheless, this localized effect may still be enough to have impact on crop production.

There is a need to determine when to apply a foliar application of micronutrients to maximize each nutrient's uptake and mobilization characteristics. Knowledge of the dynamics of micronutrient accumulation to sink organs and the fate of foliar-applied micronutrients at specific growth stages would provide a useful tool to deliver micronutrients more efficiently to meet demand, thus improving nutrient management and sustainable intensification. For many crops, soil micronutrient recovery efficiency ranges from only 5-10%, however there is a lack of data on the recovery efficiency of foliar-applied micronutrients applied at different rates and growth stages in maize production (Alexander and Schroeder, 1987; Mortvedt, 1994). There is also a lack of data regarding the fate of micronutrients applied to the leaf surface of maize. Do the applied micronutrients stay in the leaf only having localized effect or do they mobilize to other metabolically active sink cells in other plant tissues? Do micronutrients applied to older, more mature leaves have similar effects as micronutrients applied earlier in the growing season to immature leaves? As leaves develop, they transition from sink organs which import nutrients, to source organs which export nutrients to other plant tissues. Mature leaves also become less capable of importing nutrients while immature leaves are entirely dependent on the import of nutrients and are physiologically incapable of exporting nutrients (Koontz and Biddulph, 1957). It can be theorized that applications to immature leaves would be more likely to take-up the applied nutrients but less likely to be a source of the micronutrients to other plant organs, at least until maturity. Applications to older mature leaves may have reduced recovery efficiency but may be more capable of

becoming a source of foliar absorbed nutrients. These data would be valuable for understanding variation in yield response to specific nutrients applied at specific times and further direct application guidelines.

Studies have shown that cuticular penetration of foliar-applied nutrients is largely a diffusion process, though ions can also be transported into the leaf by facilitated diffusion (Yamada et al., 1965). Foliar solutes can also enter the leaf through cuticle cracks and imperfections and through the stomata, leaf hairs, trichomes, and other specialized epidermal cells (Fernández and Eichert, 2009). After passing through the cuticle, nutrients can accumulate in the intercellular space, a region outside of the cell wall of the leaf before moving to metabolically active sink cells (Baligar and Duncan, 1990). Once inside the leaf, nutrients have two pathways to reach vascular tissues: apoplastic or symplastic transport. The free space between cells provides a pathway for apoplastic movement of nutrients. Cells can also actively or passively transport nutrients through the cytoplasmic continuum, (specific ion channels and aquaporins) thereby directly moving nutrients from cell to cell through symplastic transport (Baligar and Duncan, 1990). The rate of translocation depends on the specific nutrient and the plant species (Bukovac and Wittwer, 1957). Once assimilated into a metabolic role, micronutrients have limited remobilization to other new growth plant organs which makes timing of micronutrient supply with demand even more important (Fernández and Brown, 2013).

The objective of this study was to determine the fate of micronutrients applied to the leaf surface, determine the recovery efficiency of the foliar-applied micronutrients, and evaluate the effect of foliar-applied micronutrients on maize grain yield when applied

at key growth stages at high yielding locations where maize plants have low (i.e. near critical levels but not necessarily below) plant tissue or soil concentrations of the applied micronutrient but may not have a confirmed micronutrient deficiency. High yielding crops have greater micronutrient demand and thus were targeted for inclusion in this trial (Xue et al., 2014). These data will be useful to compare to the conventional deficiency correction theory to the temporal deficiency theory. B, Fe, Mn, and Zn application were considered most agronomically important to Nebraska maize production based on a soil and plant tissue sampling survey (Chapter 2) and agronomic testing laboratory data and thus were evaluated.

4.3 Material and Methods

4.3.1 Experimental Design and Site Selection

Five multi-location randomized complete block design (RCBD) field trials were performed in 2014 and 2015 in Nebraska (Table 4-1). Replications at each location were blocked by soil type using Web Soil Survey (USDA, 2013). All locations had nine treatment combinations of treatment rates and application times (see Section 4.3.2 and Table 4-3 for treatment combinations) and nine replications for yield and six replications for whole plant sampling except the foliar Fe/Zn location which had twelve treatment combinations and four replications for yield and whole plant sampling. Locations were selected prior to foliar treatment based on having a past maize yield history in excess of 12.5 Mg ha^{-1} and having spring soil and/or plant tissue samples (V5-6) (Abendroth et al.,

2011) indicating “deficient” or “low” levels of the target micronutrient according to industry standards and Mills et al. (1996) (Table 4-2). The upper most fully collared leaf from nine V5-6 plants were combined for each plant tissue sample in each block. Similarly, nine soil cores, 20 cm deep, were collected from each block and combined for each soil sample in each block (Oakfield Apparatus Company, Oakfield, WI, 2.5 cm diameter).

Pre-season nitrogen applications varied by source and rate but all locations had applied nitrogen at a rate sufficient for 13-16.0 Mg ha^{-1} maize grain production (Shapiro et al., 2003). Additionally, a onetime application of 100 kg N ha^{-1} was applied to the Mn, Zn only, and Fe/Zn locations in the form of urea at R1 in response to mid-season heavy rainfall and hail damage at these locations. This late season application of N was supplied in an attempt to remediate any N losses due to adverse weather conditions. Three of the five locations were fully irrigated by center pivot irrigation and two locations had no irrigation; however the two locations without irrigation had rainfall approximately 250 mm greater than their ten-year averages. All locations had rainfall greater than their ten year averages, maize as their previous crop, and had 0.76 m row spacing (Table 4-1). Other agronomic practices were chosen by the producer as to best mediate pest, weed, or other fertility issues. Management practices and relevant site information can be found in Table 4-1 and 4-2.

4.3.2 Micronutrient Foliar Treatments and Whole Plant Sampling

Micronutrient foliar treatments were assigned at each site based on micronutrient recommendations from in-season V5-6 plant tissue samples and/or spring soil samples (0-20 cm) (Table 4-2 and 4-3). Foliar treatments were applied by backpack sprayer (R and D Sprayers, Opelousas, LA) using a XR 11003VS flat-fan nozzle tip (TeeJet Technologies, Spray Systems Co., Wheaton, IL) at 140 L ha^{-1} and a pressure of 207 kPa. Foliar treatments were applied to four row plots 9.1 m long by 3.05 m wide (i.e. four rows with 0.76 m spacing) approximately 0.3 m above the canopy. The center two rows were harvested for grain yield determination, and destructive whole plant samples were collected from the outer two rows. A buffer row and 0.6 m alleys bordered each plot to prevent cross-contamination by spray drift. Micronutrient foliar treatments were applied at three growth stages (1: early (V6-11), 2: middle (V15-18), 3: late (R1-2)) and two rates (1X rate: lower level of industry recommendation and 2X rate: upper level of industry recommendation) (Table 4-3). These rates were within the "usual application rates range" on a nutrient bases for B and Mn (i.e. < 1 kg ha^{-1} B and 1-10 kg ha^{-1} Mn) and slightly below for Zn and Fe (i.e. 1-10 kg ha^{-1} Zn or Fe) as reported by Mortvedt (1994). The Fe/Zn location had treatments applied at four growth stages which added a R4 application. The foliar micronutrient treatments were: MAX-IN® Boron (WinField Solutions: St. Paul, MN) 8.0% B derived from boric acid, MAX-IN® Ultra Manganese (WinField Solutions: St. Paul, MN) 15.62% $MnSO_4$, Origin® Zinc 9% (WinField Solutions: St. Paul, MN) 9.0% ZnEDTA (zinc-ethylenediaminetriacetate), and ULTRA-CHE IRON 4.5% HEDTA (WinField Solutions: St. Paul, MN) 4.5% FeHEDTA (iron-

hydroxyethylenediaminetriacetate). All treatments contained CornSorb® proprietary surfactants, saccharides, and antifoaming solvents. The Fe/Zn location used a custom blend of both ULTRA-CHE IRON 4.5% HEDTA (4.5% FeHEDTA) and Origin® Zinc 9% (9.0% ZnEDTA). The micronutrient foliar treatment rates, mass of applied nutrient, and concentrations are provided in Table 4-3.

To evaluate the fate, partitioning, and mobility of the micronutrient foliar treatments, six plants were sampled from each plot at five growth stages: (1) V6-7 prior to foliar treatments, (2) V13-15 following the "early (T1)" foliar treatments, (3) V17-VT following the "middle (T2)" foliar treatments. (4) R2-3 following the "late (T3)" foliar treatments, (5) R6 final collection (following a R4 application (T4) only at the Fe/Zn location). Six plants were cut at the soil surface from 8:00 to 11:00 AM and separated into four components and are reported as stalk, leaf, reproductive (tassel, cob, and husk), and grain tissues (Bender et al., 2013). The sampling and partitioning protocol was similar to Bender et al. (2013). However, in our trial we imposed treatments of foliar micronutrients whereas Bender et al. (2013) did not impose fertilizer treatments. Each component was weighed no longer than five hours following harvest. Stalk tissue for reproductive stage plants were shredded with a commercial chipper (MacKissic Inc., Mighty Mac 12P Shredder-Chipper) to obtain a representative sub-sample, reduce the amount of matter needed for drying, and insure uniform dry-down. Leaf, reproductive, and grain six plant samples were not sub-sampled as the entire quantity of matter had uniform dry-down. Partitioned samples were oven-dried at 65°C to constant mass, weighed, and foliage analyzed for nutrient concentrations (Midwest Laboratories, Omaha, NE). A ratio of water content to dry matter content was calculated using the

initial subsample weight and the final dry sub-sample weight. This ratio was used to calculate the weight of dry matter in the initial harvested stalk sample. All units are expressed on a dry weight (0 g kg^{-1} water content) basis. Grain nutrient analysis was performed from the six partitioned plants, whereas yield estimates were harvested at physiological maturity (R6) from the middle two rows of each plot with a plot combine (Almaco, SPC40) and standardized to 155 g kg^{-1} water content.

Foliar-treated plant tissue samples were not washed. Arkley et al. (1960) determined that the micronutrient concentrations of leaf tissue treated with foliar micronutrient sprays were significantly altered between washed and unwashed leaf samples using various washing methods, and thus we would expect some wash-off to occur due to an irrigation or rain event, though the amount of nutrient wash-off depended on the nutrient, adjuvants, and plant species. In some nutrient*plant species cases, the nutrient wash-off was reported to reduce the applied nutrient to pre-treatment levels. However, Arkely et al. also found that the internal concentrations of the applied micronutrients were not reduced. In scenarios following a foliar micronutrient treatment where there is a spike in the uptake of the applied nutrient followed by a decrease, this will be discussed as wash-off of the applied nutrient from the leaf surface. Laboratory analysis of plant tissue phosphorous (P), potassium (K), sulfur (S), calcium (Ca), Fe, Mn, Zn, and B were completed using microwave nitric acid digestion and concentrations determined using inductively-coupled plasma (ICP) spectroscopy. The percent nitrogen (N) was determined using the Dumas Method with a Leco FP-428 (Horwitz and Latimer Jr, 1920). Laboratory analysis of grain tissue B, Ca, Cu, Fe, Mg, Mn, P, K, Na, S, and Zn was prepared using ME PROC 69 which is based on AOAC 935.13. The analysis of these

data followed ME PROC 29 which is based on AOAC 985.01 (Horwitz and Latimer Jr, 1920). Samples were treated with a combination of heat and mineral acids to dissolve the minerals and destroy organic materials. The extract was analyzed for mineral content by Inductively-Coupled Argon Plasma Emission Spectrometer (ICAP-ES). Boron was not detected in grain samples at the 3 mg kg^{-1} detection limit. Spring soil samples (0-20 cm) were analyzed for Mn, Fe, and Zn concentration using DPTA (diethylenetriaminepentaacetic acid) extraction along with ICAP-ES detection. B concentration was measured using DPTA and used sorbitol ICAP-ES detection.

Apparent nutrient recovery (ANR) at the end of the growing season (R6) in the whole plant, above the soil surface, was calculated to reflect the efficiency of maize to recover the applied foliar micronutrient(s). ANR (%) = [(nutrient uptake fertilized (g ha^{-1}) - nutrient uptake control (g ha^{-1})) / (quantity of nutrient applied (g ha^{-1}))] X 100.

4.3.3 Statistical Analysis

Grain yield, biomass yield, nutrient uptake and nutrient concentration for all partitioned tissues (i.e. leaf, stalk, reproductive, and grain), and ANR were analyzed using PROC GLIMMIX SAS 9.3 software (SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513-2414, USA) (Littell et al., 2006). Block was designated as a random effect. A mean comparison test using the Dunnett Adjustment was used to compare treatment effects to the "control." Orthogonal contrasts for grain yield and nutrient quantity in partitioned and total foliage at differing growth stages and at individual locations were performed using the CONTRAST statement of SAS and were planned and

selected prior to analysis (Appendix Code 4-1). Nutrient uptake and partitioning graphs were generated by SigmaPlot (SigmaPlot v11.0; Systat Software Inc. San Jose, CA). Means generated from Excel (Microsoft Excel 2013, Microsoft Corp. Santa Rosa, CA) were imported into SigmaPlot and uptake curves were generated with the simple spline curve option with smoothed data points similar to Bender et al. (2013). An ANOVA for overall treatment effects on total uptake was conducted for non-applied nutrients at each location (N, P, K, S, Mg, Ca, Mn, B, Fe, and Zn). The ANOVA test for each non-foliar-applied nutrient (i.e. N, P, K, Mg, Ca, S, Cu, and Na) at each location reported no significant treatment main effects at $p \leq 0.05$ hence no further analysis was conducted.

4.4 Results and Discussion

4.4.1 Trial Locations and Selection

All selected trial locations had V5-6 plant tissue concentrations of the applied micronutrient near but not below critical levels except in the case of Fe at the combined Fe and Zn location (Winside) which was in excess (Table 4-2). Plant tissue macronutrient concentrations were also above critical concentrations in V5-6 plant tissue (Table 4-2) as reported by Mills et al. (1996) thus indicating macronutrients were likely not limiting according to Liebig's Law of the Minimum (van der Ploeg and Kirkham, 1999). All soil nutrient concentrations were also above critical levels as reported by Wortmann (2009) and Ward (2015) though lime would be recommended at the foliar B, Zn, and Fe/Zn mix

sites (Table 4-2). All locations also had a history of being relatively high yielding (i.e. maize yield history in excess of 12.5 Mg ha^{-1}).

Only the 2015 foliar Fe application location had visual signs of deficiency (i.e. interveinal chlorosis in the upper most new growth leaves) though neither soil or plant analysis Fe was below critical levels of 4.5 mg Fe kg^{-1} as reported by Ward (2015) and 50 mg Fe kg^{-1} for maize <0.3 m tall as reported by Mills et al. (1996) (i.e. 4.9 mg Fe kg^{-1} and 181 mg Fe kg^{-1}, respectively). pH was alkaline (i.e. 7.5) which likely contributed to reduced micronutrient availability and the subsequent visual signs of deficiency. This location was fully irrigated and high yielding (i.e. 14.2 Mg ha^{-1} in control plots) (Table 4-4).

The foliar B location for control and untreated plots had V6 leaf tissue below critical B concentrations (i.e. 4.0 mg B kg^{-1} in leaf tissue for maize prior to tassel) as reported by Mills et al. (1996) (Table 4-5 a.) but V5 leaf tissue analysis reported B at 6.0 mg B kg^{-1} (Table 4-2), and there were no visual signs of B deficiency. The soil DTPA extractable B concentration of 0.7 mg B kg^{-1} was also marginally above the 0.5 mg B kg^{-1} critical level as reported by Ward (2015). This location was fully irrigated and was high yielding (i.e. 14.9 Mg ha^{-1} in control plots) (Table 4-4).

The location receiving foliar Zn only had V5 plant tissue concentrations of 22.3 mg Zn kg^{-1}, which were above the Zn critical level of 20 mg Zn kg^{-1} (i.e. as reported by Mills et al. (1996). The soil analysis for this location reported DTPA extracted Zn at 0.9 mg Zn kg^{-1}, which was also marginally above the critical level of 0.75 mg Zn kg^{-1} as reported by Voss (1998), Wortmann et al. (2013), and Ward (2015). This location had

hail on July 7th with an estimated 5-10% yield reduction (Klein and Shapiro, 2011) but was still high yielding (13.7 Mg ha^{-1} in control plots) (Table 4-4).

The location receiving foliar Mn application was well above the 20 mg Mn kg^{-1} leaf tissue critical value at V5 (i.e. 62.0 mg Mn kg^{-1}), however, plant tissue Mn was relatively low compared to other sampled locations in Nebraska (Table 4-2). DTPA extracted soil Mn was 22 mg kg^{-1} which was above the 2.0 mg kg^{-1} critical level as reported by Ward (2015). The foliar Mn location had heavy rainfall (i.e. 202 mm from June 20th to July 4th) and standing water for nearly two weeks which correlated with a consistent drop in Mn plant tissue concentration and Mn uptake (Table 4-5 b. and 4-6 b.) prior to the foliar Mn treatment. Although this location had a history of yields more than 12.5 Mg ha^{-1}, heavy rainfall reduced the control yield to 7.9 Mg ha^{-1} (Table 4-4).

The location receiving both foliar Fe and Zn had plant tissue Zn at the Zn critical level of 20 mg Zn kg^{-1}, however, plant tissue Fe, 403 mg Fe kg^{-1}, was greater than the upper value of the Fe sufficiency range of 50-250 mg Fe kg^{-1} as reported by Mills et al. (1996). Both soil Zn and Fe (i.e. 1.4 mg Zn kg^{-1} and 110 mg Fe kg^{-1}) were above the soil critical levels of 0.75 and 4.5 as reported by Ward (2015). This location also had hail on July 7th with an estimated 5-10% yield reduction (Klein and Shapiro, 2011) but was still high yielding (12.7 Mg ha^{-1} in control plots) (Table 4-4).

Though none of the targeted micronutrients were below critical levels during early season leaf tissue sampling (V5-6), these nutrients fluctuate throughout the day and growing season as related to environmental stresses and in some samplings, micronutrient concentrations fell below their respective critical levels (i.e. B location) during the growing season. Leaf tissue nutrient concentrations are known to fluctuate throughout the

growing season due to environmental factors (i.e. soil water, temperature) between periods of adequate soil supply of micronutrients and periods of insufficient soil supply of the applied micronutrient. Further, the time of day for plant sampling effects plant concentrations of Fe, Mn, and Zn and can decrease nutrient concentration by as much as 243, 26, and 5 mg kg^{-1}, respectively, due to mid-day sampling as compared to morning sampling, but time of day has less effect on B concentration (Mundorf et al., 2015) and thus, these locations were still considered suitable sites that met the study objectives. . The goal was to find sites that were both high yielding and not overly supplied with micronutrients. Following the deficiency correction hypothesis, yield increases may not be expected if micronutrient concentrations do not fall below critical levels at any point during the growing season.

4.4.2 Effect of Foliar Micronutrients on Grain Yield in Relationship with Plant Nutrient Concentrations

The foliar-applied B, Mn, and Zn experiments showed these nutrients had limited effect on grain yield for most application time by rate level combinations though there was a 19% yield increase ($p=0.006$) due to a V18 application of 0.73 kg Mn ha^{-1} and a 4.5% yield decrease ($p=0.02$) due to a split application of foliar 0.84 kg Zn ha^{-1} applied at V11 and V15 compared to the control (Table 4-4).

The foliar Fe location (Imperial) had an average pH of 7.5, low plant tissue, and soil Fe concentrations, and showed visual signs of deficiency throughout the entirety of the trial growing season. Yields were consistently increased due to either a single foliar

application or a split application of foliar 0.22 kg Fe ha^{-1} (i.e. 2X). There was a 14.6% increase ($p=0.04$) due to the 2X application at V6 (i.e. T1R2), a 14.2% increase ($p=0.04$) due to a split application of the 2X rate at V6 and V15 (i.e. T1R1 and T2R1), and a 13.5% increase ($p=0.05$) due the 2X application at R2 (i.e. T3R2). The 2X rate consistently outperformed the 1X rate across all treatments and time of application did not have a significant effect on yield ($p\leq0.05$). Further, the single 1X application of foliar Fe (i.e. 0.11 kg Fe ha^{-1}) was consistently greater than the control and less than the 2X rate but not significantly. Because there were only three Fe rates no response function could be calculated. Therefore, it is unknown if a greater rate of Fe application would have greater response. These data highlight the importance of confirming a micronutrient deficiency prior to applying a foliar micronutrient treatment and is consistent with the deficiency correction theory.

At the B location (Meadow Grove), there were no significant yield effects. The B location for the control plots and untreated plots also reported V6 leaf tissue below critical B concentrations (i.e. 4.0 mg B kg^{-1} in leaf tissue for maize prior to tassel) as reported by Mills et al. (1996). Additionally, the B location control plots had end of the season R6 B leaf concentrations below critical B concentrations (i.e. 5.0 mg B kg^{-1} in leaf tissue for maize after tassel) (Table 4-5 a.). Both the T2R1 and T3R2 treatments increased B concentrations above the critical level (6.0 and 5.7 mg B kg^{-1}, respectively) in leaf tissue but this was not associated with a significant increase in grain yield ($p\leq0.05$).

The Mn location (Oakland) had significant grain yield increase due to the T2R1 treatment (i.e. V18 application of foliar 0.73 kg Mn ha^{-1}). This yield increase may be due

to heavy rainfall (i.e. 202 mm rainfall from June 20 – July 4th) where there was standing water on the experiment for approximately 2 weeks (Table 4-1) causing reduced uptake and concentration of Mn (Figure 4-2 d., Table 4-5 b., and 4-6 b.) prior to the foliar Mn treatment. Figure 4-2 d. indicates that the V18 treatments were applied during the period of reduced soil supply, therefore, preventing the dip in Mn uptake as seen in the control plots and all other non V18 treated plots. The concentration of Mn in the leaf tissue following the V18 treatment significantly increased the control from 83.5 mg Mn kg^{-1} to 108.7 mg Mn kg^{-1}; however, both of these leaf concentrations are well within the sufficiency range for maize prior to tassel (i.e. 15-300 mg leaf Mn kg^{-1}) as reported by Mills et al. (1996). Though each of the V18 treatments had yields greater than the control, none was significantly greater than the control at $p \leq 0.05$.

Applying the right rate at the right time during periods of insufficient soil Mn supply was likely the primary driver for the significant 19% increase of 1.52 Mg ha^{-1} grain yield when compared to the control (p=0.006) at the foliar Mn location. Overall, the 1X rate of 0.73 kg Mn ha^{-1} had greater effect on grain yield than did the 2X rate of 1.46 kg Mn ha^{-1}. Planned selected contrasts confirmed that the 1X rate had significantly greater effect (p=0.0008) on grain yield than the 2X rate and two separate applications of the 1X rate (i.e 0.73 kg Mn ha^{-1}) and had significantly greater effect (p=0.005) on yield than one application at the 2X rate (i.e 1.46 kg Mn ha^{-1}) (Appendix Table 4-1).

Inversely, when there is excess soil supply of the foliar-applied micronutrient, such as in the case of the foliar Zn only location (Winside), there may be yield reduction. This is supported by the significant 4.5%, 0.62 Mg ha^{-1} yield decrease as compared to the control (p=0.03). Mills et al. (1996) report that maize prior to tassel has a Zn leaf

concentration sufficiency range of 15-60 mg Zn kg^{-1}. At the Zn only location, the split application of 0.84 kg Zn ha^{-1} at V11 and V15 significantly increased the leaf Zn concentration from the control from 24.7 at V14 and 27.3 mg kg^{-1} at V17 to beyond the upper limit of the sufficiency range to 62.0 mg kg^{-1} ($p<0.0001$) at V14 and 91.3 mg kg^{-1} ($p<0.0001$) at V17 (Table 4-5 c. and Figure 4-1 g.). Though maize is relatively tolerant to high levels of soil Zn, maize can experience Zn toxicity (Takkar and Mann, 1978). Takkar and Mann (1978) report leaf tissue Zn above 81.0 kg mg^{-1} can cause grain yield reduction. This threshold was crossed by this treatment likely causing the significant yield reduction.

4.4.3 Foliar Micronutrient Uptake, Partitioning, and Translocation

Nutrient concentrations and uptake were measured in leaf and stalk prior to VT, leaf, stalk, and reproductive tissue at VT, and leaf, stalk, reproductive tissue, and grain during reproductive stages and is described as such in Tables 4-5 (a.-e.) and Table 4-6 (a.-e.). The effect of the foliar-applied nutrient on the respective nutrient concentration, biomass, and uptake (i.e. a function of both nutrient concentration and biomass) in each of the partitioned tissues through the growing season will be discussed. Overall, foliar applications of B, Mn, and Zn were effective at increasing their respective micronutrient concentration and uptake in leaf, stalk, reproductive tissues, and immature grain for Mn and Zn throughout the growing season when applied alone (Table 4-5 a.-c., d. 4-6 a.-c., and Figures 4-1, 4-2, and 4-3).

The control B uptake and partitioning was consistent with the B uptake and partitioning reported by Bender et al. (2013) though grain B was not detected at 3 mg kg^{-1} detection limit. Partitioned plant sampling following foliar B treatments at rates of both 0.14 kg B ha^{-1} and 0.28 kg B ha^{-1} and at all application times showed significant increase ($p \leq 0.05$) in B uptake and B concentration in leaf, stalk, and reproductive tissues; however, for all treatments by R6 leaf, stalk, and total B uptake had declined and were not different than the control at $p \leq 0.05$ (Table 4-6 a., and Figure 4-3). These data are evidence of no additional uptake and mobilization and the likely wash-off of late season (i.e.V15 and R1) foliar applications of B. This was not the case for early season applications of B.

Foliar application of B at V10 increased B uptake and mobilization. The T1R1 foliar B application (0.14 kg B ha^{-1} applied at V10) increased the R6 B uptake to the reproductive tissues as compared to the control by 10.3 g B ha^{-1} ($p=0.001$) which was due to a 3.7 mg B kg^{-1} concentration increase ($p=0.0005$) (Table 4-5 a. and 4-6 a.). These data suggest that earlier applications of B have greater penetration and mobility as compared to late foliar B applications. This pattern is consistent with (Bender et al., 2013; Karlen et al., 1988). Bender et al. (2013) report that stored B in leaf tissue appears to serve as a source of mobilized B to reproductive tissues which is further evident in these data (Figure 4-3 a.). Boron is known to play a critical role in flower production, pollen tube elongation, and germination and increases seed and fruit development so this mobilization to reproductive tissues is consistent with the expected B physiological demands (Dell and Huang, 1997). Though B is usually considered relatively immobile in cell wall components (Brown and Shelp, 1997), our data support previous claims that

there is a brief period leading up to VT of B mobilization from the leaf tissue to reproductive tissues (Table 4-6 a.). B is known to crosslink two chains of pectic polysaccharides through borate-diester bonding and thus form a network of pectic polysaccharides in cell walls which is then immobile. However, a majority of the water soluble B is localized in the apoplastic region or vacuoles as boric acid, and we theorize that this is the source of the mobilized B to reproductive tissues (Matoh, 1997). V10 or earlier is likely an important target growth stage for foliar B application and may be more successful at inducing grain yield response under more B deficient scenarios.

Foliar Mn and Zn studies had similar effects on their respective nutrient uptake and concentration in stalk and leaf tissue and are therefore discussed together for leaf and stalk components. Across all application stages, the 2X rate (i.e. 1.46 kg Mn ha^{-1} and 0.84 kg Zn ha^{-1}) foliar applications of Mn and Zn significantly increased ($p \leq 0.05$) the R6 total Mn and Zn uptake as compared to the control (Table 4-6 b., c.). The 1X rate was not significant. Further, analysis of R6 total plant tissue increases in Zn and Mn uptake due to foliar applications reveals that the foliar Zn and Mn stayed in the leaves and had limited mobility out of the leaves as evident by leaf tissue being the only organs to maintain significant levels ($p \leq 0.05$) of Zn and Mn uptake at R6 as compared to the control (Table 4-6 b., c.). The significant increase in total Zn uptake was largely due to increases in concentration and not biomass, whereas significant increase in total Mn uptake was due to both an increase in concentration and biomass (Table 4-5 b., c. and 4-7). It can be theorized that Zn was not limiting, unlike Mn, since the increase in Zn concentration was not associated with an increase in biomass.

Unlike Zn, foliar applications of Mn had infrequent significant effect on Mn uptake and concentration in reproductive tissues and grain (Table 4-5 b., c. and 4-6 b., c.). However, the significant increase in reproductive tissue Mn uptake was associated with the only significant increase in maize grain yield. Foliar Zn had greater effect on reproductive tissues and grain than did foliar Mn, especially late season applications. The V15 and R1 applications of foliar Zn increased R2 grain concentration by as much as 10.5 mg Zn kg^{-1} ($p<0.0001$) and reproductive tissues Zn concentration by as much as 18.8 mg Zn kg^{-1} ($p<0.0001$) as compared to the control (Table 4-5 c.).

Foliar applications of Zn followed a similar trend as Mn uptake in leaf and stalk tissue; however, Zn uptake and mobilization differed from Mn uptake and mobilization late in the growing season from VT to R6 (Figure 4-1 (ex. a.) and 4-2 (ex. a.)). During the reproductive stages (R1-6), the control Mn uptake plateaued sharply whereas Zn uptake continued to increase and partition to the grain which follows the same trend reported by Bender et al. (2013). The foliar Zn applications did not significantly increase Zn uptake in the R6 grain and reproductive tissues under these growing conditions as was previously reported (Table 4-6 c.) (Cakmak, 2008). The foliar Mn treated plots had large reductions in Mn uptake during reproductive stages that can be attributed to wash-off of foliar Mn from the leaf surface which was not assimilated during the reproductive stages (Figure 4-2 (ex. a.)). These data provide strong evidence that Mn applications after vegetative growth stages will likely be of no benefit. The sharp Mn uptake plateau during the reproductive growth stages in the control plots follows the same trend reported by Bender et al. (2013).

Though no yield response was observed due to reproductive stage applications of Zn under these conditions, reproductive foliar treatments of Zn can be theorized as having potential to affect grain yield. Zn uptake also had foliar Zn wash-off during vegetative growth stages, as evident by in-season spikes in treatment Zn uptake followed by a decrease (Figure 4-1 (ex. a.)), but had non-significant wash-off (i.e. no increase followed by a decrease in Zn uptake following a foliar treatment) for foliar Zn applications applied to maize at reproductive growth stages. This was possibly due to more rapid assimilation during high demand reproductive stages (Figure 4-1 (ex. e.)). At R6, both Mn and Zn uptake significantly increased due to all foliar rate and time treatments. These data suggest that the Zn and Mn, applied to the leaf surface, stayed in the leaf throughout the entirety of the growing season to R6.

The combined applications of Fe and Zn caused significant suppression of Fe uptake in both leaf and stalk tissues (Table 4-6 e. and Figure 4-5 (ex. a.)). The suppression of Fe due to the combined foliar application of Fe and Zn highlights the well-documented Zn-Fe-Mn antagonism as previously documented in maize by Warnock (1970). Zn uptake was not suppressed due to the combined foliar application of Fe and Zn and had similar uptake properties as observed at the foliar Zn only location (Table 4-6 c., d., e. and Figure 4-4 (ex. a.) and 4-1 (ex. a.)). Additional investigation confirms that there was no reduction in biomass as compared to the control driving the reduction in Fe uptake, rather the reduction in Fe uptake was driven by a reduction in plant tissue concentrations of Fe in treated plots (Table 4-5 e. and 4-7). Bender et al. (2013) also reported significant reduction in Fe uptake after VT and they theorized that this was due to pollen and silks (styles) shed which contains Fe (Pfahler and Linskens, 1974) being

greater than the uptake rate. Additionally, foliar Fe applications have also been shown to depress the plant's Fe stress mechanisms by preventing the increase in the Fe-reducing capacity of the roots that would normally occur during Fe deficiency (Römheld and Marschner, 1986). Investigation of the biomass data from this location indicates that the reduction in Fe uptake was not due to biomass reduction (i.e. no treatment biomass was significantly different ($p<0.05$) than the control biomass) (Table 4-7).

4.4.4 Recovery Efficiency of Foliar Micronutrients

Foliar applications of Mn and Zn had similar but slightly higher ANR than reported soil applied Mn and Zn ANR (Mortvedt, 1994). Mortvedt (1994) reports soil Mn and Zn ANR to range from 5-10%. For all locations, there were no ANR treatment main effects at $p\leq0.05$. There was a consistent trend for both Zn and Mn ANR at the 2X rate to always have a greater ANR than the 1X rate for treatments applied at the same growth stage (Table 4-8). Since there were no differences between treatment rates, ANR for all treatment rates were combined. Foliar Mn, Zn, and B only application had ANR least square means of 9.5, 16.9, and 2.5%, respectively with standard errors of 3.7, 9.6, and 2.9, respectively. The foliar application of a mix of Fe and Zn had negative ANR indicating suppression of Fe and Zn uptake which is consistent with the findings of Römheld and Marschner, (1986) who found reduced Fe uptake in grasses due to foliar Fe. At the foliar Fe/Zn location, the least square mean ANR for Zn was -1.3% with a standard error of 6.1 and the ANR of Fe was -9.1% with a standard error of 20.5 (Table 4-8).

The low ANR of each of the applied micronutrients implies that a majority of the foliar application was either sprayed directly onto the soil or was washed-off the leaf surface. Figures 4-1 a., 4-2 a., 4-3 a., and 4-4 a. all show a spike in their respective nutrient uptake immediately following foliar application; however, by the time of the next foliage sampling, there was a large reduction in the nutrient uptake likely due to wash-off of the treatment from the leaf surface. All foliar treatments were on the leaf surface for at least 24 hours prior to an irrigation or rain event. Our data also indicates that in some cases there may be suppression of the applied micronutrient under conditions of micronutrient toxicity (i.e. as in the case of Fe uptake following an Fe/Zn treatment under excessive Fe tissue concentrations), the actual amount of foliar-applied micronutrient being recovered by maize may actually be higher but causing suppression of the applied micronutrient in the plant tissue. For example, the maize plants may have recovered a higher percent of the foliar-applied micronutrient, but the treatment may have suppressed soil uptake thereby reducing the total amount of the micronutrient in the plant tissue. Whether a majority of the foliar treatment is falling to the soil or being recovered by the plant and suppressing soil uptake remains unresolved. What these data show is that foliar applications of micronutrients have a low ANR and the overall micronutrient status of the plant tissue per unit of applied micronutrient is usually less than 20% which similar but slightly higher than soil applications (Alexander and Schroeder, 1987; Mortvedt, 1994). However, this small increase in ANR may be critical if maize micronutrient status is near the critical level at a critical growth stage.

4.5 Conclusions

The five experiments are qualitatively summarized by the soil and plant status of the target nutrient in Table 4-9. Each column indicates with + and – notation whether the condition was conducive to micronutrient response. For example, at the B site, the initial leaf tissue was deficient (indicated by + +); there was no indication of high levels of tissue B, and the soil was low but above the critical value, B additions increased both leaf concentration, and nutrient uptake, but did not increase yield. These plant responses occurred at the pre-V10 growth stage and under conditions with confirmed B deficency, we would recommend foliar B applications at V10 or earlier.

Putting the specific experiments into perspective will explain some of the results. Though grain yields were lower than historic yields due to abnormal conditions at the Zn, Mn, and Zn/Fe locations (i.e. hail and sporadic rain events), all locations were high yielding and had yields greater than 12.5 Mg ha^{-1} (i.e. control yields of 14.9, 14.2, 13.7, 12.7 Mg ha^{-1}, respectively, for B, Fe , Zn , and Fe/Zn mix locations.) The foliar Mn location had a control yield of 7.9 Mg ha^{-1}. There was no evidence that greater grain and foliage yield and therefore greater demand for B, Mn, Fe, or Zn was associated with the increased likelihood of response to foliar micronutrient application. Under similar field conditions, foliar applications of Zn, B, or combined Zn and Fe treatments would not be expected to have predictable significant grain yield increases. There was some evidence of significant yield decreases when concentrations of the applied nutrient are above its sufficiency range, as was found in the case of the Zn only and Zn/Fe locations. Under

conditions of reduced Mn or Fe availability, applications of foliar Mn or Fe may increase grain yield which is consistent with the deficiency correction theory.

Of greatest interest, these data indicate that foliar applications of 0.22 kg Fe ha^{-1}, applied at any growth stage, to maize with confirmed Fe deficiency increased grain yield by approximately 14%. Fe application rates greater than 0.22 kg Fe ha^{-1} may be of greater benefit and are worth investigating as this study did not have enough rates to establish a rate-response curve. The foliar Fe only location was high yielding (approx. 16 Mg ha^{-1}) and this high yielding situation may have further contributed to the strong response. Greater grain and foliage production requires greater demand on soil nutrients, thus, plant Fe demand may have been greater than available soil Fe supply.

Though there was limited yield response to foliar B, Mn, and Zn under our study conditions, these data provide evidence for target growth stages to increase micronutrient uptake and mobilization of the applied micronutrient to tissues with physiological demand. Our data suggest foliar B applications prior to the V10 growth stage are effective at mobilizing B from leaf tissues to reproductive tissues post VT and late season (V15 and R1) applications of foliar B are less effective. Similarly, foliar Mn treatments during reproductive growth will likely have little effect on maize as evident by the sharp plateau in Mn uptake during reproductive growth and limited Mn uptake due to foliar applications applied after V18. Inversely, late applications of foliar Zn applied from V15 to R1 are effective at increasing Zn concentration in immature grain by as much as 10.5 mg Zn kg^{-1} and reproductive components by as much as 18.8 mg Zn kg^{-1} and theoretically could have effect on grain yield or biofortification under differing field

conditions. This increase in Zn concentration was not associated with an increase in Zn uptake.

There should also be caution when applying mixes of foliar micronutrients as there can be significant reductions in micronutrient uptake as evident by Fe suppression due to the combined application of Fe and Zn. Applying micronutrients to locations with sufficient to high levels of the applied micronutrient may also have significant yield reduction. Further, ANR for individually applied micronutrients were similar to reported soil recovery efficiencies and in some cases were almost double (i.e. ANR were largely less than 25%). These data could be used to calculate application rates with specific goals of increasing micronutrient concentrations in plant tissue from below critical values to above critical values. In conclusion, this study showed that foliar applications of B, Mn, Zn, and Fe had limited effect on grain yield in regions with soils and conditions similar to those of this study unless there is a confirmed micronutrient deficiency.

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Table 4-1. Background information and cultural practices for the five foliar micronutrient experimental locations

			Soil Classification		Rainfall†		GDD‡		
Year	Treatment	Nearest City	Soil Series	Great Group	Season	Avg.	Season	Till§	Row Spacing
					mm	mm	GDU		cm
2014	Boron	Meadow Grove	Belfore	Udic Haplustolls	728	646	3187	CT	76.2
2014	Manganese	Oakland	Zook	Vertic Endoaquolls	850	630	3432	CT	76.2
2014	Zinc	Winside	Moody	Udic Haplustolls	856	608	3138	NT	76.2
2014	Iron and Zinc	Winside	Moody	Udic Haplustolls	856	608	3138	NT	76.2
2015	Iron	Imperial	Rosebud-Canyon	Ardic Argiustolls & Ustic Torriorthents	535	463	3374	CT	76.2

	Previous Crop	Irrigation	Environmental Factors	Hybrid	Plant Date	Harvest Date
		Y/N				
	Maize	Y	-	Croplan 6274	25 April	8 Nov.
	Maize	Y	Standing water at 7/6 (V12) (2 weeks standing water)	Golden Harvest G14R38 Agrisure 3000GT	7 May	7 Nov.
	Maize	N	Hail 7/6 (V9) (5-10% yield reduction)	Pioneer 1625	20 May	18 Nov.
	Maize	N	Hail 7/6 (V9) (5-10% yield reduction)	Pioneer 1625	20 May	9 Nov.
	Maize	Y	-	Pioneer 1151	25 April	20 Nov.

† Observed and average (2005-2015) rainfall during growing season (from April-October)

‡ Observed growing degree days during the grwoing season (from April-October)

§ Till = tillage system including conventional tillage consisting of disk or chisel plow tillage (CT) or no-till (NT)

Table 4-2. Site-year mean leaf (V5-6) and soil analysis characteristics collected concurrently from each statistical block (i.e. nine samples averaged for each mean (foliar Zn & Fe had four samples averaged for each mean)) at each location in the spring and leaf analysis critical levels from Mills et al. (1996) and soil analysis critical levels are from Wortmann et al. (2009) and Ward (2015).

Treatment	Sampling	Leaf Analysis								Soil Analysis (0-20 cm)												
	Stage	N	P	K	S	Mn	B	Zn	Fe	Texture†	SOM	CEC	pH	NO_3–N	P Bray 1	P Bray 2	K	S	Ca	Mn	B	Zn	Fe
		(%)				(mg kg^{-1})					(%)	meq./1	-log(H+)	mg kg^{-1}	(mg kg^{-1})							
Foliar B	V5	4.19	0.26	3.83	0.33	74.3	6.0	22.5	270.3	SiCL	2.8	18.2	5.6	6.3	34	52.8	348	12	1980	20	0.7	2.43	101
Foliar Mn	V5	3.23	0.25	2.38	0.36	62.0	8.0	20.0	265.5	SiCL	3.2	27.1	6.4	13.3	28.8	89	243	78	3721	22	0.9	1.68	70.5
Foliar Zn	V5	4.24	0.38	3.93	0.34	130.5	6.8	22.3	308.3	SiCL	3.2	27.1	5.1	2.7	49	71.3	261	12	2325	37	0.7	0.9	53.5
Foliar Zn & Fe	V5	4.13	0.37	3.54	0.30	180.0	9.8	20.0	403.3	SiCL	3.6	29.4	5.0	19.6	58.8	75	276	14	2480	44	1	1.43	110
Foliar Fe	V6	4.03	0.43	3.85	0.30	129.3	12.4	102.0	181.1	L	2.4	16.2	7.5	20.9	31	102.2	454	17	2761	1.9	0.8	4.3	4.9
Critical Level‡		3.00	0.25	2.00	0.15	15.0	4.0	15.0	10.0	-	-	-	-	-	15	-	125	8.0	-	2.0	0.5	0.75	4.5

† Soil texture classes include silty clay loam (SiCL), and loams (L)

‡ Leaf analysis critical levels are from Mills and Jones (prior to tassel) and soil ananlysis critical levels are from Wortman et al. (2009) and Ward (2015)

Table 4-3. Schedule of foliar treatments† applied at various rates and times and schedule of whole plant sampling used for uptake, partitioning, and translocation analysis

Foliar Treatment (Time and Rate) and Sampling Date	2014				2015
	Location 1 (Foliar B#)	Location 2 (Foliar Mn‖)	Location 3 (Foliar Zn**)	Location 4 (Foliar Fe and Zn††)	Location 5 (Foliar Fe‡‡)
	Date (Growth Stage)				
Planting Date	April 25	May 7	May 13	May 13	April 25
Diagnostic Soil and Leaf Sample‡	June 18 (V5)	June 18 (V5)	June 18 (V5)	June 18 (V5)	June 26 (V6)
Whole Plant Sampling 1§	June 23 (V6)	June 21 (V7)	June 21 (V6)	June 21 (V6)	-
Control No Foliar Treatment	N/A	N/A	N/A	N/A	N/A
T1R1¶	July 2 (V10)	July 2 (V11)	July 8 (V11)	July 8 (V10)	June 26 (V6)
T1R2¶	July 2 (V10)	July 2 (V11)	July 8 (V11)	July 8 (V10)	June 26 (V6)
Whole Plant Sampling 2§	July 10 (V13)	July 11 (V15)	July 15 (V14)	July 14 (V14)	-
T2R1¶	July 13 (V15)	July 13 (V18)	July 16 (V15)	July 16 (V15)	July 17 (V15)
T2R2¶	July 13 (V15)	July 13 (V18)	July 16 (V15)	July 16 (V15)	July 17 (V15)
T1R1 and T2R1‡	July 2 (V10) & July 13 (V15)	July 2 (V11) & July 13 (V18)	July 8 (V11) & July 16 (V15)	July 8 (V10) & July 16 (V15)	June 26 (V6) & July 17 (V15)
Whole Plant Sampling 3§	July 21 (V17)	July 20 (VT)	July 25 (V17)	July 24 (V17)	-
T3R1¶	July 28 (R1)	July 26 (R2)	July 28 (R1)	July 28 (R1)	August 8 (R2)
T3R2¶	July 28 (R1)	July 26 (R2)	July 28 (R1)	July 28 (R1)	August 8 (R2)
T2R1 and T3R1¶	July 13 (V15) & July 28 (R1)	July 13 (V18) & July 26 (R2)	July 16 (V15) & July 28 (R1)	-	July 17 (V15) & August 8 (R2)
Whole Plant Sampling 4§	August 5 (R2)	August 4 (R3)	August 7 (R2)	August 6 (R2)	-
T4R1¶	-	-	-	August 19 (R4)	-
T4R2¶	-	-	-	August 19 (R4)	-
T1R1 and T4R1¶	-	-	-	July 8 (V10) & August 19 (R4)	-
T3R1 and T4R1¶	-	-	-	July 28 (R1) & August 19 (R4)	-
Whole Plant Sampling 5§	October 20 (R6)	October 6 (R6)	October 9 (R6)	October 10 (R6)	-
Harvest Date	November 8 (R6)	November 7 (R6)	November 18 (R6)	November 9 (R6)	November 25 (R6)

† All foliar treatments were applied to 9.1m x 3.05m plots (4 rows with 0.76 m spacing) with a backpack sprayer with four nozzles at a height of 0.3m above the canopy with a band width of 0.38m

‡ Initial soil and leaf samples were collected to determine if treatments were needed and which nutrient may be needed

§ Whole plant samples were collected and partitioned into leaf, stalk, reproductive, and grain where applicable

¶ T = Time of foliar application (1 : early (V6-11), 2: middle (V15-18), 3: 4: late (R1-4)), R = Treatment rate (rate 1: lower level of industry recommendation & rate 2: upper level of industry recommendation)

Boron rate 1: 1,000 mg kg^{-1} or 0.14 kg B ha^{-1} rate 2: 2,000 mg kg^{-1} or 0.28 kg B ha^{-1}; Manganese rate 1: 5,207 mg kg^{-1} or 0.73 kg Mn ha^{-1} rate 2: 10,413 mg kg^{-1} or 1.46 kg Mn ha^{-1};

Zinc rate 1: 3,000 mg kg^{-1} or 0.42 kg Zn ha^{-1} rate 2: 6,000 mg kg^{-1} or 0.84 kg Zn ha^{-1}; Iron rate 1: 750 mg kg^{-1} 0.11 kg Fe ha^{-1} rate 2: 1500 mg kg^{-1} 0.22 kg Fe ha^{-1}

8.0% Boron derived from boric acid and contains proprietary surfactants, saccharides, and antifoaming solvents CornSorb: MAX-IN® Boron (WinField Solutions: St. Paul, MN)

‖ 15.62% Manganese sulfate in addition to proprietary surfactants, saccharides, and antifoaming solvents CornSorb: MAX-IN® Ultra Manganese (WinField Solutions: St. Paul, MN)

** 9.0% ZnEDTA (zinc-ethylenediaminetriacetate) and contains proprietary surfactants, saccharides, and antifoaming solvents CornSorb: Origin® Zinc 9% (WinField Solutions: St. Paul, MN)

†† Contains both 4.5% FeHEDTA and 9.0% ZnEDTA

‡‡ 4.5% FeHEDTA (iron-hydroxyethylenediaminetriacetate) in addition to proprietary surfactants, saccharides, and antifoaming solvents CornSorb: ULTRA-CHE IRON 4.5% HEDTA (WinField Solutions: St. Paul, MN)

Table 4-4. Multiple comparison test of LSmean yields (Mg ha^{-1}) comparing foliar-applied micronutrient treatment effects applied at different rates and growth stages with the control using Dunnett's Test

	----------Foliar Micronutrient Treatment Locations----------				
	----------2014----------				----2015----
Treatment	B Only	Mn Only	Zn Only	Fe & Zn	Fe Only
Control	14.91(0.20)†	7.90(0.38)	13.70(0.20)	12.70(0.28)	14.22(0.68)
T1R1‡	14.56	8.83+	13.55	12.57	14.69
T1R2	14.71	7.17	13.49	12.69	16.29*
T2R1	14.68	9.42**	13.21+	12.54	14.61
T2R2	14.62	7.90	13.41	12.37	15.64
T1R1 and T2R1	15.00	8.66	13.08**	11.63+	16.24*
T3R1	14.37+	8.03	13.49	12.69	16.06+
T3R2	14.68	7.90	13.26+	12.26	16.14*
T2R1 and T3R1	14.54	8.72	13.30	-	15.46
T4R1	-	-	-	13.48+	-
T4R2	-	-	-	12.71	-
T1R1 and T4R1	-	-	-	12.86	-
T3R1 and T4R1	-	-	-	12.47	-

† Least square mean yield (kg ha^{-1}) followed by (SE for all values in the same column) and significant F test: Not Significant >0.10; + >0.05; *>0.01; **>0.001; ***<0.001

‡ T = Time of foliar application (1: early (V6-11), 2: middle (V15-18), 3: 4: late (R1-4)), R = Treatment rate (rate 1: lower level of industry recommendation & rate 2: upper level of industry recommendation)

Table 4-5 (a-e). Multiple comparison test of LSmeans of nutrient concentrations (mg kg^{-1}) in partitioned plant tissues at various stages comparing treatment effects with control

a. Treatment	Stage and Component	Control	T1R1	T1R2	T2R1	T2R2	T1R1 & T2R1	T3R1	T3R2	T2R1 & T3R1
Boron	V6 Leaf B	3.3(0.7)†,‡	3.3‡	3.0‡	3.5‡	4.0‡	3.0‡	4.8‡	5.0+‡	4.5‡
	V13 Leaf B	6.8(0.8)‡	7.5§	8.3+§	6.5‡	7.3‡	8.2+§	6.0‡	7.0‡	7.0‡
	V17 Leaf B	8.8(1.7)‡	9.7	10.3	14.0**§	14.7***§	14.5**§	8.8‡	9.2‡	14.0**§
	R2 Leaf B	7.3(0.8)‡	7.2	8.2	8.7+	8.0	8.3	8.8+§	12.2***§	9.0*§
	R6 Leaf B	4.8(0.6)‡	4.5	4.8	6.0*	4.5	5.0	5.0	5.7	4.8
	V6 Stalk B	5.5(0.4)‡	4.8‡	5.5‡	5.0‡	5.8‡	5.3‡	5.0‡	4.5+‡	5.0‡
	V13 Stalk B	5.2(0.9)‡	6.0§	5.7§	4.8‡	5.3‡	7.0+§	4.8‡	5.7‡	4.2‡
	V17 Stalk B	3.8(0.8)‡	5.0	5.8*	5.2§	6.7**§	6.5**§	4.3‡	4.7‡	5.8*§
	R2 Stalk B	4.2(0.6)‡	5.7*	4.8	5.5*	4.8	6.0**	5.3+§	5.8**§	4.8§
	R6 Stalk B	1.0(1.0)‡	1.0	1.2	2.3	1.0	1.2	1.0	2.5	1.2
	R2 Reproductive B	6.0(2.2)‡	5.5	6.5	6.2	6.0	5.3	6.3§	7.8§	10.3+§
	R6 Reproductive B	2.3(1.0)‡	6.0***	2.2	2.7	2.7	2.8	3.0	3.3	2.5

b.

Treatment	Stage and Component	Control	T1R1	T1R2	T2R1	T2R2	T1R1 & T2R1	T3R1	T3R2	T2R1 & T3R1
Manganese	V7 Leaf Mn	76.8(6.4)‡	72.0‡	76.3‡	75.3‡	68.3‡	69.3‡	67.8‡	74.3‡	83.8‡
	V15 Leaf Mn	65.7(13.8)‡	118.5***§	141.3***§	79.3‡	68.8‡	101.7**§	69.0‡	78.3‡	77.2‡
	VT Leaf Mn	83.5(10.4)‡	102.7+	123.8***	108.7*§,¶	118.2**§	129.7***§	79.8‡	76.8‡	115.7**§
	R3 Leaf Mn	104.7(11.8)‡	116.3	174.2***	106.7	133.7*	136.5**	132.7*§	161.2***§	153.2***§
	R6 Leaf Mn	150.0(9.9)‡	163.7	203.8***	159.7	182.2**	184.2***	177.3**	206.8***	195.2***
	V7 Stalk Mn	75.5(6.5)‡	79.8‡	90.0‡	85.0‡	77.3‡	77.0‡	82.3‡	72.5‡	84.0‡
	V15 Stalk Mn	77.7(7.5)‡	86.7§	101.0**§	68.7‡	76.2‡	83.3§	75.8‡	70.2‡	72.3‡
	VT Stalk Mn	87.5(6.6)‡	81.3	80.2	84.7§,¶	87.8§	87.3§	97.3‡	76.7‡	86.5§
	R3 Stalk Mn	78.2(7.8)‡	86.0	93.5+	80.3	104.3**	99.7**	94.7*§	120.8***§	101.5**§
	R6 Stalk Mn	40.8(6.0)‡	34.3	43.7	27.3*	42.8	49.2	43.2	46.7	43.3
	VT Reproductive Mn	79.3(13.6)‡	88.8	83.3	114.0*§,¶	89.5§	94.3§	86.0‡	87.7‡	89.0§
	R3 Reproductive Mn	23.3(5.4)‡	21.3	25.7	25.0	24.0	26.7	31.5§	36.5*§	26.2§
	R6 Reproductive Mn	15.2(5.2)‡	18.7	17.7	15.8	14.0	18.0	22.8	22.0	21.3
	R3 Grain Mn	15.9(1.8)‡	13.7	12.0*	12.3*	13.9	13.7	15.3§	13.6§	14.1§
	R6 Grain Mn	5.8(0.3)‡	6.0	6.1	5.9	6.1	5.7	5.9	6.3+	5.8

c.

Treatment	Stage and Component	Control	T1R1	T1R2	T2R1	T2R2	T1R1 & T2R1	T3R1	T3R2	T2R1 & T3R1
Zinc Only	V6 Leaf Zn	26.3(0.9)‡	27.5‡	26.5‡	25.0‡	25.8‡	25.3‡	26.8‡	27.8‡	27.8‡
	V14 Leaf Zn	24.7(5.9)‡	71.8***§	101.0***§	22.7‡	21.7‡	62.0***§,#	24.2‡	22.0‡	22.5‡
	V17 Leaf Zn	27.3(9.5)‡	58.2**	85.5***	78.2***§	108.8***§	91.3***§,#	27.3‡	28.5‡	73.3***§
	R2 Leaf Zn	29.2(22.0)‡	49.0	80.0*	54.5	74.2*	123.2***	63.0§	84.0*§	88.5**§
	R6 Leaf Zn	34.2(7.6)‡	47.8+	73.5***	57.8**	73.3***	68.0***	50.7*	77.2***	71.2***
	V6 Stalk Zn	55.3(3.2)‡	52.0‡	46.5+‡	50.0‡	53.3‡	47.5‡	60.0‡	53.3‡	55.5‡
	V14 Stalk Zn	26.8(5.0)‡	39.3*§	73.3***§	37.5*‡	32.3‡	42.7**§,#	29.5‡	30.5‡	35.3+‡
	V17 Stalk Zn	23.7(3.0)‡	27.0	45.8***	36.2***§	55.5***§	44.0***§,#	24.7‡	24.7‡	35.2***§
	R2 Stalk Zn	19.0(3.5)‡	26.0+	31.5***	27.3*	41.0***	36.0***	27.5*§	44.0***§	41.0***§
	R6 Stalk Zn	27.0(6.3)‡	25.8	28.5	29.8	37.8+	30.3	31.3	41.2*	37.0
	R2 Reproductive Zn	26.5(2.8)‡	28.7	30.3	29.3	34.7**	32.8*	38.2***§	45.3***§	41.2***§
	R6 Reproductive Zn	34.7(5.9)‡	34.3	43.0	30.0	35.2	34.2	30.3	39.8	39.7
	R2 Grain Zn	47.5(2.3)‡	48.8	52.1+	50.9	49.9	52.6*	55.2**§	53.9**§	58.0***§
	R6 Grain Zn	19.3(0.6)‡	19.8	20.3	18.6	19.4	19.7	20.5+	19.9	20.1

d.

Treatment	Stage and Component	Control	T1R1	T1R2	T2R1	T2R2	T1R1 & T2R1	T3R1	T3R2	T4R1	T4R2	T1R1 & T4R1	T3R1 & T4R1
Iron / Zinc (Zinc Values)	V6 Leaf Zn	25.8(0.9)‡	25.5‡	25.5‡	26.0‡	28.5*‡	28.0+‡	25.0‡	26.5‡	26.0‡	27.0‡	24.8‡	24.8‡
	V14 Leaf Zn	22.3(13.6)‡	74.8**§	53.8§	51.5‡	40.0‡	68.5*§	30.5‡	24.0‡	29.0‡	51.0‡	50.8§	27.3‡
	V17 Leaf Zn	31.3(6.1)‡	63.5***	65.0***	56.5**§	80.0***§	80.3***§	26.3‡	27.8‡	24.8‡	25.3‡	49.3*	25.0‡
	R2 Leaf Zn	34.5(7.8)‡	55.5+	86.0***	49.5	73.8**	69.3**	40.0§	66.8**§	31.3‡	34.0‡	71.0**	55.5+§
	R6 Leaf Zn	29.5(2.7)‡	32.3	50.5***	32.0	43.3***	44.8***	30.3	36.8+	30.3§	39.3*§	40.8**§	37.5*§
	V6 Stalk Zn	51.0(3.2)‡	44.8‡	50.3‡	46.0‡	62.3*‡	52.5‡	42.4+‡	54.0‡	49.0‡	52.8‡	44.8‡	54.3‡
	V14 Stalk Zn	35.3(4.4)‡	52.3**§	51.3*§	50.8*‡	37.3‡	44.0§	32.5‡	29.5‡	40.0‡	45.8+‡	48.3*§	48.5*‡
	V17 Stalk Zn	26.3(3.3)‡	29.0	38.8**	31.3§	31.0§	31.0§	26.3‡	23.0‡	23.8‡	28.3‡	34.3+	21.5‡
	R2 Stalk Zn	21.0(2.8)‡	18.8	22.5	19.8	24.8	23.8	23.0§	23.5§	18.0‡	17.8‡	22.5	25.3§
	R6 Stalk Zn	18.8(2.8)‡	15.0	15.8	16.5	12.0+	15.5	15.8	10.8	12.5§	16.8§	12.8§	15.5§
	R2 Reproductive Zn	29.5(2.8)‡	31.3	31.8	37.0+	32.0	35.3	36.8+§	37.3+§	36.8+‡	31.0‡	34.0	35.3§
	R6 Reproductive Zn	27.8(2.8)‡	28.0	32.3	28.3	31.8	34.8+	28.3	31.3	26.5§	32.3§	30.3§	29.8§
	R2 Grain Zn	48.4(5.9)‡	65.2+	56.1	55.1	61.9	68.7*	77.2**§	66.8*§	52.3‡	58.2‡	63.5+	66.2*§
	R6 Grain Zn	19.4(1.0)‡	18.5	19.3	17.8	19.5	20.8	18.5	18.9	18.3§	18.4§	18.7§	19.5§

e.

Treatment	Stage and Component	Control	T1R1	T1R2	T2R1	T2R2	T1R1 & T2R1	T3R1	T3R2	T4R1	T4R2	T1R1 & T4R1	T3R1 & T4R1
Iron / Zinc (Iron Values)	V6 Leaf Fe	305.0(18.9)‡	281.5‡	284.3‡	265.3‡	321.8‡	306.5‡	275.8‡	316.8‡	286.5‡	282.0‡	275.8‡	282.0‡
	V14 Leaf Fe	133.3(17.0)‡	170.0§	142.0§	159.8‡	152.3‡	171.5§	128.5‡	138.5‡	162.0‡	139.8‡	143.3§	119.5‡
	V17 Leaf Fe	143.0(13.5)‡	163.0	163.0	153.8§	183.3*§	184.0*§	131.8‡	120.3‡	132.3‡	116.9‡	137.8	124.3‡
	R2 Leaf Fe	330.3(38.2)‡	277.8	319.3	253.0	350.3	248.5	189.8**§	264.3§	274.5‡	213.3*‡	197.0*	286.8§
	R6 Leaf Fe	98.5(7.5)‡	94.3	107.3	94.3	103.3	99.3	82.5	98.8	84.8§	94.5§	89.0§	103.5§
	V6 Stalk Fe	177.5(25.9)‡	187.3‡	159.5‡	157.0‡	166.8‡	194.5‡	152.3‡	211.3‡	176.0‡	160.3‡	194.3‡	158.8‡
	V14 Stalk Fe	33.5(5.3)‡	34.5§	38.0§	35.0‡	42.5‡	40.3§	32.0‡	39.8‡	40.5‡	44.5‡	34.5§	37.5‡
	V17 Stalk Fe	37.3(10.8)‡	35.0	32.3	32.3§	50.3§	34.5§	40.8‡	59.3‡	40.5‡	38.0‡	32.8	38.5‡
	R2 Stalk Fe	86.8(12.7)‡	50.0*	52.3+	44.0*	74.8	72.3	69.0§	56.8§	58.0‡	53.8+‡	63.0	46.0*§
	R6 Stalk Fe	23.5(5.5)‡	29.5	28.3	24.8	28.0	33.3	33.5	40.5*	36§	26.8§	30§	25.8§
	R2 Reproductive Fe	73.3(15.7)‡	110.8	76.5	61.5	58.0	74.3	69.8§	52.8§	61.3‡	51.0‡	64.5	80.3§
	R6 Reproductive Fe	33.8(6.8)‡	30.0	27.8	31.0	33.5	42.8	30.5	34.8	23.8§	26.3§	31.8§	29.8§
	R2 Grain Fe	33.5(5.0)‡	42.1	38.3	36.0	47.5*	45.0	49.5*§	42.8§	36.0‡	44.9‡	42.3	43.1§
	R6 Grain Fe	19.0(1.1)‡	17.6	21.6	17.8	19.2	19.4	18.3	18.0	17.0§	16.9§	17.6§	18.7§

† Least square mean plant nutrient concentration (mg kg^{-1}) followed by (SE for all values in the same row) and significant F test: Not Significant >0.10; + >0.05; *>0.01; **>0.001; ***<0.001

‡ No foliar treatment had been applied at this stage

§ First sampling following foliar treatment

¶ First sampling following foliar treatment for plots with significant increase on grain yield

First sampling following foliar treatment for plots with significant decrease on grain yield

Table 4-6 (a-e). Multiple comparison test of LSmeans of nutrient quantity (g ha^{-1}) in partitioned plant tissues at various stages comparing treatment effects with control**.**

a. Treatment	Stage and Component	Control	T1R1	T1R2	T2R1	T2R2	T1R1 & T2R1	T3R1	T3R2	T2R1 & T3R1
Boron	V6 Leaf B	2.7(0.5)†,‡	2.2‡	2.2‡	2.5‡	2.7‡	1.8‡	2.8‡	3.3‡	3.3‡
	V13 Leaf B	18.5(2.3)‡	21.0§	21.7§	16.9‡	19.6‡	22.2+§	15.4‡	18.8‡	18.4‡
	V17 Leaf B	31.7(6.3)‡	32.9	34.9	50.3**§	48.5**§	51.1**§	31.3‡	31.3‡	45.5*§
	R2 Leaf B	24.1(2.1)‡	25.3	27.0	28.9+	26.9	29.0+	30.0*§	38.9***§	28.9+§
	R6 Leaf B	17.5(2.1)‡	15.1	16.8	21.3+	15.1	18.2	17.2	18.7	17.0
	V6 Stalk B	1.8(0.3)‡	1.4‡	1.7‡	1.6‡	1.5‡	1.6‡	2.0‡	1.4‡	1.5‡
	V13 Stalk B	14.0(3.8)‡	17.1§	13.1§	11.2‡	13.0‡	19.8+§	12.5‡	14.0‡	11.7‡
	V17 Stalk B	20.9(4.9)‡	26.1	28.8+	27.3§	30.9**§	34.6**§	24.1‡	26.0‡	28.9+§
	R2 Stalk B	26.9(3.0)‡	39.5**	31.4	34.8+	32.1	42.2**	34.1+§	36.0*§	32.5§
	R6 Stalk B	5.3(4.7)‡	5.4	5.8	11.9	5.1	6.3	5.3	12.6+	6.1
	R2 Reproductive B	15.2(3.1)‡	16.4	18.6	16.3	16.3	15.2	17.7§	18.6§	22.9+§
	R6 Reproductive B	6.5(2.9)‡	16.8***	5.9	8.1	7.5	8.4	7.8	9.9	7.0
	V6 Total B	4.5(0.5)‡	3.6‡	3.9‡	4.1‡	4.1‡	3.4‡	4.7‡	4.7‡	4.8‡
	V13 Total B	32.5(5.0)‡	38.1§	34.9§	28.1‡	32.6‡	42.0+§	27.9‡	32.8‡	30.1‡
	V17 Total B	52.6(8.2)‡	59.0	63.7	77.5**§	79.4**§	85.7***§	55.4‡	57.3‡	74.4**§
	R2 Total B	66.2(5.0)‡	81.2*	77.0	80+	75.4	86.4**	81.7*§	93.5***§	84.2*§
	R6 Total B	29.3(7.0)‡	37.3	28.5	41.3+	27.8	32.9	30.3	41.2+	30.0

b.

Treatment	Stage and Component	Control	T1R1	T1R2	T2R1	T2R2	T1R1 & T2R1	T3R1	T3R2	T2R1 & T3R1
Manganese	V7 Leaf Mn	56.7(7.3)‡	59.2‡	58.4‡	66.2‡	58.1‡	58.0‡	52.6‡	58.4‡	73.4‡
	V15 Leaf Mn	125.9(32.7)‡	225.1**§	272.4***§	167.3‡	135.1‡	202.5*§	149.3‡	145.5‡	162.7‡
	VT Leaf Mn	147.0(24.6)‡	189.9+	227.1**	213.1**§,¶	216.8**§	237.6***§	146.1‡	151.1‡	210.2**§
	R3 Leaf Mn	219.8(25.0)‡	251.1	412.5***	248.3	288.1+	335.1**	287.3+§	326.2**§	346.1***§
	R6 Leaf Mn	295.4(30.9)‡	344.6+	442.44***	347.4+	380.1**	416.8***	346.7+	428.0***	403.4***
	V7 Stalk Mn	27.3(5.8)‡	35.4‡	38.5‡	40.8‡	35.8‡	35.7‡	39.4‡	30.1‡	38.9‡
	V15 Stalk Mn	197.0(28.1)‡	251.6+§	249.0+§	206.4‡	189.7‡	225.8§	178.8‡	168.0‡	202.1‡
	VT Stalk Mn	259.4(37.3)‡	305.7	267.9	339.1*§,¶	293.3§	325.8+§	280.1‡	264.4‡	303.7§
	R3 Stalk Mn	257.3(26.4)‡	320.8+	353.8**	303.1	359.8**	414.0***	329.2+§	406.4***§	377.3**§
	R6 Stalk Mn	118.5(23.2)‡	97.4	133.5	84.2	123.9	163.1+	115.7	141.5	134.9
	VT Reproductive Mn	5.3(1.4)‡	6.9	6.2	8.7**§,¶	6.4§	6.5§	5.7‡	7.2‡	7.6+§
	R3 Reproductive Mn	79.3(12.8)‡	75.8	87.1	88.6	79.0	92.8	98.2§	103.8§	92.5§
	R6 Reproductive Mn	31.1(11.6)‡	42.1	39.0	35.8	29.3	40.6	45.9	47.8	53.6+
	R3 Grain Mn	14.4(1.7)‡	13.0	13.2	16.5	12.9	18.0	15.5§	11.7§	14.7§
	R6 Grain Mn	72.4(6.5)‡	85.1+	76.2	83.8+	77.7	84.8+	81.3	85.4*	78.0
	V7 Total Mn	84.0(11.6)‡	94.6‡	96.9‡	107.0‡	93.9‡	93.7‡	92.0‡	88.5‡	112.3+‡
	V15 Total Mn	322.9(47.8)‡	476.7**§	521.4***§	373.6‡	324.8‡	428.3**§	328.1‡	313.5‡	364.8‡
	VT Total Mn	411.7(57.0)‡	502.5	501.0	561.0**§	516.4+§	569.9**§	432.0‡	422.7‡	521.6+§
	R3 Total Mn	570.8(52.4)‡	660.8	845.2***	681.0	739.7*	857.6***	730.2*§	850.6***§	830.6***§
	R6 Total Mn	517.4(52.9)‡	569.1	691.2**	551.2	611.0+	705.2***	589.5	702.7***	669.9**

c.

Treatment	Stage and Component	Control	T1R1	T1R2	T2R1	T2R2	T1R1 & T2R1	T3R1	T3R2	T2R1 & T3R1
Zinc Only	V6 Leaf Zn	10.5(0.7)‡	11.1‡	9.9‡	9.5‡	11.0‡	10.4‡	10.9‡	11.7‡	12.7*‡
	V14 Leaf Zn	51.9(13.9)‡	154.5***§	213.6***§	45.9‡	47.2‡	123.9***§,#	53.1‡	45.5‡	47.6‡
	V17 Leaf Zn	86.6(26.8)‡	175.0**	227.9***	207.4***§	298.6***§	242.0***§,#	75.9‡	77.5‡	206.8***§
	R2 Leaf Zn	74.6(52.9)‡	125.0	192.3*	135.5	174.8+	292.4***	152.5§	209.6*§	213.3**§
	R6 Leaf Zn	102.1(23.7)‡	153.1*	210.9***	169.7**	216.1***	200.5***	148.5+	224.3***	213.1***
	V6 Stalk Zn	10.5(1.3)‡	10.3‡	7.3‡	8.9‡	11.9‡	10.2‡	11.6‡	10.3‡	13.8+‡
	V14 Stalk Zn	94.9(36.4)‡	135.2§	210.7**§	117.9‡	98.6‡	134.2§,#	78.0‡	89.8‡	96.6‡
	V17 Stalk Zn	101.0(15.6)‡	123.8	192.2***	148.0**§	244.9***§	178.5***§,#	109.5‡	100.7‡	151.0**§
	R2 Stalk Zn	115.0(25.2)‡	150.3	181.1**	159.0+	213.8***	200.9**	147.6§	256.8***§	234.9***§
	R6 Stalk Zn	140.1(36.0)‡	137.9	136.5	145.4	197.3	164.2	155.5	214.5*	193.8
	R2 Reproductive Zn	54.6(6.1)‡	57.1	61.3	58.4	64.2	60.7	70.9**§	91.1***§	80.4***§
	R6 Reproductive Zn	82.6(15.0)‡	81.4	108.9+	71.2	78.2	75.5	72.6	89.8	87.6
	R2 Grain Zn	20.0(2.0)‡	17.7	15.2*	15.3*	17.2	17.6	16.2+§	20.0§	20.1§
	R6 Grain Zn	243.2(12.4)‡	252.3	242.0	232.9	229.1	230.9	265.5+	246.7	249.2
	V6 Total Zn	21.1(2.2)‡	21.4‡	14.9*‡	18.4‡	22.9‡	20.6‡	24.5‡	22.0‡	26.5*‡
	V14 Total Zn	146.8(44.5)‡	289.7*§	424.3***§	163.8‡	145.9‡	258.1**§	131.1‡	135.3‡	144.1‡
	V17 Total Zn	187.7(32.6)‡	298.8**	420.1***	355.7***§	543.5***§	420.6***§	185.4‡	178.2‡	357.9***§
	R2 Total Zn	264.1(60.6)‡	350.2	449.9*	368.3+	470.0**	571.5***	387.2*§	577.6***§	548.7***§
	R6 Total Zn	568.0(51.9)‡	624.6	698.2*	619.2	720.7**	671.2*	642.1	775.2***	743.7**

d.

Treatment	Stage and Component	Control	T1R1	T1R2	T2R1	T2R2	T1R1 & T2R1	T3R1	T3R2	T4R1	T4R2	T1R1 & T4R1	T3R1 & T4R1
Iron/Zinc (Zinc Values)	V6 Leaf Zn	12.0(0.9)‡	10.0+‡	10.9‡	12.2‡	15.1**‡	13.7‡	11.8‡	11.5‡	11.9‡	12.7‡	10.6‡	11.2‡
	V14 Leaf Zn	40.6(25.0)‡	138.0**§	93.4§	96.0+‡	72.5‡	124.9*§	55.6‡	44.9‡	49.1‡	92.3‡	88.1§	51.6‡
	V17 Leaf Zn	75.3(14.0)‡	145.3***	149.9***	123.2*§	189.3***§	186.0***§	53.2‡	66.5‡	55.3‡	59.8‡	102.6	59.6‡
	R2 Leaf Zn	73.9(17.0)‡	118.4+	184.4***	103.6	158.8***	149.7**	82.7§	139.3**§	64.8‡	71.2‡	148.4**	120.9+§
	R6 Leaf Zn	64.3(6.8)‡	66.8	104.9***	66.7	95.1**	100.9***	63.2	77.0	62.7§	82.6+§	84.6*§	81.7+§
	V6 Stalk Zn	13.7(1.8)‡	8.8+‡	11.0‡	10.7‡	18.4+‡	11.9‡	8.6*‡	11.0‡	11.9‡	11.6‡	10.1‡	9.4+‡
	V14 Stalk Zn	67.2(8.5)‡	90.4+§	87.2+§	97.5*‡	66.7‡	93.4*§	64.9‡	58.5‡	71.4‡	85.3+‡	79.8§	98.5**‡
	V17 Stalk Zn	119.0(14.7)‡	116.3	154.8+	124.2§	140.01§	132.3§	102.6‡	103.6‡	86.7+‡	123.0‡	119.9	88.0‡
	R2 Stalk Zn	102.0(13.5)‡	85.7	107.9	91.9	121.0	115.0	109.2§	107.7§	79.1‡	83.4‡	101.7	125.3§
	R6 Stalk Zn	94.7(14.6)‡	65.6	73.5	70.4	48.6*	74.8	77.9	45.5*	59.4+§	69.6§	56.7+§	81.4§
	R2 Reproductive Zn	65.8(5.7)‡	64.9	66.0	74.9	70.0	72.1	74.3§	72.0§	72.3‡	69.0‡	67.2	72.7§
	R6 Reproductive Zn	54.4(6.7)‡	56.4	63.8	56.6	65.7	77.3*	58.1	69.7+	53.9§	66.0§	64.1§	62.6§
	R2 Grain Zn	20.0(2.0)‡	18.7	19.3	17.3	18.9	22.5	30.0**§	20.0§	14.7+‡	14.3+‡	15.6+	24.8+§
	R6 Grain Zn	210.0(14.3)‡	206.9	213.4	194.7	212.3	241.6+	209.6	220.4	206.5§	206.7§	216.7§	215.5§
	V6 Total Zn	25.8(2.4)‡	18.8*‡	22.7‡	22.9‡	33.6*‡	24.9‡	20.4‡	22.5‡	23.8‡	24.3‡	20.6‡	20.6‡
	V14 Total Zn	107.7(28.5)‡	228.5**§	180.6+§	193.5*‡	139.2‡	218.3**§	120.5‡	103.4‡	120.5‡	177.5+‡	168.0§	150.1‡
	V17 Total Zn	194.3(23.6)‡	261.5*	304.8**	247.4§	329.4***§	318.4***§	155.8‡	170.1‡	141.9‡	182.8‡	222.5	147.6‡
	R2 Total Zn	261.7(25.0)‡	287.8	377.6**	287.7	372.5**	359.3**	307.4§	339.2*§	230.8‡	238.0‡	333.0*	343.7*§
	R6 Total Zn	423.5(31.2)‡	395.7	455.5	388.4	421.7	494.6	408.7	416.1	382.5§	425.0§	422.1§	441.1§

e.

Treatment	Stage and Component	Control	T1R1	T1R2	T2R1	T2R2	T1R1 & T2R1	T3R1	T3R2	T4R1	T4R2	T1R1 & T4R1	T3R1 & T4R1
Iron / Zinc (Iron Values)	V6 Leaf Fe	144.9(12.1)‡	110.2*‡	121.6‡	124.4‡	170.4‡	145.8‡	130.1‡	136.8‡	130.8‡	132.8‡	118.0+‡	127.4‡
	V14 Leaf Fe	247.7(32.9)‡	311.8§	248.0§	294.3‡	276.4‡	315.0‡§	235.4‡	259.5‡	281.4‡	255.1‡	252.6§	233.2‡
	V17 Leaf Fe	351.0(32.1)‡	380.6	374.3	339.7§	434.7+§	426.9+§	266.3+‡	289.7‡	293.8‡	283.0+‡	289.6	298.7‡
	R2 Leaf Fe	724.9(123.3)‡	589.3	686.2	528.7	763.9	542.7	391.9**§	547.3§	575.7‡	443.1*‡	407.5*	612.0§
	R6 Leaf Fe	213.0(18.2)‡	196.0	224.9	196.5	227.6	227.0	171.3+	207.6	175.8§	199.0§	185.0§	223.9§
	V6 Stalk Fe	47.2(7.0)‡	37.7‡	35.5‡	35.3‡	46.8‡	44.6‡	29.8+‡	41.8‡	44.7‡	38.5‡	39.5‡	27.4*‡
	V14 Stalk Fe	64.8(12.6)‡	61.6§	65.2§	69.7‡	86.0‡	85.9§	66.5‡	87.8‡	71.6‡	86.2‡	61.7§	77.8‡
	V17 Stalk Fe	167.8(51.5)‡	141.8	131.0	129§	228.5§	152.1§	154.7‡	287.7+‡	155.6‡	150.2‡	116.0	157.1‡
	R2 Stalk Fe	436.0(63.1)‡	230.5*	251.6*	208.1*	372.4	341.7	330.2§	260.6+§	269.7+‡	259.1+‡	281.8+	224.3*§
	R6 Stalk Fe	116.1(24.8)‡	125.0	130.8	108.0	114.4	164.8	171.9+	169.2+	170.9+§	110.7§	133.4§	130.2§
	R2 Reproductive Fe	160.5(33.0)‡	222.5	163.5	126.1	128.8	147.5	143.1§	103.9§	124.7‡	113.2‡	128.4	167.3§
	R6 Reproductive Fe	66.2(17.1)‡	60.4	54.8	62.1	70.1	101.6	62.3	76.8	48.6§	53.9§	68.0§	62.2§
	R2 Grain Fe	14.2(1.9)‡	12.4	13.6	11.6	15.7	15.6	18.4§	12.9§	10.0‡	12.1‡	10.7	16.6§
	R6 Grain Fe	203.7(15.2)‡	196.2	235.4	193.7	208.4	221.7	205.7	212.5	190.4+§	189.3§	203.0§	203.3§
	V6 Total Fe	192.1(15.0)‡	147.9*‡	157.2‡	159.7‡	217.1‡	189.5‡	159.9‡	178.6‡	175.5‡	171.3‡	157.5‡	154.8+‡
	V14 Total Fe	312.5(35.8)‡	373.4§	313.2§	364‡	362.4‡	400.9+§	301.9‡	347.3‡	353.0‡	341.3‡	314.3§	311.0‡
	V17 Total Fe	518.8(69.0)‡	522.4	505.3	468.8§	663.2§	579.0§	421.0‡	577.4‡	449.3‡	433.2‡	405.6	455.8‡
	R2 Total Fe	1335.6(38.2)‡	1054.7	1114.9	874.5*	1190.4	1047.5	923.5+§	924.7*§	980.1+‡	827.5**‡	786.6**	1020.2+§
	R6 Total Fe	599.1(32.7)‡	577.5	645.8	560.6	620.5	715.2*	611.2	630.5	585.7§	552.9§	589.5§	619.6§

† Least square mean plant nutrient quantity (g ha^{-1}) followed by (SE for all values in the same row) and significant F test: Not Significant >0.10; + >0.05; *>0.01; **>0.001; ***≤0.001

‡ No foliar treatment had been applied at this stage

§ First sampling following foliar treatment

¶ First sampling following foliar treatment for plots with significant increase on grain yield

First sampling following foliar treatment for plots with significant decrease on grain yield

Table 4-7. Multiple comparison test of LSmean R6 Biomass (Mg ha^{-1}) comparing treatment effects with control using Dunnett's Test

	----------Foliar Micronutrient Treatment Locations----------			
Treatment	B Only	Mn Only	Zinc Only	Fe & Zn
Control	31.70(0.99)†	19.38(1.11)	31.35(1.19)	19.47(0.63)
T1R1‡	32.16	22.74**	29.69	20.86
T1R2	32.14	20.98	30.61	19.89
T2R1	31.43	21.54+	31.79	20.51
T2R2	32.76	21.80*	30.58	20.12
T1R1 and T2R1	31.01	20.41	31.48	19.83
T3R1	31.86	19.92	29.68	20.13
T3R2	30.90	19.81	30.01	20.04
T2R1 and T3R1	31.75	20.65	30.43	-
T4R1	-	-	-	19.16
T4R2	-	-	-	19.89
T1R1 and T4R1	-	-	-	20.92
T3R1 and T4R1	-	-	-	20.25

† LSmean R6 biomass (Mg ha^{-1}) followed by (SE for all values in the same column)
and significant F test: Not Significant >0.10; + >0.05; *>0.01; **>0.001; ***<0.001
‡ T = Time of foliar application (1: early (V6-11), 2: middle (V15-18), 3: 4: late (R1-4)),
R = Treatment rate (rate 1: lower level of industry recommendation & rate 2: upper level

Table 4-8. LSMeans for Apparent Nutrient Recovery (ANR) at the end of the growing season (R6). ANR(%) = [(nutrient uptake fertilized (g ha^{-1}) - nutrient uptake control (g ha^{-1}))/(quantity of nutrient applied (g ha^{-1})))] X 100. ANR was used to reflect the efficiency of maize to obtain applied foliar micronutrients. The ANOVA for treatment effects reported no treatment effects at $p \leq 0.05$. Therefore, no treatment comparisons are reported.

	Location				
Treatment	B Only	Mn Only	Zinc Only	Fe/Zn (Zn Values)	Fe/Zn (Fe Values)
	ANR				
T1R1†	5.8(2.9)‡	7.1(3.7)	13.5(9.6)	-6.6(6.1)	-15.5(20.5)
T1R2	-0.3	11.9	15.5	3.8	5.4
T2R1	8.6	4.6	12.2	-8.3	-15.0
T2R2	-0.5	6.4	18.2	-0.2	6.6
T1R1 and T2R1	1.3	12.9	12.3	8.5	6.4
T3R1	0.8	9.9	17.6	-3.5	-38.0
T3R2	4.3	12.7	24.7	-0.5	-2.5
T2R1 and T3R1	0.3	10.4	20.9	-	-
T4R1	-	-	-	-9.8	-33.8
T4R2	-	-	-	0.2	-6.4
T1R1 and T4R1	-	-	-	-0.2	-12.7
T3R1 and T4R1	-	-	-	2.1	4.9

† T = Time of foliar application (1: early (V6-11), 2: middle (V15-18), 3: 4: late (R1-4)),
R = Treatment rate (rate 1: lower level of industry recommendation & rate 2: upper level of industry recommendation)
‡ ANR Least Square Mean (%) followed by (SE for all values in the same column) The ANOVA for treatment effects reported no treatment effects at $p \leq 0.05$.

Table 4-9. Summary location characteristics and results to each foliar micronutrient treatment

Experimental Focus	Low-Deficient Leaf Tissue	High-Excessive Leaf Tissue	Low-Deficient Soil	Change in applied nutrient concentration due to treatment	Change in applied nutrient uptake due to treatment	Yield Response	Target Growth Stage‡
B	+ +†	-	- -	+ +	+ +	0	Pre-V10
Mn	+	-	- - -	+ +	+ +	+	Vegetative
Zn only	0	+	- - -	+ +	+ +	-	Any
Fe / Zn	- - / +	+ + +/ -	- - - / - - -	- - / + +	- - / + +	0	Undefined
Fe only	+ + +	-	- -	no data	no data	+ + +	Any

† +/0/- indicates direction of agreement with statement and number of characters indicates magnitude. (i.e. + indicates agreement and - indicates disagreement whereas 0 indicates neutral)

‡ Under conditions of confirmed deficiency of the target micronutrient, the listed growth stage would be the recommended stage for foliar application of the specified micronutrient.

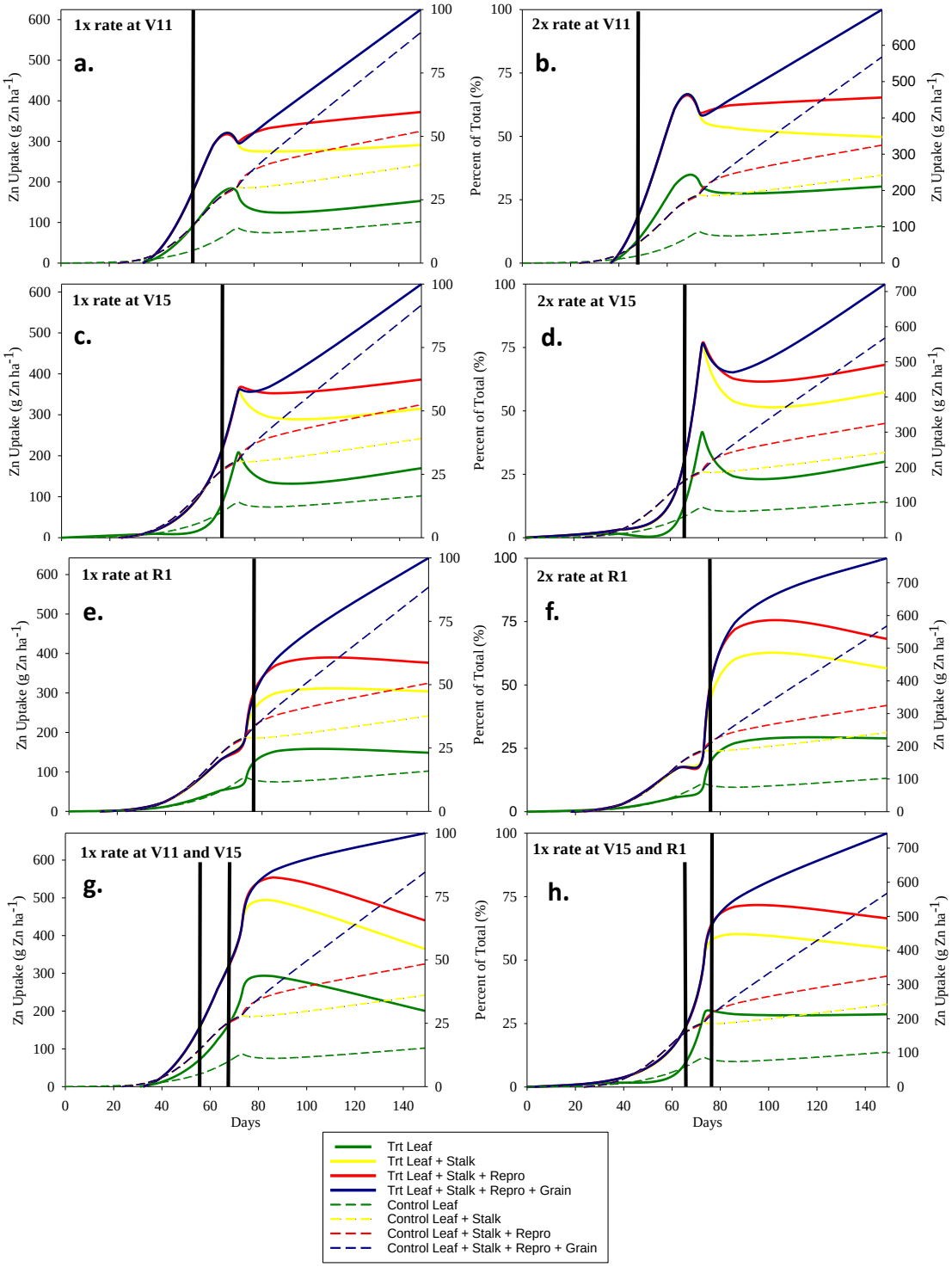

Figure 4-1. Zn uptake (g Zn ha^{-1}) and partitioning graphs with solid line graphs representing foliar Zn treated plots overlaying dashed line graphs representing the control plot. Solid vertical lines represent the time of application.

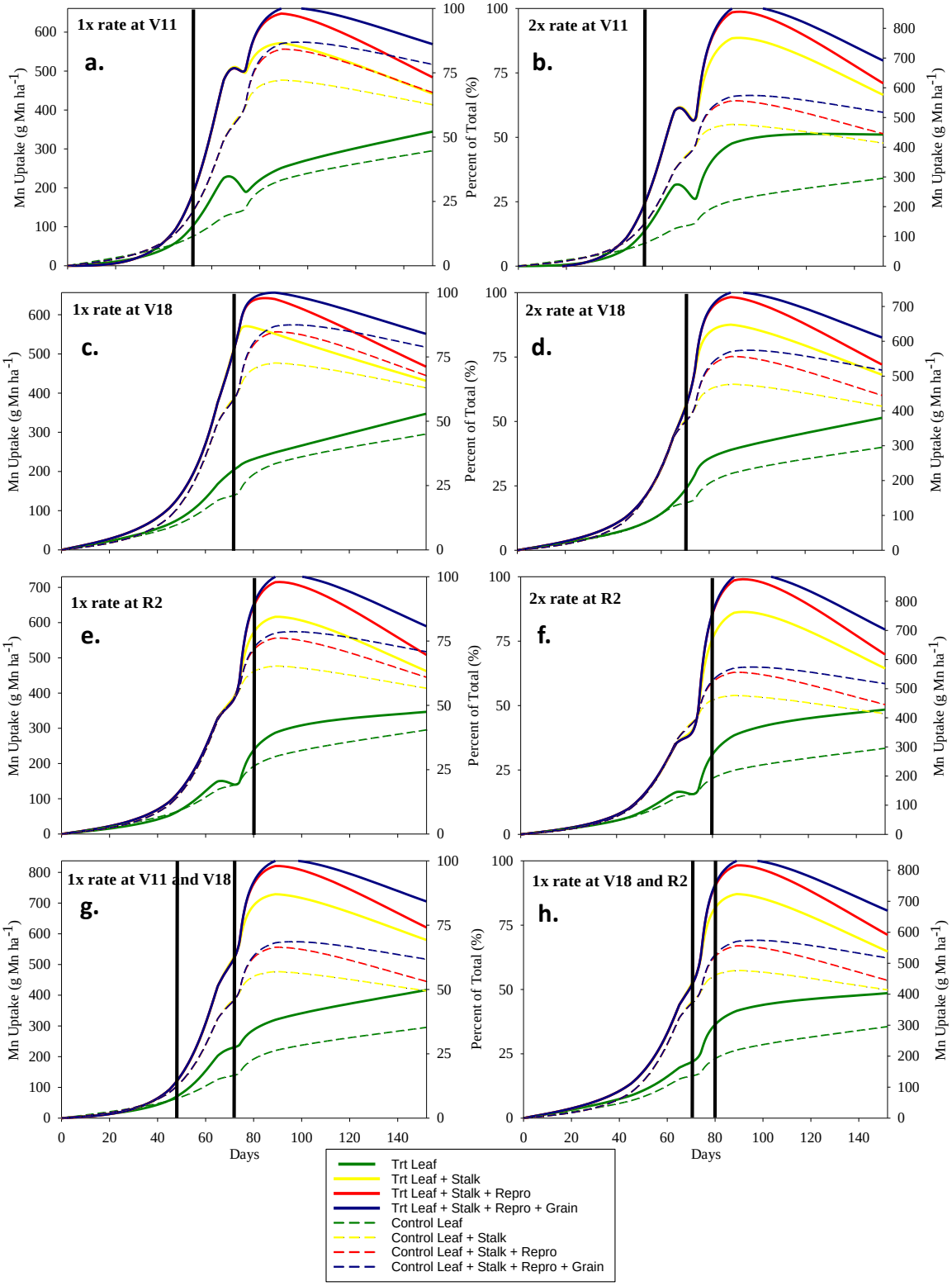

Figure 4-2. Mn uptake (g Mn ha^{-1}) and partitioning graphs with solid line graphs representing foliar Mn treated plots overlaying dashed line graphs representing the control plot. Solid vertical lines represent the time of application.

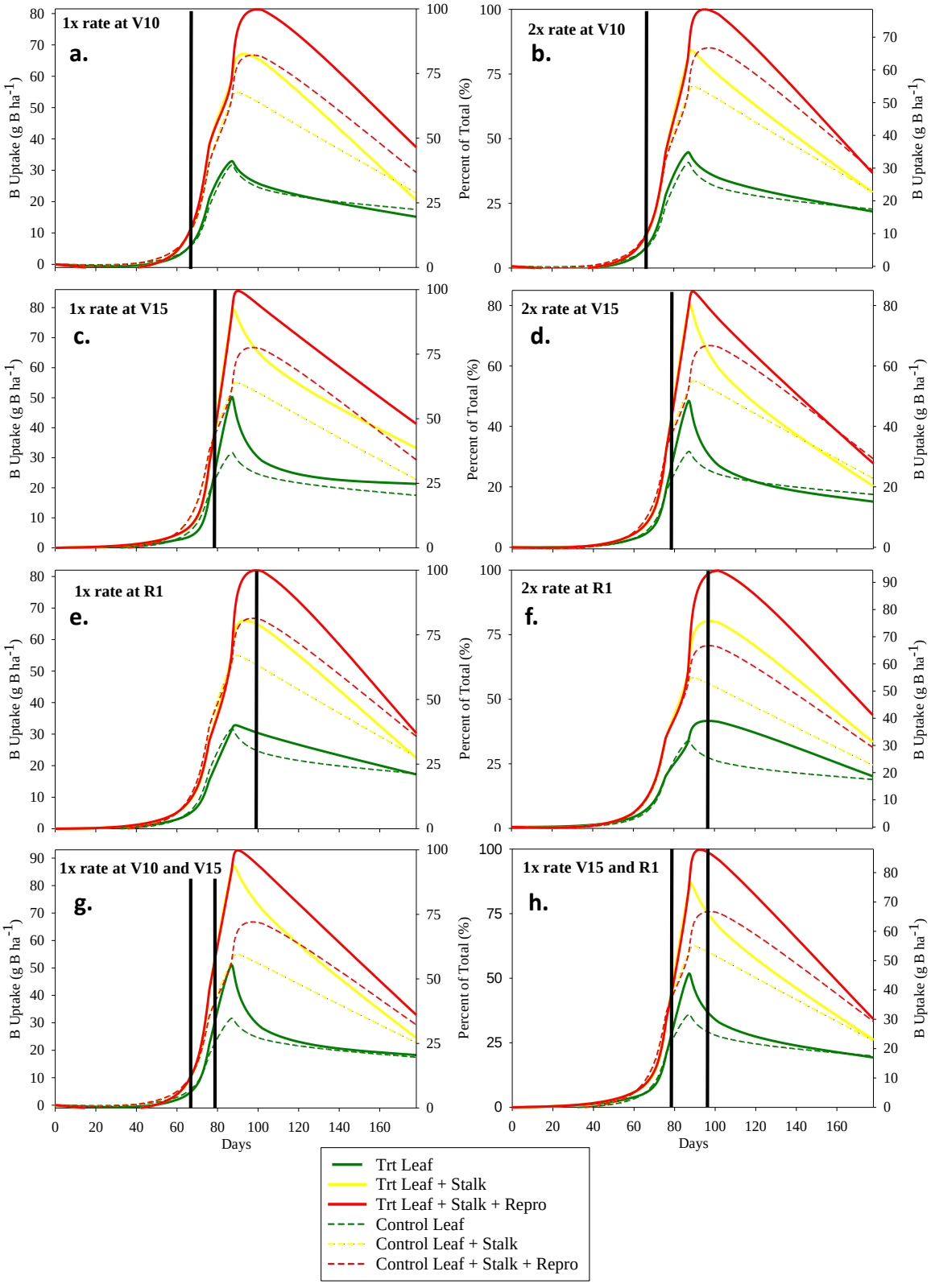

Figure 4-3. B uptake (g B ha^{-1}) and partitioning graphs with solid line graphs representing foliar B treated plots overlaying dashed line graphs representing the control plot. Solid vertical lines represent the time of application.

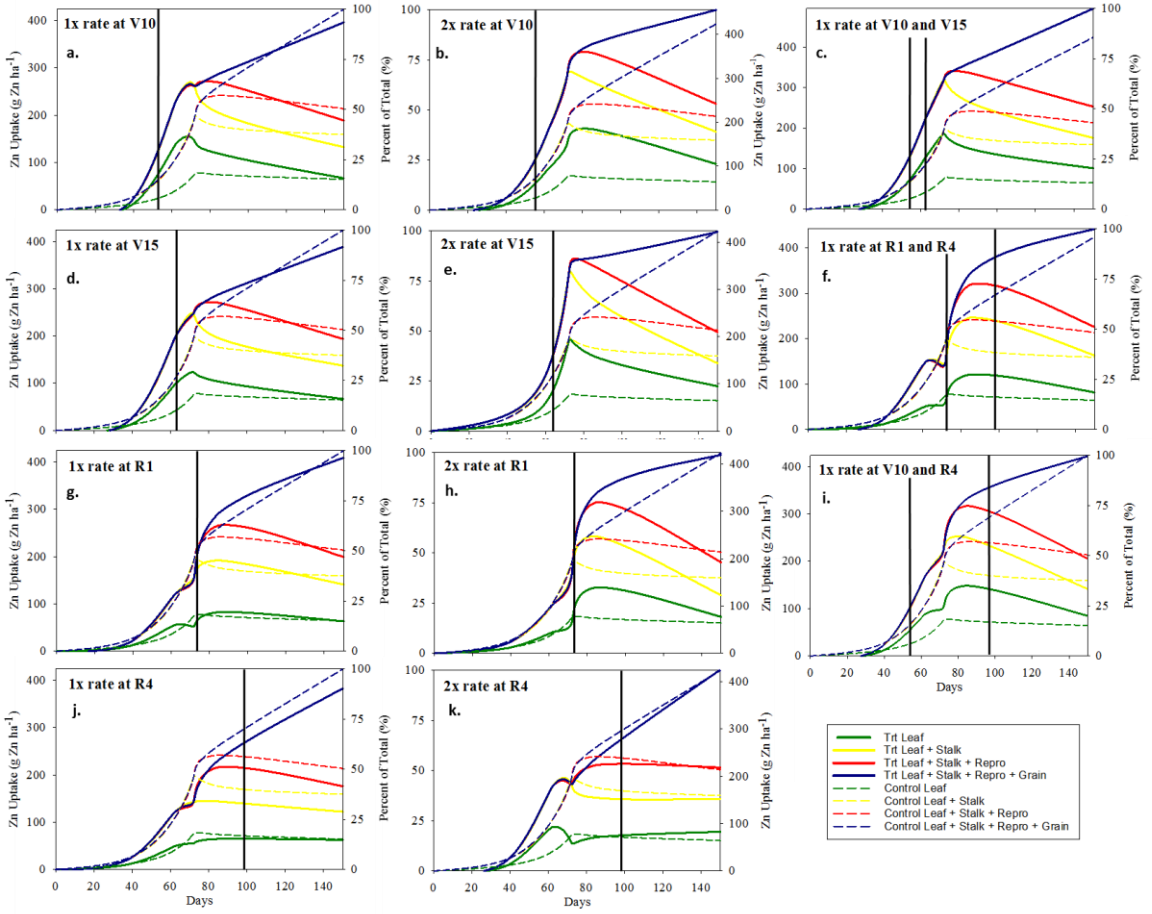

Figure 4-4. Zn uptake (g Zn ha^{-1}) and partitioning graphs with solid line graphs representing foliar Fe/Zn treated plots overlaying dashed line graphs representing the control plot. Solid vertical lines represent the time of application.

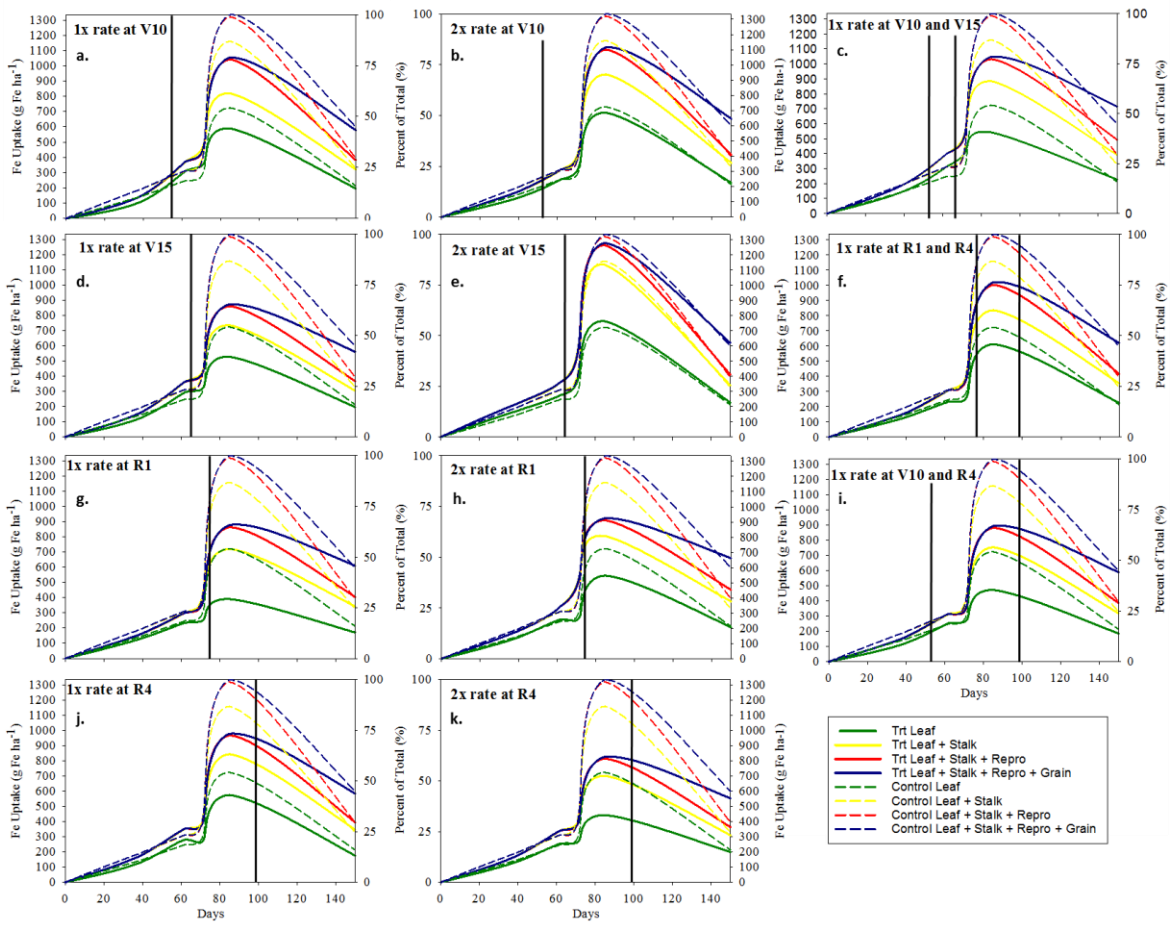

Figure 4-5. Fe uptake (g Fe ha^{-1}) and partitioning graphs with solid line graphs representing foliar Fe/Zn treated plots overlaying dashed line graphs representing the control plot. Solid vertical lines represent the time of application.

CHAPTER 5: EFFECT OF IRON AND ZINC NANOPARTICLE, CHELATE, AND SULFATE FOLIAR APPLICATIONS TO DEFICIENT MAIZE[1]

5.1 Abstract

Improving uptake, mobility, and utilization of foliar-applied iron (Fe) and zinc (Zn) is essential for increasing plant biomass and grain yield. The objective of this study was to compare the effect of foliar-applied Pheroid nanoparticle, chelate, and sulfate forms of Fe and Zn, (0.11; 0.22 kg Fe ha^{-1} and 0.45; 0.90 kg Zn ha^{-1}) on biomass, nutrient uptake and mobilization on Fe and Zn-deficient maize (*Zea mays* L.) grown in a hydroponics greenhouse study. Nanotechnology is a recent methodology that reports to improve dermal penetration, timed-release, and mobility of active ingredients in both animal and plant systems. These properties have potential to increase the effect of foliar-applied nutrients. (-)Fe and (-)Zn hydroponics solutions reduced Fe and Zn biomass, 79 and 11 percent and reduced nutrient concentrations 37 and 55 percent from V5 to V9 in their respective trials to below reported critical values thus establishing ideal experimental parameters for testing the effect of foliar-applied compounds. The upper rate of foliar-applied Fe and Zn in nanoparticle, chelate, or sulfate form increased their respective concentrations in foliage by 90, 120, and 42 percent in the Fe study and by 158, 183, and 120 percent in the Zn study. There was no effect of the Fe or Zn foliar treatments in any form or rate on foliage or root biomass, or mobilization of the applied nutrient.

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Abbreviations: ANOVA, analysis of variance, EDTA, ethylenediaminetriacetate, HEDTA, hydroxyethylenediaminetriacetate, NUE, nutrient use efficiency, Pheroid, Pheroid^{TM}, RCBD, randomized complete block design, and V2/5/9, vegetative second/fifth/and ninth growth leaf stage

Keywords: Foliar, Nanoparticles, Maize, Hydroponics, Iron, Zinc

5.2 Introduction

Nanoparticles are particles between 1 and 100 nm diameter and show properties that are not evident in their bulk counterpart (Auffan et al., 2009). Nanotechnology has potential to improve the effectiveness of active ingredients, and there is a growing body of literature reporting improved dermal penetration, timed-release and mobility of the active ingredients in both animal and plant systems (Zhao et al., 2012). Each of these observed properties would be beneficial in improving the effect of foliar-applied nutrients, improving nutrient use efficiency (NUE), and sustainable intensification (El-Ramady, 2014; Naderi and Danesh-Shahraki, 2013). Nanofertilizers deliver plant nutrients to crops through the encapsulation of nutrients inside nanomaterials, such as nanotubes or nanoporous materials, the coating of nutrients with a thin film, or delivered as emulsions with nanoparticles (DeRosa et al., 2010).

Numerous nanofertilizers have shown promise to improve nutrient utilization through foliar and soil applications. In pot studies, foliar supplementation of 20 mg ml^{-1} zinc oxide nanoparticles on tomato plants improved growth and biomass production as compared to control plants. The nanoparticle application improved uptake and penetration of zinc oxide into the leaf enabling increased utilization of the applied Zn (Panwar et al., 2012). In some studies, the nanoparticle alone has also been shown to exert a positive effect on plant growth. The treatment of tomato seeds with carbon nanotubes (10-40 mug mL^{-1}) dramatically increased germination and growth rate. Analytical methods indicated that the carbon nanotubes were able to penetrate the seed

coat and improve water uptake (Khodakovskaya et al., 2009). The Pheroid™ (Pheroid) (AnnGro®, Bloemfontein, South Africa) nanoparticle is another emerging nanomaterial that has been shown to be an effective delivery vehicle for foliar nutrient (Grobler, 2009).

Pheroid nanoparticles are composed of an organic carbon backbone and fatty acids that result in vesicles and nano-sponges that can be manipulated to entrap hydrophilic, hydrophobic, or amphiphilic compounds for transport across numerous biological membranes (Grobler, 2009). Due to its lipid structure, it is theorized that Pheroids easily associate with the plant membrane and thereby enhance the transport of compounds across the cell wall and possibly enhance the translocation of the compound throughout the plant to metabolically active sink cells. Once inside the cell, it is further theorized that the Pheroid complex is metabolized, releasing the substance and possibly acting as a plant growth stimulator (Pretorius, 2009). Furthermore, Pheroids can be manipulated to alter the release characteristics of the packaged compound (Grobler et al., 2008). These properties make Pheroids a suitable candidate for improving the efficiency of foliar-applied nutrients, such as iron (Fe) and zinc (Zn), in maize production.

Fe and Zn are essential to plant metabolism and are needed in relatively small but critical amounts by maize (Marschner 2012). In order to produce 23 Mg ha^{-1} of total maize biomass with 12.0 Mg ha^{-1} of grain, maize roots must absorb 1.4 kg Fe and 0.5 kg Zn (Bender et al., 2013). Fe and Zn can be supplemented to the plant either through soil or foliar amendments. Though plant leaves are specialized in capturing light and CO_2, their ability to absorb nutrients has long been recognized and used in nutrient management (Fernández and Eichert, 2009). Even as above-ground plant parts are

protected against uncontrolled exchange of nutrients from the environment, nutrients may still penetrate through the cuticle, cuticular cracks and imperfections or through stomata, trichomes, and lenticels (Marschner, 2012).

Foliar-applied micronutrients are widely used in agricultural production, commonly as a complementary strategy to soil fertilization. However, the effectiveness of foliar micronutrient treatments varies significantly among plant species and in relation to their composition such as: salts, complexes, or chelates and with/without additives such as: surfactants and saccharide stickers (Brown et al., 1993; Fernández and Ebert, 2005; Wojcik, 2004) and more recently, nanomaterials. Recent trials of Fe and Zn foliar treatments on maize have shown inconsistent and mixed results with one trial reporting an increase in maize grain yield of nearly 18% for a three-year average with the application of 1.0 to 1.5 kg foliar Zn ha^{-1} (Potarzycki and Grzebisz, 2009), while others report no significant increase in yield due to foliar Fe and Zn supplementation (Arif et al., 2007; Bukvić et al., 2003; Godsey et al., 2003; Mueller and Diaz, 2011; Ziaeyan and Rajaie, 2009) (Chapter 3 and 4). Regarding the absorption and overall effectiveness of foliar-applied chelate and sulfate forms of Fe and Zn, several studies have concluded that chelate forms outperform sulfate forms though this may not be cost effective (Brennan, 1992; Hsu et al., 1982).

There are several challenges that reduce the efficacy of foliar-applied micronutrients. First, foliar-applied micronutrients often have reduced absorption into plant tissues due to factors such as droplet surface tension, retention of applied droplets to the leaf surface, hydrophilic properties, molecular size and molecular charge (Fernández

and Brown, 2013). Second, after entering the plant, adsorption often occurs when micronutrients cross the plant cuticle and move apoplastically to sink cells. Thus, even though the concentration of the micronutrient has increased in the plant tissue, a physiological plant response is not induced (Fernández and Brown, 2013). Third, absorbed micronutrients that do mobilize to metabolically active cells and are utilized by plant cells then become immobilized and therefore become unavailable for subsequent plant growth (Wojcik, 2004). Without repeated foliar applications of micronutrients, the plant will often quickly become deficient once again due to the inability of micronutrients to mobilize or remobilize to new growth.

Current foliar enhancers have focused on the enhanced absorption of micronutrients using various surfactants, stickers (often various saccharides) and molecular forms, such as sulfate, phosphate and chelated forms (Fernández and Brown, 2013). However, current agronomic enhancers have had limited success in maximizing the mobility and utilization of foliar-applied micronutrients to metabolically active sink cells throughout the plant (Chapter 3 and 4). These limitations highlight the importance of developing and investigating technologies, such as nanomaterials, which could enhance dermal penetration, timed-release and mobilization of the applied compound.

The objective of this study was to compare the effect of foliar-applied Pheroid nanoparticles, chelate and sulfate forms of Fe and Zn on biomass, nutrient uptake and mobilization in maize grown under Fe and Zn deficiency scenarios. We hypothesize that the Pheroid nanoparticle, applied as a foliar application of Fe or Zn, will increase biomass, nutrient uptake and concentration of the applied nutrient, increase the quantity

and concentration of Fe or Zn in the upper new growth, and cause greater leaf re-greening as compared to conventional chelated and sulfate forms of Fe and Zn. These hypotheses are based on the assumption that Pheroid nanoparticles may enhance the dermal penetration, slow-release and/or mobilization of Fe and/or Zn to metabolically active cells which would in turn cause an increase in plant biomass with a goal of increasing grain yield (grain yield was not measured in this trial). We anticipate that a foliar application of a Fe or Zn pheroid nanoparticle complex will penetrate and mobilize through the leaf tissue more efficiently than conventional applications (chelated and sulfate forms) of Fe and/or Zn and will therefore cause a greater increase in plant biomass. We also anticipate an increase in the amount of Fe and Zn absorbed into the plant and an increase in the amount of Fe and Zn in the top leaves possibly due to increased availability of the Pheroid Fe or Zn complex or a delayed release of this complex. Visual re-greening of previously chlorotic / nutrient deficient tissue due to Fe and Zn containing foliar treatments is also expected but were not quantified (Hecht-Buchholz and Ortmann, 1986).

5.3 Materials and Methods:

5.3.1 Experimental Design

Two hydroponics greenhouse trials were performed from February 6 to June 12 of 2015 in a University of Nebraska Research Greenhouse. Each trial was a randomized

complete block design (RCBD) with nine treatment combinations and three replications. Greenhouse temperatures ranged from 23.8 to 25.6 °C during the day and 19.4 to 20.6 °C during the night. Supplemental light (approximately 420-460 nm blue and 625-680 nm red wavelengths) was provided by Lumigrow Pro 325 (LumiGrow, Notato, CA) from 630 to 730 hr and then again from 1700 to 1800 hr. Trial 1 and trial 2 consisted of an Fe-deficiency study and a Zn-deficiency study, respectively. Plants were blocked to control for a known temperature and light gradient running east and west across the hydroponics bench. Both trials were conducted under the same experimental setup except for different study times, treatments and hydroponics nutrient solutions (Table 5-1; Table 5-2). For both trials, maize seed (hybrid: Pioneer P9690) was germinated for 14 days in a 1:1 perlite and vermiculite soilless mix and misted every 6 minutes with 12 second burst during the day. After emergence, all mix was removed from the seedling roots before transfer to the hydroponics system (Figure 5-1 b.). Seedlings were transferred to a total of 27-8.5 L pots. The seedlings were held in place by polyisocyanurate rigid foam insulation boards cut to 0.3 x 0.3 m sections (Figure 5-1 a.). Each pot was the experimental unit and contained two seedlings.

5.3.2 Nutrient Solutions and Foliar Treatments

The nutrient solutions used in trial 1 were a complete control (all nutrients) and minus (-)Fe for all other treatments. The nutrient solutions used in trial 2 were a complete control (all nutrients) and minus (-)Zn for all treatments. (Table 5-1; Table 5-2). Both

were adapted from Clark (1982). The nutrient solutions were changed every seven days to maintain nutrient concentrations at desired levels (Table 5-1) and appropriate pH levels (-log(H+) 4.5-6.5). Each pot received compressed air administered by tubing suspended in the nutrient solution and connected to a central hose via large gauge hypodermic needles (Figure 5-1). During the Fe study, a one-time application of the complete nutrient solution was supplied hydroponically to all treatments over the course of a week at the V4 growth stage to prevent plant mortality and leaf desiccation (Abendroth et al., 2011).

Foliar treatments were applied at the V5 growth stage within a spray chamber (Research Track Sprayer; DeVries, Hollandale, MN) using a TP8001E flat-fan nozzle tip (TeeJet Technologies, Spray Systems Co., Wheaton, IL) at 140 L ha^{-1} and at a pressure of 207 kPa (30 psi). The treatments were applied to individual plants at a speed of 3.7 kph (2.3 mph) and height of 0.3 m above the canopy with a band width of 0.38 m (15 in.). Two rates of Fe and Zn were applied at the upper and lower level of industry recommendations to improve likelihood of measuring response. In trial 1 (i.e. Fe-deficiency scenario), the foliar treatments consisted of two rates of Fe(II) sulfate encapsulated by Pheroid nanoparticles at a rate of 120 ml ha^{-1}; two rates of Fe HEDTA (hydroxyethylenediaminetriacetate); and two rates of Fe(II) sulfate (Table 5-2). All Fe containing treatments received the same rates of Fe at rates of 0.11 kg Fe ha^{-1} and 0.22 kg Fe ha^{-1}, which are standard industry rates for maize production. Both the Fe HEDTA and Fe(II) sulfate treatments contained proprietary surfactants, saccharides and antifoaming solvents CornSorb™ (CornSorb) (WinField Solutions®, Shoreview, MN) which are standard additives in industrial foliar treatments. In addition, there was a Pheroid

nanoparticle only treatment applied at a rate of 120 ml ha^{-1} of Pheroid nanoparticles and two controls receiving no foliar treatments, one with the complete hydroponics nutrient solution and the other receiving no Fe in the nutrient solution (Table 5-2).

In trial 2 (i.e. Zn-deficiency scenario), the foliar treatments consisted of two rates of Zn sulfate encapsulated by Pheroid nanoparticles at a rate of 120 ml ha^{-1}; two rates of Zn EDTA (ethylenediaminetriacetate); and two rates of Zn sulfate (Table 5-2). All Zn containing treatments received the same rates of Zn at rates of 0.45 kg Zn ha^{-1} and 0.9 kg Zn ha^{-1}, which are standard industry rates for maize production. Both the Zn EDTA and Zn sulfate treatments contained proprietary surfactants, saccharides and antifoaming solvents, CornSorb, which are standard additives in industrial foliar treatments. Again, there was a Pheroid nanoparticle only treatment applied at a rate of 120 ml ha^{-1} of Pheroid nanoparticles and two controls receiving no foliar treatments, one with the complete hydroponics nutrient solution and the other receiving no Zn in the nutrient solution (Table 5-2). After receiving their respective treatments in the spray chamber, plants were transferred back to the hydroponics bench and were allowed to grow to V9 before plant sampling and analysis.

5.3.3 Treatments, Sampling, and Nutrient Analysis

Two maize seedlings were grown in each pot. After the seedlings reached the V5 growth stage, one plant was removed from each pot, partitioned into roots and foliage, oven-dried at 60°C to constant mass, weighed, and the foliage was analyzed for Fe of Zn

concentrations. The remaining plant in each pot was the experimental unit to which the foliar treatments were applied at V5. At the V9 growth stage, all plants were sampled and partitioned into three components: new growth leaves including the 10^{th} and all other unfurled leaves, bottom section below the unfurled top leaves and above the roots that included both stem and leaves, and the roots were partitioned. No nutrient analysis was performed on the roots. Each of the three components were placed into paper bags and oven-dried to constant mass at 60°C and weighed. Arkley, et al. (1960) had previously shown that the concentration of Zn and Fe in leaf tissue treated with foliar Zn and Fe sprays may be reduced due to washing and thus the fraction of nutrient in the leaf as compared to on the leaf surface cannot be distinguished. The two foliage samples were sent to Midwest Laboratories (13611 "B" St., Omaha, NE 68144) for analysis of nutrient concentrations. The laboratory analysis of leaf phosphorous, potassium, sulfur, calcium, magnesium, iron, manganese, zinc, copper and boron were conducted using microwave nitric acid digestion and concentrations were determined using inductively-coupled plasma emission spectroscopy. The percent nitrogen was determined using the Dumas Method with a Leco FP-428 (International et al., 2006).

5.3.4 Data Analysis

The experiment was analyzed as a randomized complete block design (RCBD) with nine treatments and three replications. The response variables of interest were: V5 biomass, Fe and Zn concentration and uptake, V9 foliage biomass, change in total foliage

biomass (i.e. V9 whole plant dry weight minus V5 whole plant dry weight, excluding roots); the concentration of Fe or Zn in the foliage in both the top leaves and the bottom foliage separately; total quantity of Fe or Zn, in their respective trials, in both the top leaves and the bottom foliage (i.e. concentration (mg kg^{-1}) of Fe or Zn multiplied by dry weight); and root dry weight at V9. For each trial, these data were analyzed using PROC GLM (SAS Institute Inc., Cary, NC) (Littell et al., 2006) specifically for the analysis of variance (ANOVA) for a RCBD. A mean comparison test using the Dunnett Adjustment was used to compare treatment effects to the "deficient control" and a LS means pairwise test was completed to compare nutrient containing treatments against one another (Appendix Code 5-1). Raw concentration data for N, P, K, S, Mg, Ca, Na, Mn, B, and Cu were plotted in Excel (Microsoft Excel 2013, Microsoft Corp. Santa Rosa, CA) and an exploratory analysis for a nutrient by treatment interaction showed no correlation or grouping, thus no further analysis was conducted.

5.4 Results and Discussion

5.4.1 Inducing Deficiencies with (-)Fe and (-)Zn Hydroponics Solutions

Often soils provide enough micronutrients on their own so this study was performed with greenhouse hydroponics to control nutrient levels and produce singular nutrient deficiency scenarios. The hydroponics system was effective for comparing foliar nutrient treatments under their respective nutrient-induced deficiency scenarios. The

parameters of interest for assessing nutrient deficiency were: visual signs of deficiency, biomass, nutrient concentration, and nutrient uptake. In trial 1, this system was effective at inducing rapid Fe deficiency scenarios as evident by visual signs of deficiency (i.e. chlorosis in upper leaves) in pots receiving the (-)Fe nutrient solution. Iron deficiency chlorosis was visible by the V2 growth stage and remained evident to the V5 stage when foliar treatments were applied (Figures 5-2; Figure 5-3).

Maize plant receiving the complete solution / (+)Fe had no visual signs of deficiency throughout the trial (Figure 5-2). At the V5 growth stage, the mean maize biomass of plants receiving the complete solution / (+)Fe (8.4 g) was significantly greater than that of the mean maize biomass of plants receiving the (-)Fe solution (2.2 g) $p<0.0001$). At the V9 growth stage, the mean maize biomass of plants receiving the complete solution / (+)Fe was 45.1 g compared with 9.3 g with the (-)Fe solution (Table 5-3). The mean root biomass was also significantly greater for maize receiving the complete solution / (+)Fe (21.1 g) as compared to the mean root biomass for maize receiving the (-)Fe solution (5.1 g) $p=0.0003$ (Table 5-3).

Sampled plants from the (-)Fe pots at V5 had a mean Fe concentration of 55.7 mg kg^{-1} compared with 90.7 mg kg^{-1} for the (+)Fe pots but the difference was not significant. This 55.7 mg kg^{-1} V5 whole plant tissue concentration and observed visual signs of deficiency are consistent with published critical level of 50 mg kg^{-1} for maize plants less than 0.305 m (12 in). tall (Mills et al., 1996). The supply of Fe in solution also had a significant effect on the total amount of Fe in the foliage. At the V5 growth stage, maize

receiving the complete solution / (+)Fe had 76.0 mg in the foliage compared with 13.9 mg for the (-)Fe solution ($p<0.0001$) (Table 5-4).

No visual signs of Zn deficiency were evident in maize receiving the (-)Zn deficiency nutrient solutions for trial 2 and there was no significant effect on biomass (Figure 5-2). The biomass difference between the Zn supplied and non-supplied at the V5 growth stage was only 0.2 g, which was not significant. Further, at the V9 growth stage, maize plants receiving the complete solution / (+)Zn had a greater, but not significantly greater biomass than the mean maize biomass of plants receiving the (-)Zn solution (i.e. 34.2 g > 30.4 g). Additionally, the greatest mean change (from V5 to V9) in foliage biomass occurring in the complete solution / (+)Zn control maize plants (i.e. 31.9 g increase in biomass from V5 to V9) (Table 5-4).

The supply of Zn in the solution had a significant effect on the concentration of Zn in the foliage and the total amount of Zn in the plant tissue. At the V5 growth stage, the mean Zn concentration of maize plants was 78.7 mg kg^{-1} for (+)Zn compared with 52.0 mg kg^{-1} with the (-)Zn solution (52.0 mg kg^{-1}) $p=0.01$. However, both foliage Zn concentrations were within the sufficiency range for maize of less than 0.305 m tall as reported by Mills et al. (1996). Mills et al. (1996) report the Zn sufficiency range for maize of less than 0.305 m (12 in) tall to be 20-60 mg kg^{-1} Zn. At the V9 growth stage, the mean Zn concentration of maize plants with (+)Zn was 40.2 mg kg^{-1} compared with 18.0 mg kg^{-1} for (-)Zn ($p=0.008$) (Table 5-4). Mills and Jones (1996) reported a Zn sufficiency range prior to tassel of 15-60 mg kg^{-1} Zn (Table 1-1, Chapter 1). Though the mean concentration of maize plants receiving the (-)Zn solution is within the sufficiency

range reported by Mills and Jones, this value is still near the bottom of the sufficiency range. Further, at the V5 growth stage, maize plants had foliar Zn of 18.2 mg with (+)Zn compared with 9.8 mg of Zn for the (-)Zn solution ($p=0.03$) (Table 5-4).

5.4.2 Effect of Foliar Zn and Fe Nano, Chelated, and Sulfate Forms

In both trial 1 and 2, the foliar application of Fe/Zn from all forms (i.e. Pheroid, HEDTA, and Sulfate) and rates (0.11 kg Fe ha^{-1} and 0.22 kg Fe ha^{-1} and 0.45 kg Zn ha^{-1} and 0.90 kg Zn ha^{-1}) to maize grown in (-)Fe/Zn solution had no significant effect on new growth or total foliage biomass or root biomass as compared to the "deficient control" that received no foliar applications of Fe/Zn and was also grown in (-)Fe/Zn solution. The Pheroid only treatment also had no significant effect on foliage or root biomass in both trial 1 and 2 as compared to the "deficient control." Comparisons between Zn containing treatments indicated no significant difference in their effect on foliage or root biomass when pairwise comparisons were performed (Table 5-6). Comparisons between Fe containing treatments showed that Fe-Sulfate rate 2 had the greatest change in foliage biomass which was significantly greater than the rate 2 application of FeHEDTA. Fe-Sulfate did not, however, significantly outperform FeHEDTA in increasing foliage biomass at rate 1 or any other treatments at $p \geq 0.05$ (Table 5-5). Comparisons of foliage biomass at V9 indicated none of the Fe containing treatments significantly increased biomass as compared to other Fe containing treatments at $p \geq 0.05$ (Table 5-5). Biomass was used as an indicator for measuring potential yield effect due to each treatment

(Kemanian et al., 2007). Even though samples were not grown to grain harvest, grain yield increase due to any of the treatments would not be expected.

Significant treatment effects occurred on foliage Fe and Zn concentrations and total foliage Fe and Zn in their respective trials. In both trials, this treatment effect was dependent on rate. Increases in concentration and total plant foliage Fe and Zn were achieved with increased application rates of Fe and Zn in their respective trials regardless of the nutrient source forms. This was more evident for the foliar Zn applications, which may be due to the greater application rate of Zn as compared to the application rate of Fe (Table 5-2). The Pheroid only treatments in both trial 1 and 2 had no significant effect on concentration or total nutrient levels. The application of 0.90 kg Zn ha^{-1} from all forms resulted in significantly greater mean Zn concentrations in the total foliage at V9 as compared to applications of 0.45 kg Zn ha^{-1}, which resulted in increases but did not result in significant increases in total V9 foliage Zn ($p \geq 0.05$) as compared to the (-)Zn control (Table 5-3). Zn-Pheroid and ZnEDTA rate 2 applications induced significantly greater V9 foliage Zn concentrations than their rate 1 counterpart (Table 5-6). At V9, the mean foliage Zn concentrations due to the effect of Zn-Pheroid, ZnEDTA, and Zn-Sulfate at rate 2 (i.e. 0.90 kg Zn ha^{-1}) were 46.5, 51.0 and 40.0 mg kg^{-1}, respectively (Table 5-4). Each of these Zn concentrations are well within the sufficiency range for maize Zn as compared to the mean V9 Zn foliage concentrations for the control, which was 18.0 mg kg^{-1} and is at the lower range of reported Zn sufficiency range for maize prior to tasseling (i.e maize Zn sufficiency range prior to tasseling 15-60 mg kg^{-1} (Table 1-1, Chapter 1) (Mills et al., 1996). ZnEDTA at rate 2, significantly outperformed Zn-Sulfate rate 2 in

increasing Zn concentration and total Zn in the bottom foliage at V9, but there was no significant increase in total Zn concentration and total foliage Zn for all rate 2 treatments (Table 5-6).

This trend was also evident with the Fe containing foliar treatments but was only significant for the Fe concentrations in the bottom foliage. At V9, the mean Fe concentrations in the bottom foliage due to the effect of Fe-Pheroid, FeEDTA, and Fe-Sulfate at rate 2 (i.e. 0.22 kg Fe ha^{-1}) were 59.7, 92.3 and 62.3 mg kg^{-1}, respectively (Table 5-3). The effect of FeHEDTA rate 2 on bottom foliage Fe concentration at V9 was significantly greater than the effect of rate 2 applications of Fe-Pheroid and Fe-Sulfate ($p \geq 0.05$). There were no significant differences between any rate 1 or 2 Fe treatments in increasing Fe concentration of total Fe in the total foliage (Table 5-5). Each of these Fe concentrations are well within the sufficiency range for maize Fe as compared to the mean V9 Fe foliage concentrations for the control (30.7 mg kg^{-1}), which is at the lower range of the reported Fe sufficiency range for maize prior to tasseling but is still sufficient (i.e maize Fe sufficiency range prior to tasseling 10-200 mg kg^{-1} (Table 1-1, Chapter 1) (Mills et al., 1996).

Mobility of the foliar-applied nutrient to new growth leaves was also assessed by measuring the Fe and Zn concentrations in the top new growth (un-treated) leaves in their respective trials. An increase in concentration in the top new growth leaves of Fe and Zn in their respective trials would have been speculated to have been induced by an increase in mobility or a timed-release of the foliar-applied nutrient from the leaf surface to other metabolically active sink cells throughout the growing season. However, in both trial 1

and 2, no significant increase (at $p \geq 0.05$) in concentration of Fe and Zn were observed in the top leaves. In both trial 1 and 2, there were no significant differences in mobility of Fe and Zn in their respective trials between pairwise comparisons (Table 5-5; Table 5-6). These data provide evidence of limited or no mobility provided by the Pheroid, chelate, and sulfate forms. Visual inspection of Fe treated leaves may have provided a different explanation. Leaves treated with foliar Fe all had some re-greening (Figure 5-3). This was especially visible for rate 2 applications. No visual signs of foliar Zn treatment effects (i.e. re-greening) were observed in trial 2 except leaf burn that was similarly evident for all treatments. Foliar applications of Fe-Sulfate re-greened in speckling patterns that were highly localized to the site of the droplet. FeHEDTA and Fe-Pheroid also had speckling patterns but had smoother re-greening patterns across the entire leaf surface (Figure 5-3). This may be suggestive of localized mobilization near the droplet / leaf tissue contact site.

5.5 Conclusions

There was significant effect of (-)Fe and (-)Zn hydroponics solutions in inducing Fe and Zn deficiencies in the respective trials which established a valuable experimental design for testing the effect of nanoparticle and other foliar nutrient applications. In trial 1, the removal of Fe from the nutrient solution induced rapid Fe deficiency chlorosis and a significant reduction in foliage Fe concentration. In trial 2, the removal of Zn from the nutrient solution did not induce visual signs of deficiency but did significantly reduce the

concentration of Zn in the foliage. In both trials, maize receiving the complete solution was able to grow comparable to field conditions. This study demonstrates the capabilities of the hydroponics system to induce nutrient deficiencies and in turn, establish a useful scenario for testing foliar treatments under selectively induced nutrient deficiency scenarios.

The main objective of this study was to compare the effect of Pheroid nanoparticle Fe and Zn complexes with conventional Fe and Zn chelated and sulfate forms applied foliar to Fe and Zn-deficient maize. We hypothesized that the Pheroid nanoparticle Fe and Zn complexes would increase foliar dermal penetration, mobilization and possibly timed-release of the associated Fe and Zn and would therefore induce a greater effect on maize biomass, as a predictor of increasing maize grain yield, and Fe and Zn concentration in the top new-growth leaf tissue, as a predictor of mobilization or timed-release. However, none of the foliar Fe and Zn treatments increased foliage or root biomass or increased Fe and Zn in the top new-growth foliage in Fe or Zn-deficient maize. There was evidence of increased Zn and Fe concentrations in the bottom and total foliage, bringing them above established critical levels, and some localized re-greening, but this resulted in no increase in biomass or nutrient mobilization to new growth.

Although we did not see any advantage of Pheroid nanoparticles, the theoretical benefits of nanomaterials (i.e. enhanced dermal penetration, timed-release, and mobilization of the applied nutrients to metabolically active cellular components) should continue to be investigated to assist in overcoming many of the challenges opposing foliar applications of plant nutrients and other topical treatments (i.e. herbicides and

insecticides). Similarly, soil applications of nanomaterials (i.e. hydroxyapatite nanoparticles) have also been theorized to improve fertilizer efficiency for crop production but has yet to exceed conventional nutrient sources (Montalvo et al., 2015). Research on the effect of foliar Fe and Zn applications on maize have had limited success, therefore it may be more advantageous to test the effect of foliar nanomaterials on more responsive crops (Arif et al., 2007; Bukvić et al., 2003; Godsey et al., 2003; Mueller and Diaz, 2011; Ziaeyan and Rajaie, 2009) (Chapter 2 and 4).

Overall, this study demonstrates the inability of foliar nutrient applications in various forms to mobilize to metabolically active sink cells and be available to new growth plant tissues and thereby elicit an increase in maize biomass. In attempt to improve the effect of foliar applications of nutrients, there is pressing need for technologies that enhance the dermal penetration, timed-release and mobilization of the applied nutrients to metabolically active cellular components. There are numerous emerging nanomaterials that claim to do just that and should be evaluated.

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Table 5-1. Hydroponics nutrient solution compositions. Specific chemicals and mixing details in Clark, R.B., 1982. (Adapted from Clark, R.B., 1982)

	----No Zn Solution†----		---Complete Solution‡---		----No Fe Solution§----	
	mg/liter	µM	mg/liter	µM	mg/liter	µM
Ca	302	7540	302	7540	302	7540
K	283	7240	283	7240	283	7240
Mg	37.8	1550	37.8	1550	37.8	1550
N03-N	321	22900	321	22900	321	22900
NH4-N	39.0	2780	39.0	2780	39.0	2780
Cl	65.0	1940	65.0	1940	65.0	1940
S	58.5	1820	58.5	1820	58.5	1820
P	2.00	65	2.00	65	2.00	65
Fe	2.76	49	2.76	49	0	0
Mn	0.974	18	0.974	18	0.974	18
B	0.536	50	0.536	50	0.536	50
Zn	0	0	0.300	4.6	0.300	4.6
Cu	0.076	1.2	0.076	1.2	0.076	1.2
Mo	0.155	1.6	0.155	1.6	0.155	1.6
Na	4.56	200	4.56	200	4.56	200
HEDTA	13.0	47	13.0	47	0	0

† Solution administered in trial 1

‡ Solution administered in trial 1 and trial 2

§ Solution administered in trial 2

Table 5-2. Treatments applied at V5 in trial 1 (Fe deficiency scenario) and trial 2 (Zn deficiency scenario)†

Trial 1 (Fe Deficiency Scenario)		Trial 2 (Zn Deficiency Scenario)	
Foliar Treatment	Hydroponics Nutrient Solution	Foliar Treatment	Hydroponics Nutrient Solution
Control No foliar trt applied	Complete Solution	Control No foliar trt applied	Complete Solution
Control No foliar trt applied	(-)Fe	Control No foliar trt applied	(-)Zn
Pheroid Nanoparticle‡ only Foliar rate 1	(-)Fe	Pheroid Nanoparticle only Foliar rate1	(-)Zn
Fe Pheroid Nanoparticle Foliar rate 1§	(-)Fe	Zn Pheroid Nanoparticle Foliar rate 1‡‡	(-)Zn
Fe Pheroid Nanoparticle Foliar rate 2¶	(-)Fe	Zn Pheroid Nanoparticle Foliar rate 2††	(-)Zn
FeHEDTA# Foliar rate 1	(-)Fe	ZnEDTA§§ Foliar rate 1	(-)Zn
FeHEDTA Foliar rate 2	(-)Fe	ZnEDTA Foliar rate 2	(-)Zn
Fe Sulfate|| Foliar rate 1	(-)Fe	Zn Sulfate¶¶ Foliar rate 1	(-)Zn
Fe Sulfate Foliar rate 2	(-)Fe	Zn Sulfate Foliar rate 2	(-)Zn

† All treatments were applied to individual plants at a speed of 3.7kph (2.3mph) and height of 0.3m above the canopy with a band width of 0.38m (15in.) in a spray chamber

‡ The pheroid nanoparticle application rate for all pheroid containing treatments was 120mg/ha ($1.07*10^{-4}$ lbs/A)

§ Rate 1 Fe concentrations were 750 ppm 0.11 kg Fe ha^{-1} (0.1 lbs Fe/A) and 120mg/ha pheroid nanoparticle

¶ Rate 2 Fe concentrations were 1500 ppm 0.22 kg Fe ha^{-1} (0.2 lbs Fe/A) and 120mg/ha pheroid nanoparticle

4.5% FeHEDTA (iron-hydroxyethylenediaminetriacetate) in addition to proprietary surfactants, saccharides, and antifoaming solvents CornSorb

|| 6.0% Iron(II) Sulfate in addition to proprietary surfactants, saccharides, and antifoaming solvents CornSorb

‡‡ Rate 1 Zn concentrations were 3,000 ppm or 0.45 kg Zn ha^{-1} (0.4 lbs Zn/A) and 120mg/ha pheroid nanoparticle

†† Rate 2 Zn concentrations were 6,000 ppm or 0.90 kg Zn ha^{-1} (0.8 lbs Zn/A) and 120mg/ha pheroid nanoparticle

§§ 6.0% ZnEDTA (zinc-ethylenediaminetriacetate) and contains proprietary surfactants, saccharides, and antifoaming solvents CornSorb

¶¶ 6.0% Zinc Sulfate and contains proprietary surfactants, saccharides, and antifoaming solvents CornSorb

Table 5-3. Results of Trial 1 comparing foliar Fe treatments on maize grown in Fe-deficient hydroponics nutrient solution. Results are comparing LSmeans for treatment effect with the "deficient control" (Dunnett's Test).

Nutrient Solution	(-) Fe Solution								--(+) Fe Solution--
Foliar Fe Rate	No Foliar Fe	No Foliar Fe	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2	No Foliar Fe
Treaments	Deficient Control†	Pheroid Only	Fe-Pheroid	Fe-Pheroid	FeHEDTA	FeHEDTA	Fe-Sulfate	Fe-Sulfate	Complete Control
V5 foliage dry biomass (g)§	2.2	1.2	2.0	1.2	1.4	2	1.9	1.2	8.4±1.2****‡
V9 foliage dry biomass (g)	9.3	9.9	9.7	8.3	10.6	6.7	8.9	11.9	45.1±6.8****
Change in foliage dry biomass (g)¶	7.2	8.6	7.8	7.1	9.2	4.7	7.0	10.7	36.7±6.7****
V5 Fe concentration (mg kg^{-1})§	55.7	67.0	72.0	74.7	70.0	58	85.7	74.7	90.7±24.0^{+}
V9 bottom foliage Fe concentration (mg kg^{-1})#	30.7	33.3	43.0	59.7^{+}	60.7^{+}	92.3****	43.7	62.3*	65.7±18.2*
V9 top foliage Fe concentration (mg kg^{-1})‖	41.0	39.0	43.5	76.3	94.0	65.3	49.3	39	48.3±51.3
V9 total foliage Fe concentration (mg kg^{-1})	35.8	36.2	40	68	77.3	78.8	46.5	50.7	57.0±31.7
Change in total foliage Fe concentration (mg kg^{-1})**	-19.8	-30.8	-22.8	-6.7	7.3	20.8	-39.2	-24.0	-33.7±43.1
V5 foliage Fe (mg)§	13.9	9.7	15.7	9.9	11.1	12.8	18.7	9.6	76.0±10.5****
V9 bottom foliage Fe (g)	0.02	0.03	0.03	0.03	0.04	0.05	0.03	0.06	0.26±0.03****
V9 top foliage Fe (mg)	3.8	3.6	4.7	4.5	6.8	6.0	7.9	6.8	20.0±0.33
V9 total foliage Fe (g)	0.02	0.03	0.05	0.04	0.05	0.05	0.04	0.06	0.28±0.34
Change in foliage Fe (g)††	0.01	0.02	0.02	0.03	0.04	0.04	0.02	0.05	0.21±0.04****
Root dry biomass (g)§§	5.1	4.5	4.6	3.8	4.9	3.5	4.5	5.4	21.1±4.3****

† Mean comparison test using the Dunnett Adjustment to compare least square means for treatment effects with the "deficient control"

‡ Mean plant value followed by (±95% confidence interval for all treatments in the same row) and significant F test: Not Significant >0.20; + >0.10; * >0.05; **>0.01; ***>0.001; ****≤0.001

§ Parameter measured prior to foliar treatment

¶ Mean difference in foliage mass at the V9 growth stage minus the V5 growth stage foliage mass

Mean Fe concentration in the bottom foliage excluding the unfurled leaves at the V9 growth stage

‖ Mean Fe concentration in the top unfurled leaves at the V9 growth stage used as an indicator of Fe mobility

** Mean difference in the concentration of Fe in each plant at the V9 growth stage minus the V5 growth stage plant Fe concentration

†† Mean difference in total plant Fe at the V9 growth stage minus the V5 growth stage plant Fe

§§ Mean root dry biomass at the V9 growth stage

Table 5-4. Results of Trial 2 comparing foliar Zn treatments on maize grown in Zn-deficient hydroponics nutrient solution. Results are comparing LSmeans for treatment effect with the "deficient control" (Dunnett's Test).

Nutrient Solution	(-) Zn Solution								-- (+) Zn Solution --
Foliar Zn Rate	No Foliar Zn	No Foliar Zn	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2	No Foliar Zn
Treaments	Deficient Control†	Pheroid Only	Zn-Pheroid	Zn-Pheroid	ZnEDTA	ZnEDTA	Zn-Sulfate	Zn-Sulfate	Complete Control
V5 foliage dry biomass (g)§	1.9	1.9	2.0	2.4	2.1	2.2	1.9	2.1	2.3±0.5‡
V9 foliage dry biomass (g)	30.4	32.1	32.8	31.3	31.1	33.4	32	28.9	34.2±5.7
Change in foliage dry biomass (g)¶	28.5	30.2	30.7	29.0	29.0	31.1	30.1	26.8	31.9±5.5
V5 Zn concentration (mg kg^{-1})§	52.0	45.7	49.7	57.3	43.3	46.3	46.7	41.7	78.7±10.6***
V9 bottom foliage Zn concentration (mg kg^{-1})#	14.0	17.0	27.3	61.0****	34.7^{+}	76.3****	37.3*	50.3***	42.7±13.1**
V9 top foliage Zn concentration (mg kg^{-1})‖	22.0	24.7	23.7	32.0	23.0	25.8	26.3	29.7	37.7±8.9*
V9 total foliage Zn concentration (mg kg^{-1})	18.0	20.8	25.5	46.5****	28.8	51.0****	31.8^{+}	40.0**	40.2±8.4***
Change in total foliage Zn concentration (mg kg^{-1})**	-34.0	-24.8	-24.2	-10.8**	-14.5^{+}	-1.2***	-14.8^{+}	-1.7***	-38.5±11.7
V5 foliage Zn (mg)§	9.8	8.9	10.1	13.7	9.0	10.2	9.1	9.0	18.2±3.8**
V9 bottom foliage Zn (g)	0.03	0.04	0.07	0.14**	0.08	0.20***	0.09	0.10^{+}	$0.10\pm0.05^{+}$
V9 top foliage Zn (g)	0.02	0.02	0.02	0.03	0.02	0.02	0.03	0.03	0.04±0.01*
V9 total foliage Zn (g)	0.05	0.06	0.09	0.17**	0.10	0.22***	0.11	0.13^{+}	0.14±0.05*
Change in foliage Zn (g)††	0.04	0.05	0.08	0.16**	0.09	0.21±***	0.10	0.12^{+}	$0.12\pm0.05^{+}$
Root dry biomass (g)§§	11.1	12.4	12.1	10.7	11.5	11.9	10.9	9.0	11.0±3.1

† Mean comparison test using the Dunnett Adjustment to compare least square means for treatment effects with the "deficient control"

‡ Mean plant value followed by(±95% confidence interval for all treatments in the same row) and significant F test: Not Significant >0.20; + >0.10; * >0.05; **>0.01; ***>0.001; ****≤0.001

§ Parameter measured prior to foliar treatment

¶ Mean difference in foliage mass at the V9 growth stage minus the V5 growth stage foliage mass

Mean Zn concentration in the bottom foliage excluding the unfurled leaves at the V9 growth stage

‖ Mean Zn concentration in the top unfurled leaves at the V9 growth stage used as an indicator of Zn mobility

** Mean difference in the concentration of Zn in each plant at the V9 growth stage minus the V5 growth stage plant Zn concentration

†† Mean difference in total plant Zn at the V9 growth stage minus the V5 growth stage plant Zn

§§ Mean root dry biomass at the V9 growth stage

Table 5-5. Mean comparison test of trial 1 LSmean treatment effects comparing all Fe containing treatments to one another

Nutrient Solution	----------No Fe Solution----------					
Foliar Fe Rate	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2
Treaments	Fe-Pheroid	Fe-Pheroid	FeHEDTA	FeHEDTA	Fe-Sulfate	Fe-Sulfate
V9 foliage dry biomass (g)	9.7[a],†	8.3[a]	10.6[a]	6.7[a]	8.9[a]	11.9[a]
Change in foliage dry biomass (g)‡	7.8[ac]	7.1[ac]	9.2[ac]	4.7[bc]	7.0[ac]	10.7[a]
V9 bottom foliage Fe concentration (mg/kg)§	43.0[a]	59.7[a]	60.7[a]	92.3[b]	43.7[a]	62.3[a]
V9 top foliage Fe concentration (mg/kg) (mobility)¶	43.5[a]	76.3[a]	94.0[a]	65.3[a]	49.3[a]	39.0[a]
V9 total foliage Fe concentration (mg/kg)	40.0[a]	68.0[a]	77.3[a]	78.8[a]	46.5[a]	50.7[a]
Change in foliage Fe concentration (mg/kg)#	-22.8[a]	-6.7[a]	7.3[a]	20.8[b]	-39.2[a]	-24.0[a]
V9 bottom foliage Fe (g)	0.03[a]	0.03[a]	0.04[ac]	0.05[ac]	0.03[a]	0.06[bc]
V9 top foliage Fe (g)	0.01[a]	0.00[a]	0.01[a]	0.01[a]	0.01[a]	0.01[a]
V9 total foliage Fe (g)	0.05[a]	0.04[a]	0.05[a]	0.05[a]	0.04[a]	0.06[a]
Change in foliage Fe (g)‖	0.02[a]	0.03[ac]	0.04[ad]	0.04[bcd]	0.02[a]	0.05[bd]
Root dry biomass (g)††	4.6[a]	3.8[a]	4.9[a]	3.5[a]	4.5[a]	5.4[a]

† Means followed by the same letter in the same row are not significantly different at $p \geq 0.05$

‡ Mean difference in foliage mass at the V9 growth stage minus the V5 growth stage foliage mass

§ Mean Fe concentration in the bottom foliage excluding the unfurled leaves at the V9 growth stage

¶ Mean Fe concentration in the top unfurled leaves at the V9 growth stage used as an indicator of Fe mobility

Mean difference in the concentration of Fe in each plant at the V9 growth stage minus the V5 growth stage plant Fe concentration

‖ Mean difference in total plant Fe at the V9 growth stage minus the V5 growth stage plant Fe

†† Mean root dry biomass at the V9 growth stage

Table 5-6. Mean comparison test of trial 2 LSmean treatment effects comparing all Zn containing treatments to one another

Nutrient Solution	----------No Zn Solution----------					
Foliar Zn Rate	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2
Treaments	Zn-Pheroid	Zn-Pheroid	ZnEDTA	ZnEDTA	Zn-Sulfate	Zn-Sulfate
V9 foliage dry biomass (g)	32.8[a]†	31.3[a]	31.1[a]	33.4[a]	32.0[a]	28.9[a]
Change in foliage dry biomass (g)‡	30.7[a]	29.0[a]	29.0[a]	31.1[a]	30.1[a]	26.8[a]
V9 bottom foliage Zn concentration (mg/kg)§	27.3[a]	61.0[bc]	34.7[a]	76.3[b]	37.3[a]	50.3[ac]
V9 top foliage Zn concentration (mg/kg) (mobility)¶	23.7[a]	32.0[a]	23.0[a]	25.8[a]	26.3[a]	29.7[a]
V9 total foliage Zn concentration (mg/kg)	25.5[a]	46.5[b]	28.8[ac]	51.0[b]	31.8[ad]	40.0[bcd]
Change in foliage Zn concentration (mg/kg)#	-24.2[a]	-10.8[ac]	-14.5[ad]	-1.2[bc]	-14.8[ae]	-1.7[bcde]
V9 bottom foliage Zn (g)	0.07[a]	0.14[bc]	0.08[ac]	0.20[b]	0.09[ac]	0.10[ac]
V9 top foliage Zn (g)	0.02[a]	0.03[a]	0.02[a]	0.02[a]	0.03[a]	0.03[a]
V9 total foliage Zn (g)	0.09[a]	0.17[bc]	0.10[ac]	0.22[ac]	0.11[b]	0.13[ac]
Change in foliage Zn (g)‖	0.08[a]	0.16[ac]	0.09[a]	0.21[bcd]	0.10[a]	0.12[ad]
Root dry biomass (g)††	12.1[a]	10.7[a]	11.5[a]	11.9[a]	10.9[a]	9.0[a]

† Means followed by the same letter in the same row are not significantly different at $p \geq 0.05$

‡ Mean difference in foliage mass at the V9 growth stage minus the V5 growth stage foliage mass

§ Mean Zn concentration in the bottom foliage excluding the unfurled leaves at the V9 growth stage

¶ Mean Zn concentration in the top unfurled leaves at the V9 growth stage used as an indicator of Zn mobility

Mean difference in the concentration of Zn in each plant at the V9 growth stage minus the V5 growth stage plant Zn concentration

‖ Mean difference in total plant Zn at the V9 growth stage minus the V5 growth stage plant Zn

†† Mean root dry biomass at the V9 growth stage

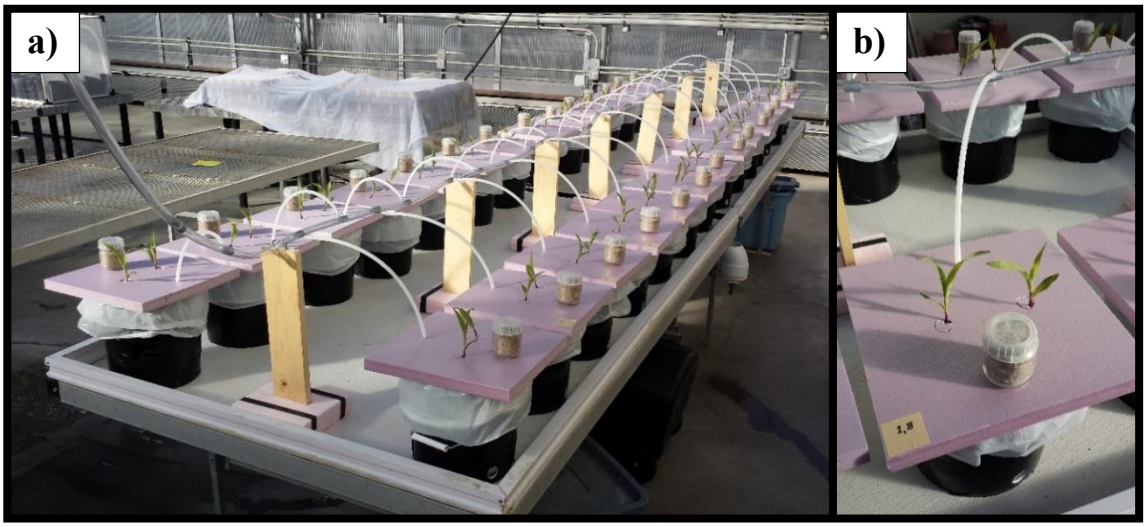

Figure 5-1. a) Experimental hydroponics design b) Image of maize seedlings held in the hydroponics solutions

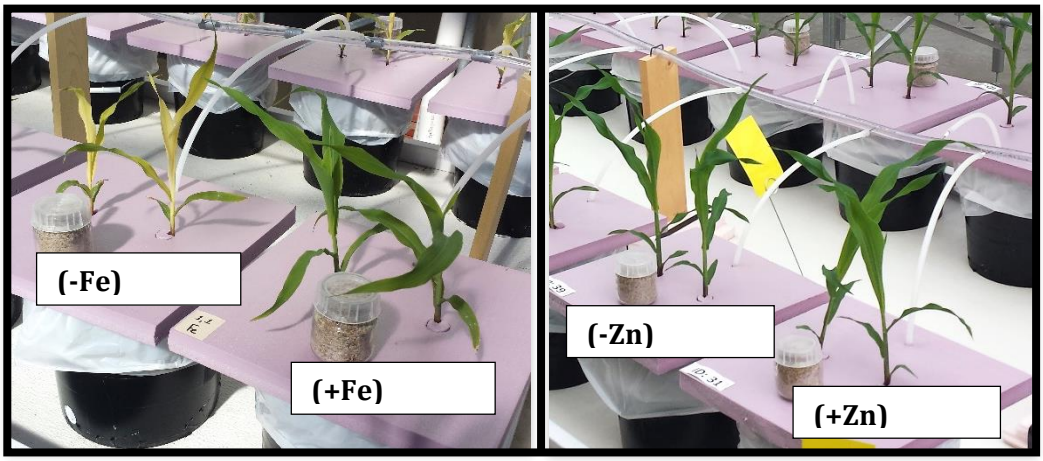

Figure 5-2. V3-V4 maize plants, prior to spraying, grown in trial 1 (i.e. (-) Fe Scenario) and trial 2 (i.e. (-) Zn Scenario). There were visual signs of Fe deficiency as evident by chlorosis in the upper leaves but no visual sign of Zn deficiency.

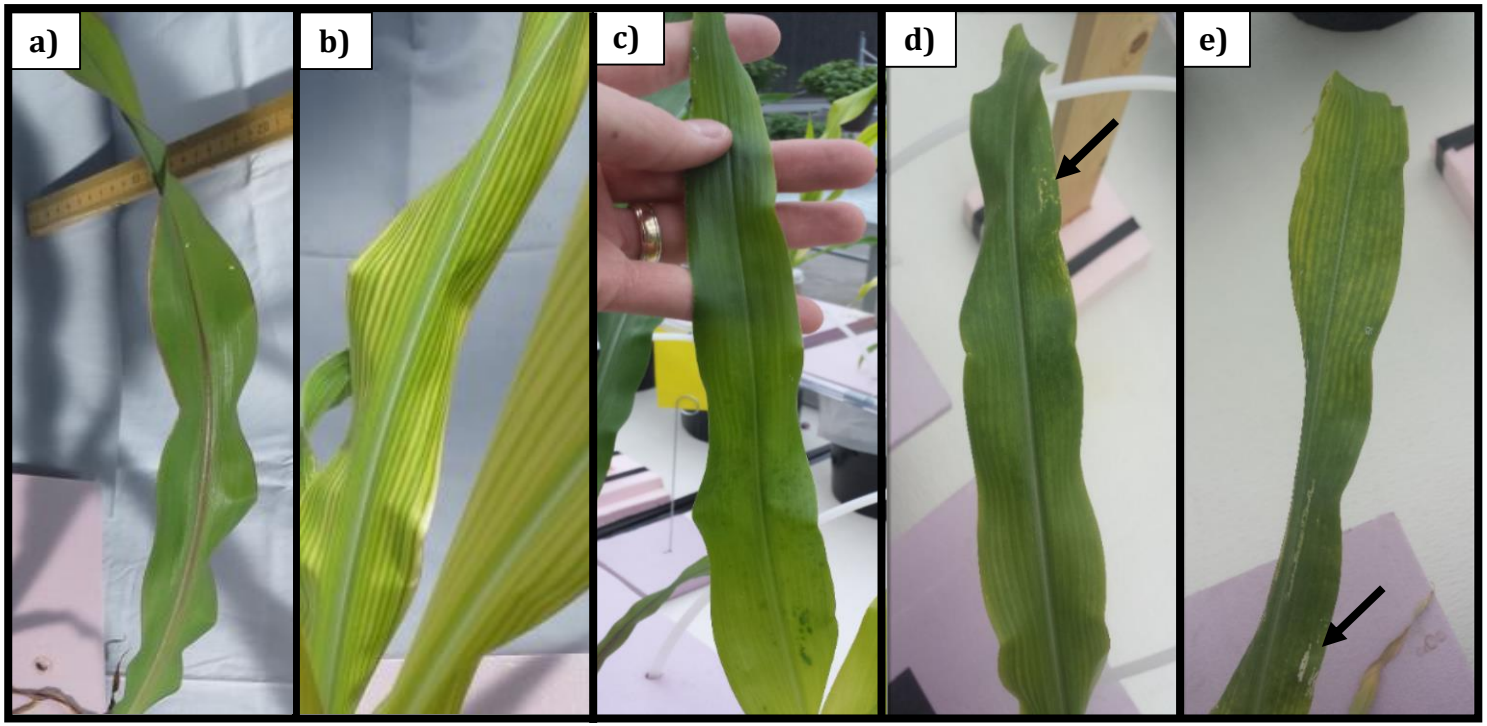

Figure 5-3. Examples of leaf re-greening characteristics of the fifth leaf of maize plants, grown in trial 1, 10 days post foliar Fe treatment applications. No visual signs of re-greening due to the foliar Zn treatments were observed in trial 2. All images were taken from the same statistical block and each of the treated images received "rate 2" (0.22 kg Fe ha^{-1}). Arrows indicate areas with leaf "burn." a) "Complete Control"b) "Deficient Control(-Fe)" c) "Fe-Pheroid Rate 2" d) "FeHEDTA Rate 2" e) "Fe-Sulfate Rate 2"

CHAPTER 6: THE CURRENT STATUS AND FUTURE OF FOLIAR MICRONUTRIENT APPLICATION IN MAIZE PRODUCTION

6.1 Summary of Findings: Where do Foliar Micronutrients Fit into Maize Production?

The previous five chapters review the current state of knowledge about micronutrient use in maize, describe research related to improving our understanding of micronutrient management in maize production, and provide guidance for micronutrient management strategies for future research. In large part, these chapters have compared the conventional deficiency correction theory with the theory that there may be temporal micronutrient deficiency in supply under high yielding, high micronutrient demand conditions even when soil or plant micronutrient concentrations are above critical levels. The following chapter will outline the findings of each chapter in this context.

Chapter 1 reviewed the literature, with emphasis on current research and conventional micronutrient management practices including the use of leaf analysis to guide micronutrient application, outlined past research of foliar micronutrient application on maize, and defined the objectives for the following studies. Based on this chapter, I developed a three-prong approach to determine how micronutrients fit into Nebraska maize production which included: (1) surveying Nebraska micronutrient status in maize production (chapter 2), (2) conducting field trials (i.e. on farm strip trials) using conventional micronutrient foliar formulations under current farmer agronomist practices

(chapter 3), and performing small-plot and greenhouse trials to improve guidelines for foliar micronutrient application (chapter 4 and 5).

The survey in chapter 2 was designed to set the context of micronutrient nutrition of maize production in Nebraska and aid in developing a research agenda for the subsequent studies. The objectives of this survey were: (1) to evaluate the nutritional status of maize production locations and develop an understanding of which nutrients may be most limiting (i.e. identify locations with soil and/or plant tissue concentrations with nutrients below critical concentrations) with particular interest in soil and plant tissue micronutrient concentrations and their relationship with yield, and (2) to explore factors influencing micronutrient concentrations of soil, plant tissue and grain samples and their relationships with other soil, plant and grain parameters and yield. Nebraska soils are largely micronutrient fertile and in most cases micronutrient applications are not recommended under the conventional deficiency correction theory. The results of the survey indicate that the mean soil B, Cu, Fe, Mn, and Zn concentrations were 0.6, 1.7, 67.5, 21.3, and 2.2 mg kg^{-1}, mean leaf B, Cu, Fe, Mn, and Zn concentrations were 8.7, 10.0, 181.0, 80.6, and 27.0 mg kg^{-1}, and mean grain Cu, Fe, Mn, and Zn concentrations were 2.8, 19.6, 5.2, and 19.9 mg kg^{-1}. These data were valuable in determining micronutrients to target in subsequent yield response trials under similar field conditions where there may be opportunity for yield increases under the high yield/ temporal deficiency in supply theory.

The survey indicated that there are few locations with soil and plant tissue samples below critical nutrient concentrations in Nebraska (Figure 2-1). However, P, S, and B each had greater than 10% of the sampled locations reporting soil concentrations

below published critical soil levels. Magnesium, S, Zn, and B each had greater than 10% of the sampled locations reporting plant tissue samples below critical plant tissue levels. In particular, Zn had over 50% of the locations reporting plant tissue Zn concentrations below critical levels. Approximately, 7% of the locations were around 10 mg kg^{-1} below the critical plant tissue Zn concentration. S and Zn had 9 and 1%, respectively, of samples with both soil and plant concentrations below critical levels.

The survey also demonstrated that for six of the eleven tested nutrients, soil extractable nutrient concentrations did not correlate well with their elemental concentration in plant tissues (i.e. only K, Mg, Ca, S, and Mn had significant correlations) and provided insight into some of the key correlations between nutrient concentrations in soil, plant tissue and grain samples (Tables 2-11). These data provided guidance on which micronutrient to target with subsequent application studies (i.e. B, Mn, Fe, Zn), highlighted the challenge in soil and plant tissue laboratory report interpretation, and provided insight into the numerous relationships between soil, plant, and grain nutrient concentrations and associated parameters (Table 2-11). An example of how these data are useful in soil and plant tissue laboratory report interpretation is that if soil and plant nutrient values do not correlate then it is inappropriate to assume that low plant nutrient concentrations indicate low soil concentrations and thus there is a need for soil application for maize.

The survey data revealed a consistent negative relationship between grain yield and grain mineral and protein density which is consistent with the findings of Fan, et al. (2008) in wheat grain. This trend has not been evaluated in maize grain. As yield increases, total nutrient uptake increases, however, these data indicated that the

concentration of essential nutrients in maize grain is negatively correlated with increasing yield. As would be expected with increased grain biomass harvest, all nutrient uptake except S had significant positive correlation with grain yield (P, K, Mg, S, Cu, Fe, Mn, Zn: 0.76, 0.65, 0.36, -0.08, 0.41, 0.53, 0.49, 0.23, p= <0.001, <0.001, 0.001, 0.47, <0.001, <0.001, <0.001, 0.04, respectively) (Table 2-7). Grain protein uptake was negatively correlated with yield (-0.34, p=0.002). Grain K, Mg, S, Fe, Mn, Zn and protein concentrations each had significant negative correlations with grain yield (-0.43, -0.37, -0.44, -0.23, -0.22, -0.26, -0.34, p= <0.001, 0.001, <0.001, 0.04, 0.02, 0.002, respectively). This negative correlation is likely an artifact of maize breeding programs and nutrient management programs that have been effective at increasing the total mass of maize grain production (i.e. likely due to increases in grain starches and saccharides) but have not been effective at maintaining mineral and protein concentrations. In the context of consuming maize as a staple crop, higher yielding maize may be less nutrient dense and therefore may increase the odds of micronutrient malnutrition; however, in the context of commercial maize production and micronutrient application, these data may indicate greater micronutrient efficiency of higher yielding maize.

Based on the survey data from 2013-4 and discussions with agronomists and farmers, 26 on-farm field trials (chapter 3) and five small-plot trials (chapter 4) were conducted testing four micronutrients. In chapter 3, foliar applications of micronutrients (i.e. boron (B), iron (Fe), manganese (Mn), and zinc (Zn)) were evaluated at the field scale under irrigation across Nebraska from the high pH soils of Western Nebraska to high yielding locations of Eastern Nebraska. The objective of these on-farm strip trials was to evaluate the effect of foliarly-applied micronutrients on grain yield and plant

tissue elemental status under current farmer-agronomist practices using commercially available foliar micronutrients.

Nebraska soils are generally micronutrient fertile and in most cases micronutrient treatments are not necessary. In the survey we averaged 0.6, 68, 21, and 2.2 mg kg^{-1} for soil B, Fe, Mn, and Zn in comparison of strip trial we averaged 0.6, 34, 9.5, and 3.6 mg kg^{-1}, respectively. Each 95% confidence interval was above their respective soil critical levels of 0.5, 4.5, 2.0 and 0.75 mg kg^{-1} for soil B, Fe, Mn, and Zn, respectively. When the survey and strip trial locations are combined there were 111 sites and 26% and 4% of the sites were at or below soil critical levels for B and Zn, respectively, and no sites were at or below Fe or Mn soil critical levels.

Soil samples and plant tissue nutrient concentrations in the strip trials were not predictive of response under these study conditions (Table 3-4 and 3-5); however, no location had soil or plant tissue micronutrient concentrations below critical levels. Nonetheless, there were three locations that did have significant yield increases of 0.75 and 0.69 Mg ha^{-1} due to foliar applications of a mix of Zn, Mn, and B and a significant yield increase of 0.63 Mg ha^{-1} due to an Fe only treatment (Table 3-4) under these study conditions. This indicates that there may be need and opportunity for predictive tools to aid producers with forecasting yield response to foliar-applied micronutrients beyond a stand-alone plant tissue or soil sample value such as those correlations discussed in chapter 2. A well-known example of this occurs with Fe, Mn, and pH. Though there may be adequate soil Fe concentrations, high soil Mn or pH may be influencing the low plant Fe concentrations. These correlations are not intended to be interpreted as causative but rather to aid in understanding nutrient relationships which may be driving nutrient

concentrations in the plant and grain thereby affecting yield. Additionally, when the yield response was correlated with the control yield, there was no correlation (r=0.03) between higher yielding locations and an increased likelihood of yield response to foliar micronutrient application (Figure 3-7). This further refutes the theory that high yielding conditions may have greater response to micronutrient applications

The strip trial results are largely supportive of the deficiency correction theory and do not support the theory of benefit due to foliar micronutrient application to high yielding maize, without evidence of need, during periods of greatest nutrient demand. The most consistent result of these trials was no increase in grain yield despite an increase in the leaf nutrient concentration of the applied micronutrient especially in the case of Zn (Table 3-5). Under conditions without visual signs of deficiency, responses of leaf micronutrient concentrations and grain yield to foliar micronutrient treatments were limited and inconsistent. The three-year combined analysis of variance showed no significant yield increase due to any treatment except for foliar Fe application applied in locations with visual signs of Fe deficiency, alkaline pH, and low but sufficient soil and plant Fe concentrations (Table 3-5). Locations receiving foliar Zn, Mn, or B application ranged in grain yield differences due to treatment from -0.38 to 0.75 Mg ha^{-1}. Of the foliar treatments containing Zn, Mn, and/or B, there were no significant yield increases ($p<0.05$) for the three-year averages for each product formulation (Table 3-5). The combined analysis of variance for identical Fe treatments at differing locations indicates that a foliar application of 123 g Fe ha^{-1} can increase grain yield ($p=0.008$) by an average of 0.4 Mg ha^{-1} under conditions of high pH (i.e. 7.2 to 8.0), sufficient but low soil Fe

concentrations (i.e. 8.0 to 13.0 mg kg^{-1}), and sufficient but low plant tissue Fe concentration prior to tassel (i.e. 68 to 86 mg kg^{-1}), and visual signs of Fe deficiency.

The strip trial data helped develop the small plot intensive (SPI) research reported in chapter 4. The objectives of the SPI research were to determine the fate and mobility of foliar-applied B, Fe, Mn, and Zn, determine the recovery efficiency of the applied micronutrients, and evaluate the effect of micronutrients, applied foliarly at multiple rates and growth stages, on maize grain yield. Foliar B, Mn, and Zn had limited effect on grain yield for most application time by rate levels though the Mn site had a 19% yield increase due to a V18 application of 0.73 kg Mn ha^{-1} and at the Zn site, a yield decrease of 4.5% due to a split application of foliar 0.84 kg Zn ha^{-1} applied at V11 and V15. Neither location had soil Mn or Zn below critical levels in their respective trials. Due to abnormal conditions at the Zn, Mn, and Zn/Fe locations (i.e. hail and sporadic rain events), grain yield was notably below its historical mean. Under scenarios of greater grain and foliage yield, demand for Zn, Mn, or Fe may exceed soil supply and could cause response to foliar applications. The only location that had consistent grain yield response was also the only location that had visual signs of micronutrient deficiency. Regardless of application time from V6 to R2, there was a 13.5-14.6% increase in grain yield due to a foliar application of 0.22 kg Fe ha^{-1}. Rate 2 (0.22 kg Fe ha^{-1}) consistently outperformed rate 1 (0.11 kg Fe ha^{-1}) across all treatments.

Though there was limited yield response to foliar B, Mn, and Zn under our study conditions, these data did provide evidence for target growth stages to increase micronutrient uptake and mobilization of the applied micronutrient to tissues with physiological demand and aided in better understanding of the fate of the foliar-applied

micronutrients. Foliar applications of B, Mn, and Zn were all effective at increasing their respective micronutrient concentration and uptake in leaf, stalk, and reproductive organs (Mn and Zn also had effect on immature grain uptake). Fe, Mn, and Zn had limited translocation. However, early season applications of B had significant mobilization to reproductive tissues at or after VT.

Our data suggest early season B applications (i.e. prior to V10) are effective at storing and mobilizing B from leaf tissues to reproductive tissues post VT and late season (V15 and R1) applications of foliar B are less effective. This was evident by a 10.3 g B ha^{-1} ($p=0.001$) increase, (3.7 mg B kg^{-1} concentration increase ($p=0.0005$)) in R6 reproductive tissue B due to a V10 application of 0.14 kg ha^{-1} foliar B. This increase in R6 reproductive tissue B was not observed for V15 or R1 applications. These data suggest that earlier applications of B have greater penetration and mobility as compared to late foliar B applications which is consistent with (Bender, et al., 2013; Karlen, et al., 1988). Bender, et al. (2013) report that stored B in leaf tissue appear to serve as a source of mobilized B to reproductive tissues which agrees with our data. B is known to play a critical role in flower production, pollen tube elongation, and germination and increases seed and fruit development so this mobilization to reproductive tissues is consistent with the expected B physiological demands (Dell and Huang, 1997).

Foliar applications of Mn during vegetative growth stages were effective at increasing Mn concentration in leaf tissue. However, similar to foliar B at reproductive growth stages, foliar Mn treatments during reproductive growth stages will likely have little effect on maize as evident by the sharp plateau in Mn uptake during reproductive growth and limited Mn uptake due to foliar applications applied after V18. Inversely, late

applications of foliar Zn applied from V15 to R1 are effective at increasing Zn concentration in immature grain by as much as 10.5 mg Zn kg^{-1} and reproductive components by as much as 18.8 mg Zn kg^{-1} and theoretically could have effect on grain yield or biofortification under differing field conditions. This increase in Zn concentration was not associated with an increase in Zn uptake.

The strip trial and SPI data illustrate the importance of confirming a micronutrient deficiency prior to applying a foliar micronutrient treatment, provide guidance for specific growth stages to target with specific micronutrients, and highlight the uncertain effects of foliar-applied micronutrients on grain yield.

Apparent nutrient recovery (ANR) could not be calculated from the strip trials or survey data. For the SPI experiments, we found that foliar applications of Mn and Zn had similar but slightly higher recovery efficiencies at the end of the growing season than reported soil applied Mn and Zn recovery efficiencies. Foliar applications of Mn and Zn ranged from 4.6% to 24.7% recovery whereas Mortvedt (1994) reported that soil applied Mn and Zn recovery efficiencies ranged from 5-10% recovery. Growth stage of application did not significantly change ANR. Foliar Mn, Zn, and B only application had ANR least square means of 9.5, 16.9, and 2.5%, respectively with standard errors of 3.7, 9.6, and 2.9, respectively. The low ANR of each of the applied micronutrients implies that a majority of the foliar application was either sprayed directly onto the soil, was washed-off the leaf surface, or possibly reduced soil uptake.

The limited effect of foliar-applied micronutrient applications in the strip and SPI trials (i.e. low ANR, limited micronutrient translocation, limited effect on grain yield or biomass) lead to the investigation of several carriers including nanoparticle technologies

which have been shown to improve dermal penetration, mobilization, and timed-release of compounds in plant and mammalian systems and was theorized to improve foliar delivery of micronutrients which was investigated in a greenhouse study reported in chapter 5. The objective of this study was to compare the effect of foliar-applied Pheroid nanoparticle, chelate, and sulfate forms of Fe and Zn, (0.11; 0.22 kg Fe ha^{-1} and 0.45; 0.90 kg Zn ha^{-1}) on biomass, nutrient uptake and mobilization on Fe and Zn-deficient maize (*Zea mays* L.) grown in a hydroponics greenhouse study.

Hydroponics solutions [(-)Fe and (-)Zn] reduced Fe and Zn biomass, 79% and 11% and reduced nutrient concentrations 37% and 55% from V5 to V9 in their respective trials to below reported critical values thus establishing ideal experimental parameters for testing the effect of foliarly-applied compounds. The upper rate of foliarly-applied Fe and Zn in nanoparticle, chelate, or sulfate form increased their respective concentrations in foliage by 90, 120, and 42 percent in the Fe study and by 158, 183, and 120 percent in the Zn study. However, there was no effect of the Fe or Zn foliar treatments in any form or rate on foliage or root biomass, or mobilization of the applied nutrient.

In conclusion, these studies showed that foliar applications of B, Mn, Zn, and Fe had limited effect on grain yield in regions with soils and plant tissues well above critical levels but may have significant benefit when soil or plant concentrations are near or below critical levels especially when there is visual signs of micronutrient deficiency. Without evidence of micronutrient need, foliar micronutrient application to high yielding maize is not recommended. Overall, (1) our basic recommendation philosophy of deficiency correction is valid at high yields, (2) Nebraska soils are well supplied with micronutrients, (3) increasing leaf nutrient concentration may not be related to increasing

yield, (4) very little of the foliar-applied micronutrients are translocating to grain and decreasing grain concentration are likely due to dilution from starch and not poor soil, and (5) there is very little evidence of temporal deficiencies in supply under high yield conditions.

6.2 Future Directions

6.2.1 Opportunities for Foliar Fe and Precision Farming Technologies

Locations receiving foliar-applied Fe supplements were the only locations that had visual signs of deficiency (i.e. interveinal chlorosis, stunting, and yellowing), had plant and soil samples near critical levels (i.e. mean plant Fe range: 60-180 ppm; mean soil Fe range: 4.0-10.0 ppm) and had consistent yield response. Plant tissue critical values for Fe are 50 ppm for maize plants 12 in. tall or less (Mills, et al., 1996) and soil Fe concentrations of less than 4.5 ppm are considered medium and less than 2.0 ppm low Fe (Buchholz, et al., 2004). Overall, response was more pronounced and statistically significant in the small-plot trial described in chapter 4 than in the foliar Fe strip trials in chapter 3.

The strip trials homogenized in-field soil pH and Fe variation (Figure 6-1) and had one of the seven locations with a significant yield increase of 0.63 Mg ha^{-1} ($p=0.01$) due to foliar-applied Fe (Chapter 3), whereas the small-plot trial was situated on a known Fe deficient location and had consistent increases in grain yield. While we did not take

yields within strips, visual observations of treated strips showed small re-greening patterns where foliar Fe droplets contacted the leaf surface.

In the small plot trial, the higher Fe rate of 0.22 kg Fe ha^{-1} 4.5% FeHEDTA consistently outperformed the lower Fe rate of 0.11 kg Fe ha^{-1} regardless of the growth stage of application (Chapter 4). Our results indicate that foliar applications of as little as 0.22 kg Fe ha^{-1}, regardless of application time from V6 to R2 to known high pH or low plant tissue Fe testing locations, can have significant effect on increasing grain yield by as much as a 14.6% increase in grain yield. Additionally, there were no treatment differences between non-control treatments at p=0.05. Fe application rates greater than 0.22 kg Fe ha^{-1} may be of greater benefit and would be worth investigating as this study did not have enough rates to establish a rate-response curve or a cost-benefit analysis. The foliar Fe only location was high yielding (approx. 16 Mg ha^{-1}) and this high yielding situation may have further contributed to the strong response. Greater grain and foliage production requires greater demand on soil nutrients, thus plant Fe demand may have been greater than soil Fe supply.

The difference in treatment response between the strip trials and the small-plot trial could be due to differences in the proprietary ingredients in the two Fe products or specific hybrid Fe-deficiency tolerances but is more likely due to the targeting of the small-plot trial to a known high pH and low Fe plant tissue testing location within the field (Figure 6-1).

This study lead to the conclusion that foliar Fe applications would be a strong candidate for precision farming technologies by applying foliar Fe only to known high pH or low Fe testing locations. Since Fe-deficiency symptomology displays chlorosis,

spectral imaging technologies would likely be the management tool of choice. However, plant or soil analysis will need to be performed first to confirm Fe deficiency, as other nutrients, such as N, also display chlorosis during deficiency. Nutrient management using less prescriptive whole field applications of foliar Fe may have yield increases that are less evident, may cause yield reduction in areas where plant Fe concentrations are already sufficient, and are likely less economical when applied at the field scale. This scenario is likely not exclusive to Fe management, and under conditions of confirmed deficiency, there would likely be similar results for other micronutrients.

6.2.2. Opportunity for a Micronutrient Yield Response Model

Under limited, prescriptive scenarios, foliar application of micronutrients may be beneficial even when there are no visual signs of deficiency. It should be noted that determining predictable times and locations to apply micronutrients to achieve a profitable yield increase has remained elusive when based solely on either a soil or plant tissue nutrient concentration and where there is no visual sign of micronutrient deficiency. Without these predictive tools, utilizing foliar micronutrient successfully and consistently will be difficult under similar conditions. The likely factors for inclusion in a predictive model would be soil and plant critical levels in addition to factors identified in chapter 2 (Table 2-11) that had significant correlation with the micronutrient of interest.

It can be theorized that locations that have both soil and plant tissue samples below or near critical values and high levels of a parameter that has a negative correlations with the micronutrient of interest or low levels of a parameter that has a

positive correlation with the micronutrient of interest as identified in chapter 2 may be the most likely scenario to induce a yield response from treatment with the nutrient of interest. The successful development of a predictive model will include more than a singular plant tissue and soil elemental concentration alone. The data presented in chapter 2, indicate which non-target nutrients or parameters may be influencing the nutrient of interest. These relationships are presented in Table 2-11. A well-known example of this occurs with Fe, Mn, and pH. Though there may be adequate soil Fe concentrations, high soil Mn or pH may actually be driving the low plant Fe concentrations. Soil Fe was not the only driver of plant Fe.

6.2.3 Opportunities for Nanomaterials and Hydroponics for Future Foliar Micronutrient Evaluation

There was significant effect of (-)Fe and (-)Zn hydroponics solutions in inducing Fe and Zn deficiencies in the respective trials which established a valuable experimental design for testing the effect of nanoparticle and other foliar nutrient applications. In trial 1, the removal of Fe from the nutrient solution induced rapid Fe deficiency chlorosis and a significant reduction in foliage Fe concentration. In trial 2, the removal of Zn from the nutrient solution did not induce visual signs of deficiency but did significantly reduce the concentration of Zn in the foliage. In both trials, maize receiving the complete solution was able to grow comparable to field conditions. This study demonstrated the capabilities of the hydroponics system to induce nutrient deficiencies and in turn, establish a useful

scenario for testing foliar treatments under selectively induced nutrient deficiency scenarios.

Although there was no benefit of Pheroid nanoparticles, the theoretical benefits of nanomaterials (i.e. enhanced dermal penetration, timed-release, and mobilization of the applied nutrients to metabolically active cellular components) should continue to be investigated to assist in overcoming many of the limitations of foliar applications of plant nutrients as well as other topical treatments (i.e. herbicides. insecticides). Similarly, soil applications of nanomaterials (i.e. hydroxyapatite nanoparticles) have also been theorized to improve fertilizer efficiency for crop production but have yet to exceed conventional nutrient sources (Montalvo, et al., 2015). Research on the effect of foliar application of micronutrients on maize have had limited success, therefore it may be more advantageous to test the effect of foliar nanomaterials on more responsive crops (Arif, et al., 2007; Bukvić, et al., 2003; Godsey, et al., 2003; Mueller and Diaz, 2011; Ziaeyan and Rajaie, 2009).

Of additional interest, these data were not consistent with the foliar Fe small-plot trial results. In both the small-plot field trial and this hydroponics greenhouse trial there were visual signs of Fe deficiency. However, while both trials used the same Fe product and rate, in the greenhouse trial, there was no significant increase in biomass, but in the small-plot field trial, there was a consistent and highly significant increase in grain yield. This inconsistency is suggestive that either biomass was not a predictor of grain yield in the greenhouse trial, the foliar treatment is actually being taken up through the roots in the field trial and not in the hydroponics study, the (-)Fe hydroponics solution possibly induced too great of deficiency for the foliar Fe treatments to overcome, or there a

possible synergism between foliar Fe application and soil that is not yet evident. Further research should address the ratio of the foliar treatment being applied to the leaf or the soil and which is contributing to the applied nutrient's plant uptake.

Overall, the hydroponics study demonstrated the inability of foliar application of micronutrients in various forms to mobilize to metabolically active sink cells and be available to new growth plant tissues and thereby elicit an increase in maize biomass. In attempt to improve the effect of foliar applications of nutrients, there is pressing need for technologies that enhance the dermal penetration, timed-release, and mobilization of the applied nutrients to metabolically active cellular components. There are numerous emerging nanomaterials that claim to do just that and should be evaluated.

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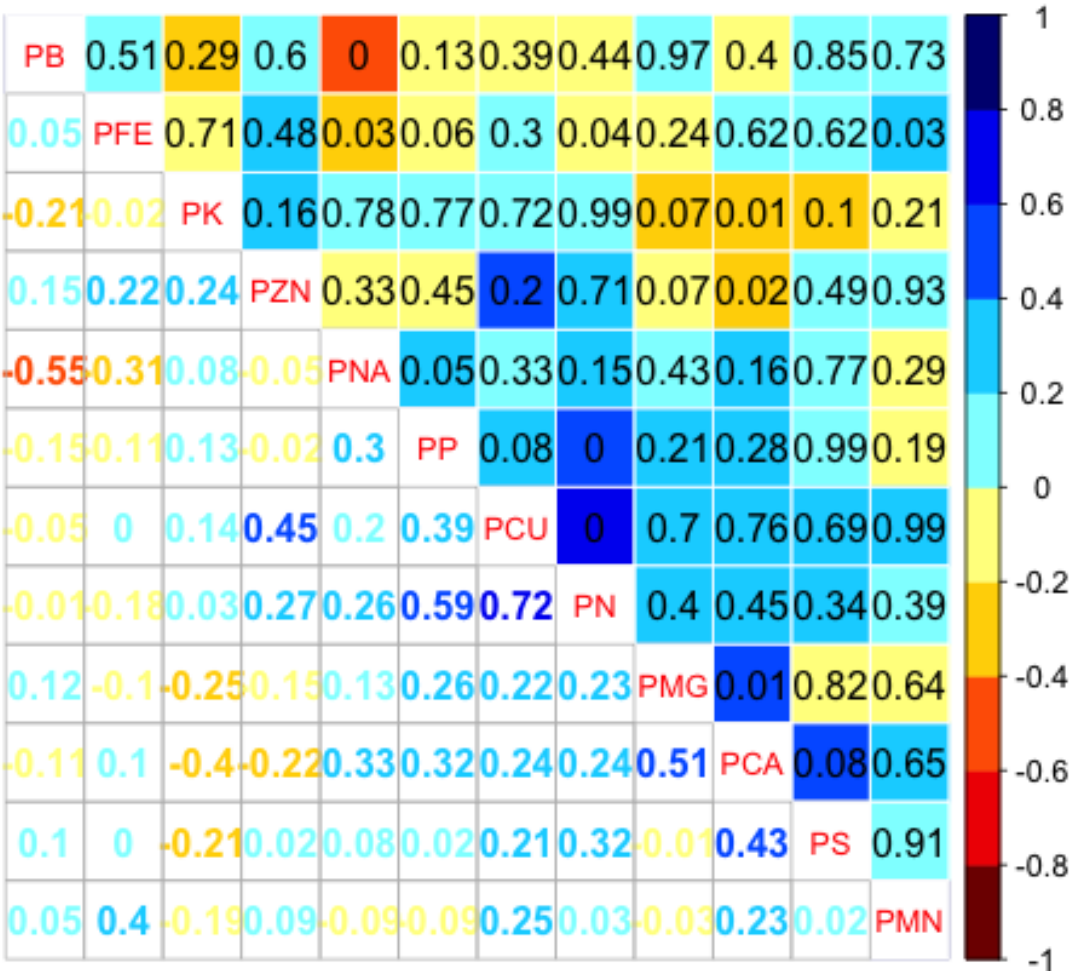
Mueller, N.D. and D.A.R. Diaz. 2011. Improving corn and soybean yields with starter and foliar fluid fertilizers. Proceedings of the 2011 Fluid Forum.: 108.

Ziaeyan, A. and M. Rajaie. 2009. Combined effect of zinc and boron on yield and nutrients accumulation in corn. Int. J. Plant Prod. 3: 35-44.

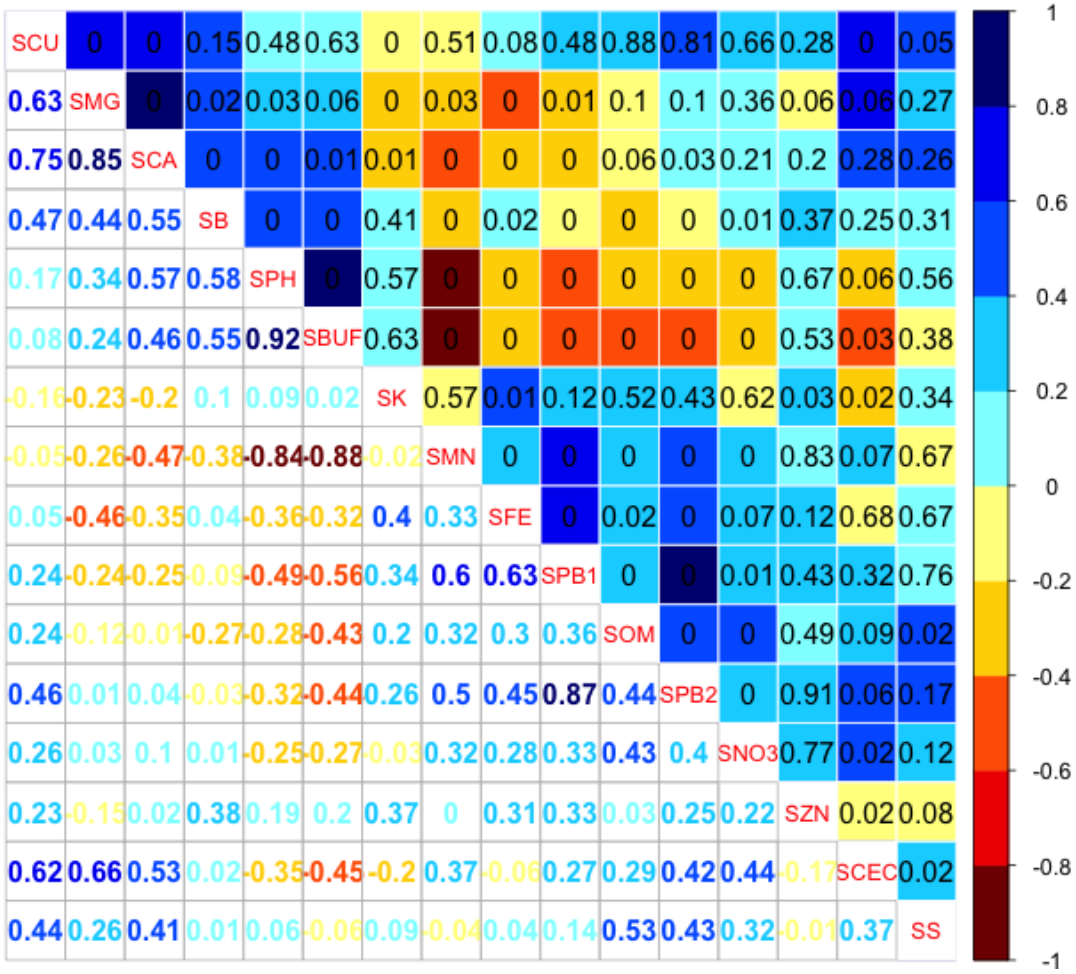
Figure 6-1. Strip trial location 2 aerial image, taken prior to treatment at location 2, showing the typical in-field variation running across strips. The grids depict the large strip trial layout running East and West across the field and the small-plot layout directly over a high pH / Fe deficient location.

APPENDIX

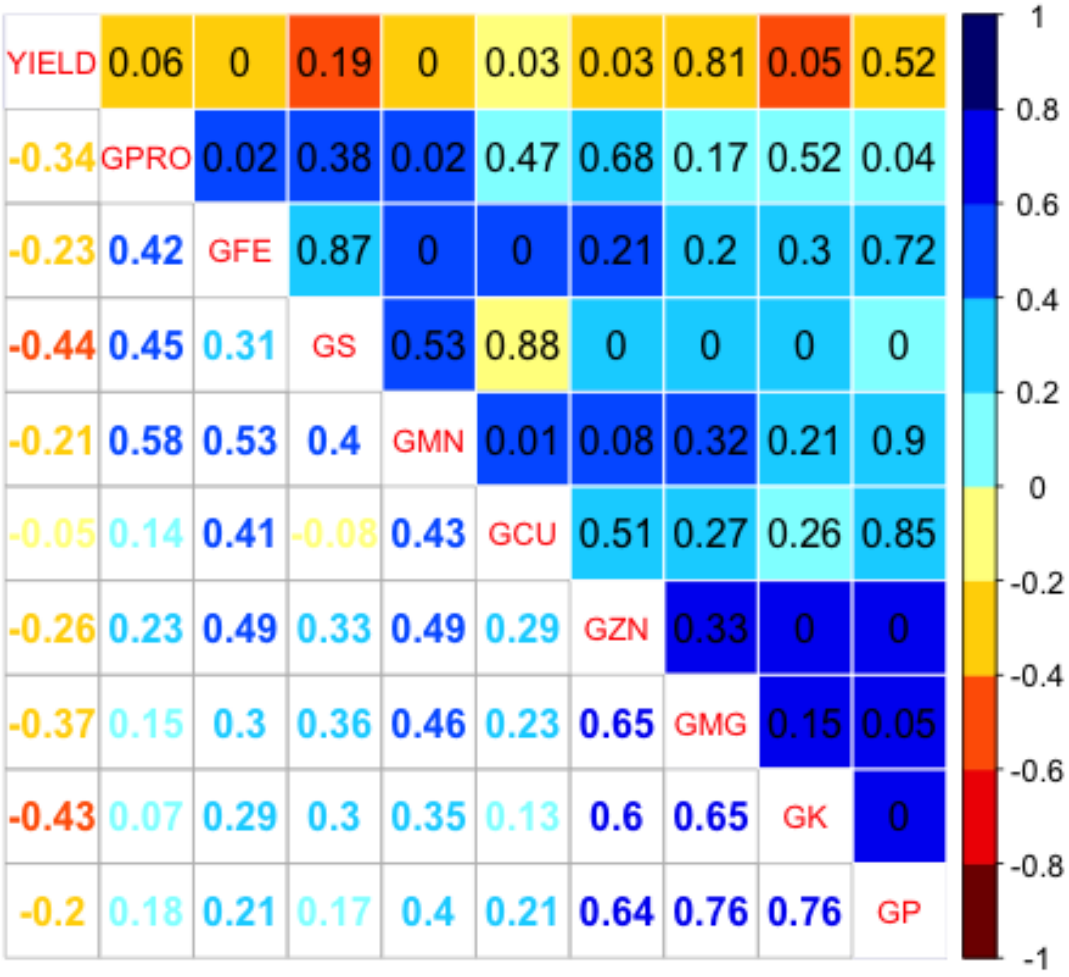
Appendix Table 2-1. Correlation coefficients among plant variables are reported in the lower left half. Coefficients were computed using the Pearson Method. P-values for each pairwise correlation are reported in the upper right half. P-values reported as 0 are <0.001. The central red diagonal labels indicate the nutrient chemical symbol of interest proceeded by a P indicating the source as plant tissue.



Appendix Table 2-2. Correlation coefficients among soil variables are reported in the lower left half. Coefficients were computed using the Pearson Method. P-values for each pairwise correlation are reported in the upper right half. P-values reported as 0 are <0.001. The central red diagonal labels indicate the nutrient chemical symbol or parameter of interest proceeded by an S indicating the source as soil. Non-chemical symbol key: PH=pH, BUF=buffer index, PB1=phosphorous Bray 1, OM=organic matter, PB2=phosphorous Bray 2, CEC=cation exchange capacity.



Appendix Table 2-3. Correlation coefficients among grain variables are reported in the lower left half. Coefficients were computed using the Pearson Method. P-values for each pairwise correlation are reported in the upper right half. P-values reported as 0 are <0.001. The red diagonal labels indicate the nutrient chemical symbol or parameter of interest proceeded by a G indicating the source as grain. Non-chemical symbol key: PRO=protein, YIELD=kg ha^{-1} at 155 g kg^{-1} water content.



Appendix Table 4-1. Planned selected contrasts for yield (kg ha^{-1}) and nutrient quantity (g ha^{-1}) in partitioned and total foliage at differing growth stages and at individual locations

Location	Selected Contrasts	-PM-	-------V6-------			----V12 to V14----			-------V17 to VT-------				---------------R2----------------					-------------R6-------------				
		Yield	Leaf	Stalk	Total	Leaf	Stalk	Total	Leaf	Stalk	Repro	Total	Leaf	Stalk	Repro	Grain	Total	Leaf	Stalk	Repro	Grain	Total
		P > F																				
Boron	Control v. Treated	†							+	*		**	*	**			**					
	Rate 1 v. Rate 2					+							+							+		
	2 Applications at Rate 1 v. 1 Application at Rate 2								*			*										
	Early (T1) and Mid. (T2) Multiple Applications v. Mid. (T2) and Late (T3) Multiple Applications	+	*		+	+	*	*						+	+								
	Early (T1) Applications v. Late Applications (T2 or T3)					**		*	+				**					+		+		
	Mid. (T2) Applications v. Late (T3) Applications								***			***	**				+					
	Interactions																	+		**		
	Early (T1) Applications v. Late (T3) Applications		+		+	**		+					***									
Manganese	Control v. Treated			+	+	*		*	**		+	***	***	***			**	***			+	
	Rate 1 v. Rate 2	***								+		**	***	*		+	**	***	*			
	2 Applications at Rate 1 v. 1 Application at Rate 2	**						+	+							*						
	Early (T1) and Mid. (T2) Multiple Applications v. Mid. (T2) and Late (T3) Multiple Applications																					
	Early (T1) Applications v. Late Applications (T2 or T3)				***	***	***		+				*									
	Mid. (T2) Applications v. Late (T3) Applications	+						**	***	+			+				+		+	+		
	Interactions					+							**									
	Early (T1) Applications v. Late (T3) Applications				***	***	***	+	***													
Zinc Only	Control v. Treated	+				***		+	***	***		***	**	***	**	+	***	***					**
	Rate 1 v. Rate 2					*			**	***		***	+	***	**		***	***	+	*	+	***
	2 Applications at Rate 1 v. 1 Application at Rate 2			+	*	+							+									
	Early (T1) and Mid. (T2) Multiple Applications v. Mid. (T2) and Late (T3) Multiple Applications		*	+	*	***		**		+		+	+		**							
	Early (T1) Applications v. Late Applications (T2 or T3)			+	*	***	***	***	*			*			**				+	+		
	Mid. (T2) Applications v. Late (T3) Applications								***	***		***			***						**	
	Interactions		+	+	*	***	+	**						+		*						
	Early (T1) Applications v. Late (T3) Applications			+	**	***	***	***	***	***		***		*	***		+		+			
Iron / Zinc (Zinc Values)	Control v. Treated					+	+	+	*				**				*	*	+			
	Rate 1 v. Rate 2		+	*	*				*	*		**	***				**	***		*		
	2 Applications at Rate 1 v. 1 Application at Rate 2			+	+		*			+						+						
	T1 & T2 Multiple Applications v. T3 & T4 Multiple Applications		*			*		+	***	*		***						+		+		
	T1 & T2 Multiple Applications v. T1 & T4 Multiple Applications	+‡	*						***			**				*		+				
	Vegetative Stage Applications v. Reproductive Stage Applications					*	*	**	***	***		***	***				**	*				
Iron / Zinc (Iron Values)	Control v. Treated													*			*					
	Rate 1 v. Rate 2		+		+		+											+				
	2 Applications at Rate 1 v. 1 Application at Rate 2																					
	T1 & T2 Multiple Applications v. T3 & T4 Multiple Applications			+		+		+	**											+		*
	T1 & T2 Multiple Applications v. T1 & T4 Multiple Applications	+‡	+					+	**			+	*			+		+				**
	Vegetative Stage Applications v. Reproductive Stage Applications								***						+			+	*			

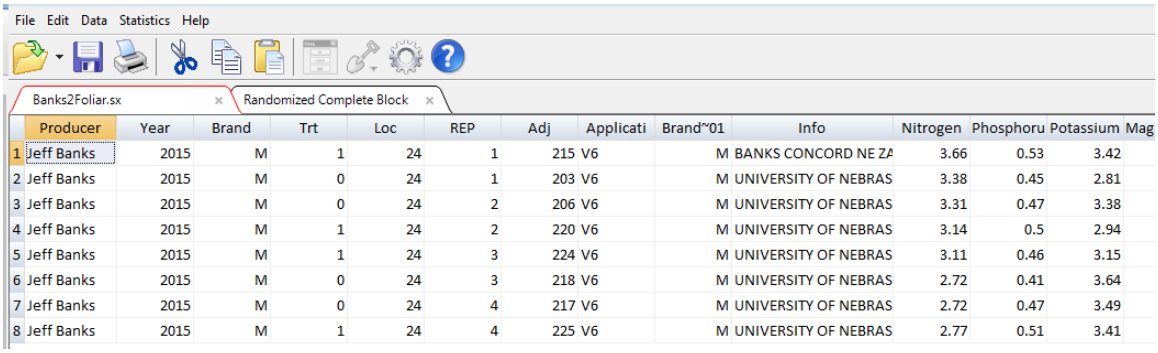
† Contrast significant F test: Not Significant >0.10; + >0.05; *>0.01; **>0.001; ***≤0.001

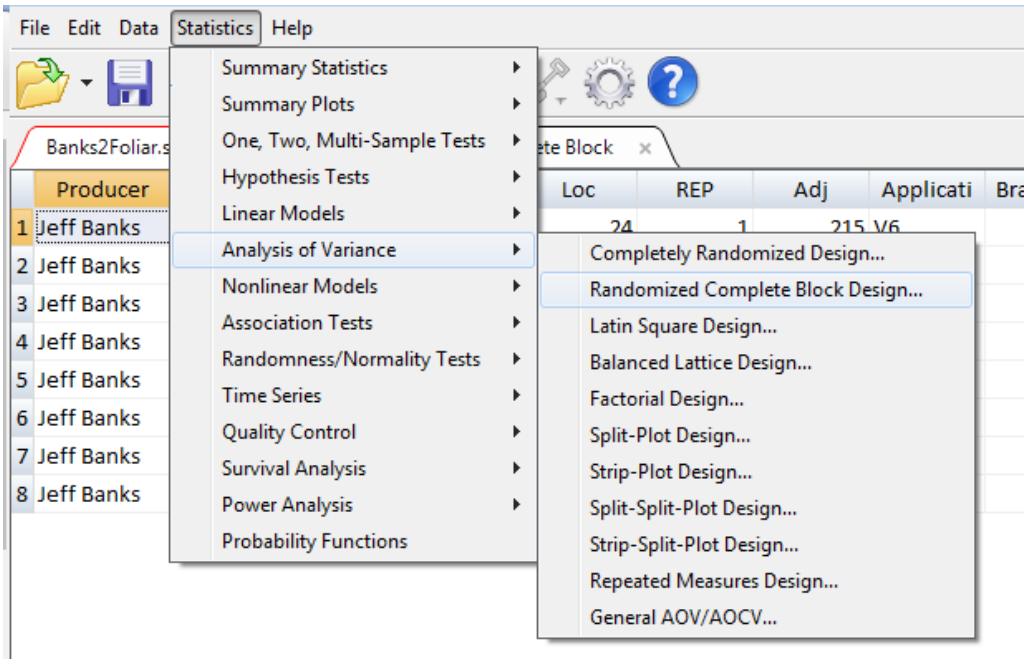
‡ Same analysis for yield at the Iron / Zinc location

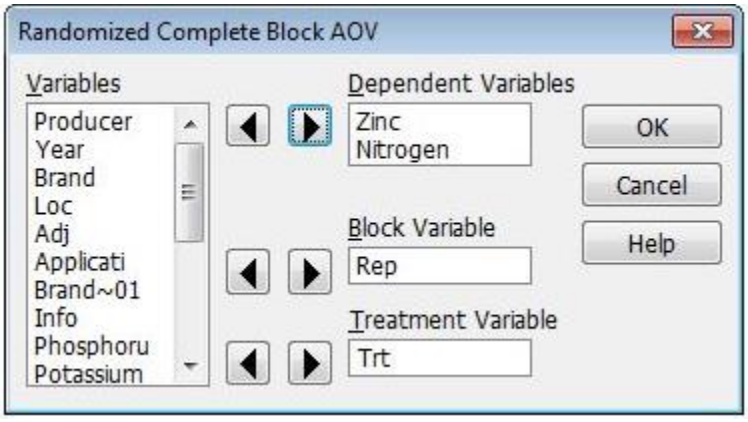
Appendix Code 2-1. Example R model statements used in Chapter 2.

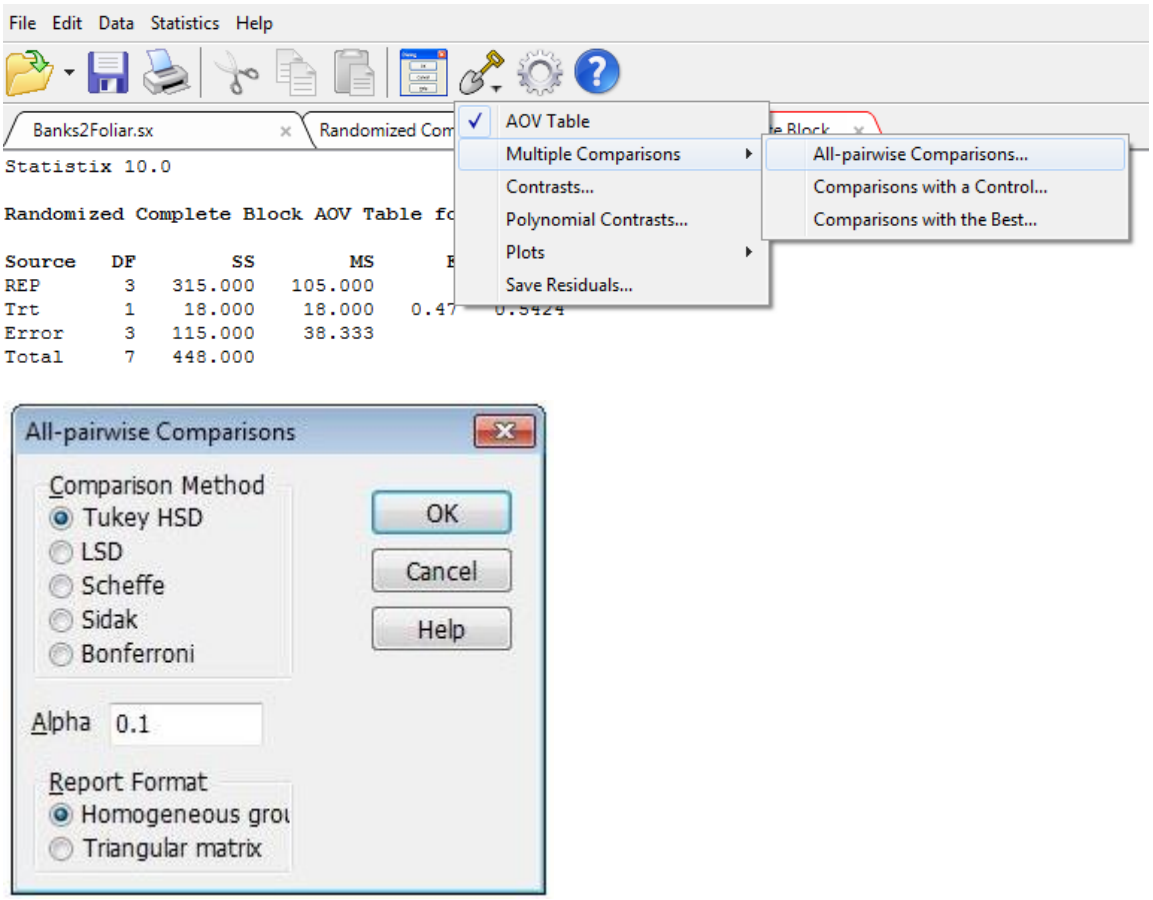
```
# Creating p-value matrix for correlation coefficients
cor.mtest <- function(mat, conf.level = 0.95){
  mat <- as.matrix(mat)
  n <- ncol(mat)
  p.mat <- lowCI.mat <- uppCI.mat <- matrix(NA, n, n)
  diag(p.mat) <- 0
  diag(lowCI.mat) <- diag(uppCI.mat) <- 1
  for(i in 1:(n-1)){
    for(j in (i+1):n){
      tmp <- cor.test(mat[,i], mat[,j], conf.level = conf.level)
      p.mat[i,j] <- p.mat[j,i] <- tmp$p.value
      lowCI.mat[i,j] <- lowCI.mat[j,i] <- tmp$conf.int[1]
      uppCI.mat[i,j] <- uppCI.mat[j,i] <- tmp$conf.int[2]
    }
  }
  return(list(p.mat, lowCI.mat, uppCI.mat))
}

#Correlation Matrix
sxpxg.cor<-cor(sxpxg, use="pairwise.complete.obs")

#Table
sjt.corr(sxpxg, corMethod = "pearson",
        title="Correlation Coefficients Among All Variables",
        showPValues = TRUE, pvaluesAsNumbers=TRUE,
        #file="/Users/lmbastos/Dropbox/Zach/Correlation tables and plots/7 sxpxg table.docx",
        CSS=list(css.thead="border-top:double black; font-weight:normal; font-size:0.9em;",
                 css.firsttablecol="font-weight:normal; font-size:0.9em;"))

#Plot
res1 <- cor.mtest(sxpxg,0.95)
corrplot(sxpxg.cor, method="color", col=col1(10),
        cl.length=11, order = "AOE", type="upper", tl.cex = .6,
        tl.col = "red", tl.pos="d", p.mat = res1[[1]], insig = "p-value", sig.level=-1) ## add all p-
values
corrplot(sxpxg.cor, method="number", order="AOE", type="lower", addCoef.col="grey",
add=TRUE, col=col1(10),
        diag=FALSE,tl.pos="n", cl.pos="n")
```

Appendix Code 3-1. Example Statistix data analysis procedures used in Chapter 3.








File Edit Data Statistics Help
Banks2Foliar.sx
Randomized Com
AOV Table
Multiple Comparisons
Contrasts...
Polynomial Contrasts...
Plots
Save Residuals...
All-pairwise Comparisons...
Comparisons with a Control...
Comparisons with the Best...
Statistix 10.0
Randomized Complete Block AOV Table fo
Source DF SS MS F
REP 3 315.000 105.000
Trt 1 18.000 18.000 0.47 0.5424
Error 3 115.000 38.333
Total 7 448.000

All-pairwise Comparisons
Comparison Method
Tukey HSD
LSD
Scheffe
Sidak
Bonferroni
OK
Cancel
Help
Alpha 0.1
Report Format
Homogeneous grou
Triangular matrix

Appendix Code 4-1. Example SAS model statements used in Chapter 4.

YIELD SAS MODEL STATEMENTS EXAMPLE

```
title1 'Sunderman B Yield Data Run';
   data RCB;
      input blk   trt   yield djyield     population;
      datalines;
example dataline: 1     6     232.58      234.15      108
   ;
proc print;
   run;
proc GLIMMIX data=RCB;
      class blk trt;
      model yield =blk trt;
      random blk;
      CONTRAST 'CHK VS OTHERS' trt -8 1 1 1 1 1 1 1 1;
      contrast '2 applications v. 1 application at Totrate2' trt 0 -1.5
-1.5 0 0 0 1 1 1;
      contrast 'V10 & V15 double application at rate 1 v. V15 & R1
double application at rate 1' trt 0 1 -1 0 0 0 0 0 0;
      contrast 'Rate 1 v. Rate 2' trt 0 0 0 1 1 1 -1 -1 -1;
      contrast 'Early(V10) applications v. later applications (V15 &
R1)' trt 0 0 0 2 -1 -1 2 -1 -1;
      contrast 'V15 applications v. R1 applications' trt 0 0 0 0 1 -1 0
1 -1;
      contrast 'Interactions' trt 0 0 0 2 -1 -1 -2 1 1;
      contrast 'V10 applications v. R1 applications' trt 0 0 0 -1 0 1 -
1 0 1;
      lsmeans trt;
      lsmeans trt / diff=control ("1") cl;
      lsmeans trt / pdiff;
      run;
      proc GLIMMIX data=RCB;
      class blk trt;
      model djyield =blk trt;
      random blk;
      CONTRAST 'CHK VS OTHERS' trt -8 1 1 1 1 1 1 1 1;
      contrast '2 applications v. 1 application at Totrate2' trt 0 -1.5
-1.5 0 0 0 1 1 1; contrast 'V10 & V15 double application at rate 1 v.
V15 & R1 double application at rate 1' trt 0 1 -1 0 0 0 0 0 0;
      contrast 'Rate 1 v. Rate 2' trt 0 0 0 1 1 1 -1 -1 -1;
      contrast 'Early(V10) applications v. later applications (V15 &
R1)' trt 0 0 0 2 -1 -1 2 -1 -1;
      contrast 'V15 applications v. R1 applications' trt 0 0 0 0 1 -1 0
1 -1;
      contrast 'Interactions' trt 0 0 0 2 -1 -1 -2 1 1;
      contrast 'V10 applications v. R1 applications' trt 0 0 0 -1 0 1 -
1 0 1;
      lsmeans trt;
      lsmeans trt / diff=control ("1") cl;
lsmeans trt / pdiff;
```

```
      run;
proc GLIMMIX data=RCB;
      class blk trt;
      model population =blk trt;
      random blk;
      CONTRAST 'CHK VS OTHERS' trt -8 1 1 1 1 1 1 1 1;
      contrast '2 applications v. 1 application at Totrate2' trt 0 -1.5
-1.5 0 0 0 1 1 1; contrast 'V10 & V15 double application at rate 1 v.
V15 & R1 double application at rate 1' trt 0 1 -1 0 0 0 0 0 0;
      contrast 'Rate 1 v. Rate 2' trt 0 0 0 1 1 1 -1 -1 -1;
      contrast 'Early(V10) applications v. later applications (V15 &
R1)' trt 0 0 0 2 -1 -1 2 -1 -1;
      contrast 'V15 applications v. R1 applications' trt 0 0 0 0 1 -1 0
1 -1;
      contrast 'Interactions' trt 0 0 0 2 -1 -1 -2 1 1;
      contrast 'V10 applications v. R1 applications' trt 0 0 0 -1 0 1 -
1 0 1;
      lsmeans trt;
      lsmeans trt / diff=control ("1") cl;
lsmeans trt / pdiff;
      run;
```

PARTITIONED PLANT COMPONENTS SAS MODEL STATEMENT EXAMPLE

```
title1 'Dunklau Zn Only Data Run R6';
   data RCB;
      input blk   trt leaf stalk repro grain;
      datalines;
example dataline: 1     1     113.9468707 118.4814611 82.71931398
      263.4740946
;
   proc print;
   run;
proc GLIMMIX data=RCB;
      class blk trt;
      model leaf =blk trt;
      random blk;
      CONTRAST 'CHK VS OTHERS' trt -8 1 1 1 1 1 1 1 1;
      contrast '2 applications v. 1 application at Totrate2' trt 0 -1.5
-1.5 0 0 0 1 1 1;
      contrast 'V10 & V15 double application at rate 1 v. V15 & R1
double application at rate 1' trt 0 1 -1 0 0 0 0 0 0;
      contrast 'Rate 1 v. Rate 2' trt 0 0 0 1 1 1 -1 -1 -1;
      contrast 'Early(V10) applications v. later applications (V15 &
R1)' trt 0 0 0 2 -1 -1 2 -1 -1;
      contrast 'V15 applications v. R1 applications' trt 0 0 0 0 1 -1 0
1 -1;
      contrast 'Interactions' trt 0 0 0 2 -1 -1 -2 1 1;
      contrast 'V10 applications v. R1 applications' trt 0 0 0 -1 0 1 -
1 0 1;
      lsmeans trt;
```

```
      lsmeans trt / diff=control ("1") cl;
      lsmeans trt / pdiff;
      run;
      proc GLIMMIX data=RCB;
      class blk trt;
      model stalk =blk trt;
      random blk;
      CONTRAST 'CHK VS OTHERS' trt -8 1 1 1 1 1 1 1 1;
      contrast '2 applications v. 1 application at Totrate2' trt 0 -1.5
-1.5 0 0 0 1 1 1; contrast 'V10 & V15 double application at rate 1 v.
V15 & R1 double application at rate 1' trt 0 1 -1 0 0 0 0 0 0;
      contrast 'Rate 1 v. Rate 2' trt 0 0 0 1 1 1 -1 -1 -1;
      contrast 'Early(V10) applications v. later applications (V15 &
R1)' trt 0 0 0 2 -1 -1 2 -1 -1;
      contrast 'V15 applications v. R1 applications' trt 0 0 0 0 1 -1 0
1 -1;
      contrast 'Interactions' trt 0 0 0 2 -1 -1 -2 1 1;
      contrast 'V10 applications v. R1 applications' trt 0 0 0 -1 0 1 -
1 0 1;
      lsmeans trt;
      lsmeans trt / diff=control ("1") cl;
lsmeans trt / pdiff;
      run;
      proc GLIMMIX data=RCB;
      class blk trt;
      model repro =blk trt;
      random blk;
      CONTRAST 'CHK VS OTHERS' trt -8 1 1 1 1 1 1 1 1;
      contrast '2 applications v. 1 application at Totrate2' trt 0 -1.5
-1.5 0 0 0 1 1 1; contrast 'V10 & V15 double application at rate 1 v.
V15 & R1 double application at rate 1' trt 0 1 -1 0 0 0 0 0 0;
      contrast 'Rate 1 v. Rate 2' trt 0 0 0 1 1 1 -1 -1 -1;
      contrast 'Early(V10) applications v. later applications (V15 &
R1)' trt 0 0 0 2 -1 -1 2 -1 -1;
      contrast 'V15 applications v. R1 applications' trt 0 0 0 0 1 -1 0
1 -1;
      contrast 'Interactions' trt 0 0 0 2 -1 -1 -2 1 1;
      contrast 'V10 applications v. R1 applications' trt 0 0 0 -1 0 1 -
1 0 1;
      lsmeans trt;
      lsmeans trt / diff=control ("1") cl;
      lsmeans trt / pdiff;
      run;
      proc GLIMMIX data=RCB;
      class blk trt;
      model grain =blk trt;
      random blk;
      CONTRAST 'CHK VS OTHERS' trt -8 1 1 1 1 1 1 1 1;
      contrast '2 applications v. 1 application at Totrate2' trt 0 -1.5
-1.5 0 0 0 1 1 1;
      contrast 'V10 & V15 double application at rate 1 v. V15 & R1
double application at rate 1' trt 0 1 -1 0 0 0 0 0 0;
      contrast 'Rate 1 v. Rate 2' trt 0 0 0 1 1 1 -1 -1 -1;
```

```
      contrast 'Early(V10) applications v. later applications (V15 &
R1)' trt 0 0 0 2 -1 -1 2 -1 -1;
      contrast 'V15 applications v. R1 applications' trt 0 0 0 0 1 -1 0
1 -1;
      contrast 'Interactions' trt 0 0 0 2 -1 -1 -2 1 1;
      contrast 'V10 applications v. R1 applications' trt 0 0 0 -1 0 1 -
1 0 1;
      lsmeans trt;
      lsmeans trt / diff=control ("1") cl;
      lsmeans trt / pdiff;
      run;
```

Appendix Code 5-1. Example SAS model statements used in Chapter 5.

FE NANOPARTICLE TRIAL SAS MODEL STATEMENTS EXAMPLE

```
DATA Fe Nanoparticle;
INPUT BLK   TRT   ITOTG FTOTG FBG   FTOPG FROOTG      IFEG  FFEG  FBFEG
      FTOPFEG     IFECONC     FFECONC     FBFECONC    FTOPFECONC
      F_I_CONCFE  F_I_Weight  F_I_TOTGFE
;
CARDS;
example dataline: 1   1     8.13  41.35 38.18 3.17  13.98 0.071544
      0.367092    0.347438    0.019654    88    76.5  91    62    -11.5
      33.22 0.295548
;

PROC SORT; by BLK TRT ;
PROC PRINT;
TITLE1 'Fe Nanoparticle';
PROC GLM;
CLASS BLK TRT ;
MODEL BLK   TRT   ITOTG FTOTG FBG   FTOPG FROOTG      IFEG  FFEG  FBFEG
      FTOPFEG     IFECONC     FFECONC     FBFECONC    FTOPFECONC
      F_I_CONCFE  F_I_Weight  F_I_TOTGFE = BLK  TRT;
lsmeans TRT;
lsmeans trt/ diff=control ("2") cl;
RUN;
```