FOLIAR APPLICATION OF IRON CHELATED FERTILIZER AND SURFACTANTS FOR MANAGEMENT OF IRON DEFICIENCY CHLOROSIS IN SOYBEANS

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Iron deficiency chlorosis (IDC) is a production challenge for farmers growing soybeans [*Glycine max* (L.) Merr.], especially in the Red River Valley. It is critical to correct this deficiency as soon as symptoms arise before growth, and ultimately yield, is negatively impacted. Field experiments of foliar applied iron fertilizers (o-o-EDDHA, o-o-EDDHSA, HEDTA, and an amino acid) and suitable adjuvants (HSOC [high surfactant oil concentrate], non-ionic surfactant, acidifier, and organosilicone surfactant), to control IDC were conducted during the 2013 and 2014 growing seasons, respectively. There was high variability among the results for both the SPAD meter readings and soil iron concentration. The yield values were greater in the treated plots than with control plots, but not significantly so. Further experiments should be conducted to gain more knowledge on the prolonged use and efficiency of these products in the correction of IDC.

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DEDICATION

This is dedicated to my family, who have stood beside and supported me in every venture I have taken. Always pushing me to achieve my goals no matter how trivial they may seem, and being the best support system I could have ever asked for.

This is dedicated to my best friend, Cassey, who not only supported my decision to go back to school, but helped out during all the long summer days of field work. Always there to lend an ear or helping hand whenever I became too stressed or burnt out to do it alone.

This is dedicated to my boyfriend, John, who is always there supporting me in everything I do. Being a great supporter of all my ideas and willing to help see them through, as well as pushing me to do my best.

This is dedicated to all the farmers in Minnesota and North Dakota I have had the pleasure of meeting through research, work, family, or as neighbors. You have all helped cultivate my love of farming and growing things. I hope this research benefits you all in some way.

This is dedicated to all the Professors and Soil Scientists who have helped me garner my interest and increase my knowledge in the world of soil science. Thank you for your guidance, nurturing, and teaching through all these years. I have learned so much from all of you.

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ORGANIZATION OF THESIS

This thesis is divided into (i) literature review, (ii) materials and methods, (iii) results and discussion, (iv) conclusion, and (v) references. The literature review discusses the history of iron deficiency chlorosis, the role of iron in plant nutrition, iron chelates, and adjuvants and surfactants. The materials and methods section explains the procedures for the field experiments, measurements, soil analyses, and statistical analyses for both growing seasons. The results and discussion section discusses the results of the field experiments. The thesis is then summarized by the general conclusions, followed by the references cited section.

LITERATURE REVIEW

History of Iron Deficiency Chlorosis

Iron (Fe) is the fourth most abundant element in the earth's crust, with North Dakota soils, on average, having 5% Fe by weight (Franzen, 2013). Deficiency of iron is becoming a common problem in many vegetal species (Ortiz et al., 2007) since it is vital for important plant functions such as photosynthesis, DNA synthesis, protein formation, biological N_2 fixation, and respiration (Caliskan et al., 2008). Iron deficiency chlorosis is a common nutritional disorder worldwide under both calcareous and alkaline soils (Rodriguez-Lucena et al., 2009, 2010; Schenkeveld et al., 2008; Ylivainio, 2010). Lindsay (1979) suggests that iron has a minimum solubility in the pH range of 7.4 to 8.5. Chelating agents can increase solubility, which is needed because most plants can develop severe deficiency at pH 5 (Lindsay, 1979; Rroco et al., 2003). While many factors such as high soil moisture, extreme temperatures, and poor aeration may cause Fe chlorosis, high pH values, elevated bicarbonate concentrations and high levels of soluble salts tend to be the most critical factors (Helms et al., 2010; Kandel, 2014; Schenkeveld et al., 2008; Wiersma, 2005). Ferric iron chelates formed in aerobic soils consist of soluble organic ligands produced by either the breakdown of organic matter or by microbial biosynthesis of iron-chelating compounds called siderophores (Jeong and Connolly, 2009).

Under calcareous soil conditions, which cover one third of the earth's surface, calcium carbonate buffers the soil solution pH between the range of 7.5 to 8.5, causing Fe to precipitate as Fe hydroxides ($Fe(OH)_x$) (Ortiz et al., 2007). Based on the low solubility of these precipitates and a high pH, Fe availability decreases (Kobayashi and Nishizawa, 2012; Rodriguez et al., 2010; Schenkeveld et al., 2008). The most soluble Fe oxide limits total Fe concentration at

around 10^{-10} M, with optimum plant growth levels at 10^{-8} M (Rodriguez-Lucena et al., 2009). Another factor contributing to deficiency is Fe^{2+}, or ferrous iron, which is very soluble in water, but upon exposure to oxygen, is oxidized to Fe^{3+}, or ferric iron, becoming less soluble (Franzen, 2013). Iron can form insoluble complexes that are not accessible under neutral or alkaline pH, decreasing the bioavailability under some soil types (Jeong and Connolly, 2009).

Advances in genetics and machinery have extended the corn-soybean rotation north and west into regions historically used for spring wheat production (Liesch et al., 2011) as yields have increased (Salvagiotti et al., 2008). Soybean acreage in North Dakota increased from 3 million acres in 2003 to nearly 4.7 million acres in 2012 (Endres and Kandel, 2014). Iron chlorosis causes roughly $120 million in lost yield annually in the north central United States (North Dakota, South Dakota, Minnesota, and Iowa) (Liesch et al., 2011). Iron deficiency chlorosis is commonly observed in soybean fields in eastern North Dakota and western Minnesota every year (Kandel, 2014; Wiersma, 2005). However, high spatial variability and different weather patterns can make Fe chlorosis prevalent to varying degrees each year (Liesch et al., 2011).

Iron deficiency chlorosis is characterized by a significant decrease of chlorophyll in leaves and can cause a decrease in crop yield (O'Rourke et al., 2007; Schenkeveld et al., 2008). This decrease of leaf chlorophyll causes a pale green or yellowing of the leaf tissue between the veins of younger leaves, a characteristic symptom of Fe-deficient plants, while under extreme conditions stunting, necrosis, and plant death can occur (Caliskan, 2008; Goos and Johnson, 2000; Kandel, 2014; Liesch et al., 2011; Rodriguez-Lucena et al., 2009; Wiersma, 2005). These symptoms can be seen once the first trifoliate leaf emerges, and can last until the seventh

trifoliate stage, with the plants sometimes greening up during the flowering and pod-filling stages (Kandel, 2014).

Various management strategies have been suggested to reduce incidence of IDC, including varietal selection (Goos and Johnson, 2000; Helms et al., 2010), application of Fe fertilizer in furrow, seed coating of Fe fertilizer (Goos and Johnson, 2001; Wiersma, 2005), foliar application of Fe fertilizer, increased planting density and row spacing (Franzen, 2013), and planting an oat cover crop (Kandel, 2014).

Addition of Fe chelated fertilizers to the soil through in-furrow applications is currently one of the most effective method under field conditions, although foliar application could be a strategy implemented to diminish chlorosis effects (Fernandez et al., 2002). There are three main classes of iron fertilizers include inorganic iron compounds (Fe salts, hydroxides, and other industrial products), synthetic iron chelates (polyaminocarboxylic acids), and natural iron complexes (humates, lignosulfonates, amino acids, and citrates) (Abadia et al., 2011). In the case of soil and foliar application of iron chelates, fertilization is done at either one or a few specific time points to increase crop-available iron (Abadia et al., 2011). In calcareous soils, severe impairment of iron stress response mechanisms may occur, causing a need for repeated applications of Fe to supply the plants (Wiersma, 2005). Planting of IDC tolerant cultivars on chlorotic soils is also an effective method, but even good cultivars can still suffer symptoms (Wiersma, 2005). Helms et al., (2010) suggest planting IDC tolerant cultivars on the parts of the field where IDC symptoms generally occur, while planting the highest yielding cultivar where IDC symptoms are absent to increase overall yield (Goos and Johnson, 2000; Goos and Johnson, 2001; Wiersma, 2005).

Roles of Iron in Crop Growth

Iron is an essential micronutrient in plants required in great abundance. Under conditions of limited concentrations, plants can mobilize and uptake iron from the soil through physiological and morphological changes to ensure there is enough for critical cellular processes (Hindt and Gueriont, 2012). Chlorophyll biosynthesis requires Fe, as well as components such as heme, the Fe-sulfur cluster, and other Fe-binding sites. (Kobayashi and Nishizawa, 2012). Iron participates in electron transfer during processes such as photosynthesis and respiration, through redox reactions, cycling between Fe^{2+} and Fe^{3+} (Kim and Guerinot, 2007).

Plant accumulation of excess Fe has to be controlled because of products from the reduction of molecular oxygen, superoxide and hydrogen peroxide, can be catalyzed by Fe^{2+} and Fe^{3+} to form reactive hydroxyl radicals which can damage most cellular components (Kim and Guerinot, 2007). Plants need to acquire sufficient amounts to meet physiological and morphological demands while also staying below the toxicity levels (Jeong and Connolly, 2009; Kobayashi and Nishizawa, 2012; Wiersma, 2005). Mobilization of Fe within the rhizosphere is different for dicots and nongraminaceous monocots and graminaceous plants (Hindt and Gueriont, 2012; Jeong and Connolly, 2009; Kobayashi and Nishizawa, 2012). For dicots, mobilization of Fe is a coordination of three activities, acidification, reduction and transport, along the plasma membrane of root epidermal cells (Jeong and Connolly, 2009). Under low Fe availability, these molecular components are also accompanied by morphological changes in the root structure and architecture like increased formation and branching of root hairs, lateral root formation, and root tip swelling, which all help in increasing root surface area (Abadia et al., 2011; Hindt and Gueriont, 2012; Kobayashi and Nishizawa, 2012).

During acidification, dicot plants extrude protons and other phenolic compounds across the plasma membrane and into the rhizosphere by a proton ATPase pump (Jeong and Connolly, 2009; Kobayashi and Nishizawa, 2012; Rroco et al., 2003) to decrease the pH of the soil solution and increase the solubility of Fe^{3+}. One unit drop in pH allows the Fe^{3+} to become more soluble by 1000-fold. Expression of ferric chelate reductases and ferrous iron transporters are also induced, which help to reduce ferric iron chelates to form soluble ferrous iron (Hindt and Gueriont, 2012; Kim and Guerinot, 2007; Jeong and Connolly, 2009). This ferrous iron is then transported into the root by IRT1, a protein based metal transporter expressed in the epidermal cells of Fe-deficient roots and localized in the plasma membrane (Kim and Guerinot, 2007). Under wet soil conditions, carbonates solubilize producing bicarbonate which neutralizes the acidity in the rhizosphere, making the Fe-reducing protein secreted by dicot plants ineffective (Franzen, 2013).

Graminaceous plants use a chelation Fe-deficiency response, releasing molecular weight compounds known as the mugineic acid family of phytosiderophores (Rroco et al., 2003). These mugineic acids are secreted under diurnal patterns, peaking in the morning (Kobayashi and Nishizawa, 2012). They efficiently bind Fe^{3+} in the rhizosphere creating complexes which are then transported into the roots through by yellow-stripe transporters (Jeong and Connolly, 2009; Kim and Guerinot, 2007; Kobayashi and Nishizawa, 2012).

Upon entering the symplast, Fe is bound to various chelators to keep it in solution and prevent it from generating hydroxyl radicals. Citrate, an organic acid, binds Fe^{3+} while nictianamine form complexes with both Fe^{2+} and Fe^{3+}. These complexes also help transport Fe both long and short distances through the plants (Jeong and Connolly, 2009; Kim and Guerinot,

2007; Ortiz et al., 2007). Translocation involves various steps including radial and symplastic transport across the root tissue and Casparian strip, xylem loading and unloading, phloem loading and unloading, and symplastic transport toward new growth. Little accumulated iron is mobilized from older to younger tissues, which causes the plants to require a continuous supply of iron to maintain proper growth (Wiersma, 2005). Upon entering the cell, it is placed in an appropriate compartment for use in cellular function as well as to reduce cytotoxicity. Approximately 80-90% of cellular Fe can be found in the chloroplasts and mitochondria (Kobayashi and Nishizawa, 2012).

Iron chlorosis is believed to cause difficulties in the transport elements within the plant due to the presence of bicarbonate ions and high pH as well as a decreased amount of Fe-complex anions in the xylem sap reducing iron mobility. There is a critical moment in which the iron is distributed from the veins and apoplast to the inside of the cells. During this time the iron is reduced before being taken up into the mesophyll, which allows time for the bicarbonate ions and high pH to inhibit any further transport, as well as cause iron precipitation (Ortiz et al., 2007).

"The paradox of iron chlorosis" is a phenomenon in which both chlorotic and non-chlorotic leaves have similar concentrations of total Fe. Although mildly chlorotic soybeans may achieve a green color, it doesn't necessarily mean that Fe is no longer a limiting factor (Wiersma, 2005). Chlorosis reduces or inhibits the growth of the leaf, which leads to a higher concentration of the element. It can even occur in leaves where the tissue has an adequate level of Fe but is still physiologically deficient because of inactivation. Inactive Fe can be found within chlorotic leaves, which could be caused by Fe not passing through the plasma membrane but instead being

deposited in the apoplast. Under calcareous soils this can be caused from alkaline pH, iron oxidation, and low enzyme activity. This immobilization may occur even with an adequate external iron supply (Ortiz et al., 2007).

Iron Chelates

Seed, soil, and foliar application of Fe chelates or fertilizers have been done for many years with varying degrees of success. These inconsistencies can be because of varying levels of chlorosis severity, the soil type, plant genetics, and low application rates (Wiersma, 2005). Application of Fe chelates may increase the rate of Fe as well as extending the period during which Fe is available to the growing plants, especially under high deficiency conditions (Wiersma, 2005).

Chelated forms of Fe are considered best over inorganic forms because they are soluble and readily available for translocation into the plant's leaves. The chelates overall stability is a good indicator as to their effectiveness, while the rapid degradation of some chelates may reduce the potential for the treatment of deficiencies (Liesch et al., 2011; Rodriguez-Lucena et al., 2010; Schenkeveld et al., 2012).

Under some highly calcareous soils, correction of Fe deficiency requires an application of chelated iron to the soil under high doses, which in some cases is very uneconomical. Foliar application of chelates with a good surfactant while the plants stomata are open, may increase the absorption of iron into plant leaves, and be more cost effective than soil application (Abadia et al., 2011; Horesh and Levy, 1981). Two drawbacks to foliar application is the limited knowledge on leaf mesophyll Fe uptake mechanisms and understanding the penetration, translocation, and bioavailability of leaf-applied Fe fertilizers (Abadia et al., 2011).

Iron complexes include both polymeric and non-polymeric molecules that are derived from many substances of different natural origins (e.g., humates, lignosulfonates, amino acids, gluconate, and citrate). With a low stability, complexes are used primarily under mild chlorotic conditions, through fertigation or foliar application. These are cheaper than synthetic chelates, so they can be used across a broader range of crops and since they are from a natural source, they also degrade easier. Since they are less stable, their efficiency is lowered and are generally recommended for foliar applications or soil-less horticulture (Abadia et al., 2011; Rodriguez-Lucena et al., 2009; Rodriguez-Lucena et al., 2010).

Lignosulfonates (LS) are by-products obtained during the sulfite pulping process, during which lignin is broken down and made water soluble by introducing sulfonic acid, producing pulp paper polymers. Hydroxyl radicals in lignin molecules can bond to carbon which allows them to behave like carboxylic groups in organic acids, while in soils lignin breakdown is similar to that of humic substances. These characteristics allow LS to complex Fe and may help correct Fe chlorosis. Under calcareous conditions, Fe-LS complexes have a low stability, so foliar application could be used as an alternative (Rodriguez-Lucena et al., 2009).

Synthetic Fe chelates are effective as fertilizers because Fe is bound to the chelating agent, remaining soluble over a wide range of pH values. While they are highly effective, they are also expensive and typically only used on high-value crops. Polyaminocarboxylate chelating agents have also been scrutinized because they can remain persistent in the soil solution and can influence metal availability and mobility, causing environmental issues (Abadia et al., 2011; Rodriguez-Lucena et al., 2009). These synthetic Fe chelates with known structures can be classified as either nonphenolic or phenolic (Rodriguez-Lucena et al., 2010).

Nonphenolic synthetic aminocarboxylate chelating agents are chemicals that form strong, stable, water soluble complexes with di- and trivalent cations. They are used in industrial, nutritional, medical, and agricultural applications to prevent precipitate formation, uptake of toxic metals, and increase bioavailability. Some common forms of these agents are NTA (nitrile triacetic acid), EDTA (ethylenediaminetetraacetic acid), and DTPA (diethylenetriaminepentaacetic acid). These products have difficulty maintaining Fe in the soil solution under calcareous or alkaline soils, and are more recommended for fertigation or foliar applications (Goos and Germain, 2001; Rodriguez-Lucena et al., 2010; Schenkeveld et al., 2012).

Of all soil applied Fe fertilizers, the most effective and commonly used are synthetic Fe^{3+} chelates, primarily consisting of polyamine-carboxylic acid with phenolic groups like ethylendiamine di(o-hydroxyphenylacetic) acid (EDDHA), ethylendiamine di(2-hydroxy-4-methylphenylacetic) acid (EDDHMA), and a less commonly used ethylendiamine di(2-hydroxy-5-sulfophenylacetic) acid (EDDHSA) (Fig. 1). These molecules were synthesized in the 1950's, and have been used to create Fe^{3+} complexes and correct Fe deficiencies among plants (Alvarez-Fernandez et al., 2005). Many micronutrient fertilizers for Fe, Zn, Cu and Mn are based on aminocarboxylate chelates, which enhance solubility and transport to the plant roots (Schenkeveld et al., 2012).

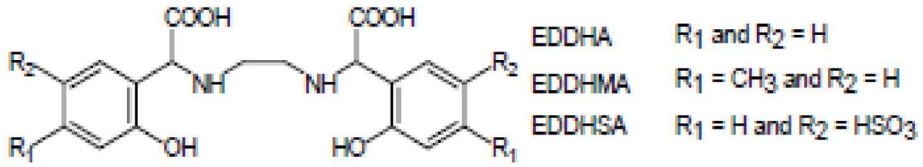

Figure 1. A molecular structure of a Fe^{3+} synthetic chelate commonly found in fertilizers (Alvarez-Fernandez et al., 2005).

Iron ethylendiamine-N, N'-bis o-hydroxyphenylacetic acid (Fe-o-o-EDDHA) and its equivalents, have two phenolic groups that replace the carboxylic groups of EDTA increasing the stability (Rodriguez-Lucena et al., 2010). Application of synthetic Fe chelates can be used to correct or avoid Fe chlorosis by increasing Fe solubility and transport through the soil solution and into plants. One of the more effective synthetic chelates under neutral and alkaline soils is FeEDDHA, and is produced through a Mannich-like reaction between ethylene diamine, glyoxylic acid, and phenol with the addition of Fe, and consists of positional isomers, diastereomers and polycondensates. There are four groups of FeEDDHA varying by their protonation and complexation: racemic o,o-FeEDDHA, meso o,o-FeEDDHA, o,p-FeEDDHA, and other unidentified compounds. These variances also affect their ability to solubilize, preserve, and transport Fe from the solution and into plants, such as soybeans or other dicot plants (Schenkeveld et al., 2008; Schenkeveld et al., 2012).

Iron solubility is lowest between pH 7.4 to 8.5, so Fe chelates are used to increase the availability to plants, especially in calcareous environments. Under alkaline soil conditions, Fe-EDTA and Fe-DTPA stability decreases, allowing other cations (Ca, Mn, Zn, Cu) to replace Fe in the chelate, thus removing iron from the solution (Lindsay, 1979). Under acidic or near-neutral soils, EDTA seems to be the most effective. Under calcareous soil Fe-EDDHA, which covers a pH range of 4 to 9, is more efficient at maintaining Fe that Fe-DTPA and Fe-EDTA. The overall efficiency of the chelates are greatly influenced by the soil type, clay content, and pH of the soil (Aboulroos et al., 1983; Ylivainio, 2010).

Foliar application of synthetic chelates and complexes can be used to overcome Fe chlorosis, although the effectiveness varies depending upon the leaf, compound applied and

surfactant. The optimal formulation for foliar fertilizer application is difficult to identify, but based on improved SPAD meter readings synthetic chelates are more efficient than complexes. Rodriguez-Lucena et al. (2010) found that EDDS (ethylenediaminedisuccinic acid), EDTA compounds, amino acids and transferrin were the most effective treatments for greening leaves. The type of surfactant used during foliar application can influence the effectiveness of the treatments (Rodriguez-Lucena et al., 2010).

In order to create an adequate Fe fertilization recommendation, the effectiveness of the individual FeEDDHA components, such as its ability to stay in solution, competition with other metal ions, ability to deliver Fe to the plant, and ability of each component to interact with Fe in the soil, needs to be understood. Commercial FeEDDHA formulation compositions varies greatly, but under European fertilizer law (Regulation (EC) No. 2003/2003; amendment (EC) No. 162/2007) quality is assured under two parameters: soluble Fe content and percentage of Fe chelated by o,o-EDDHA and o,p-EDDHA (Schenkeveld et al., 2008).

Adjuvants and Surfactants

Water molecules are bipolar, containing a partial positive and negative charge. When water molecules are put together, the opposite forces attract each other creating surface tension. Application of water to a hydrophobic surface can cause beading which is also a result of surface tension. Waxy surfaces on plant and other target species reduces the amount of water that is able to penetrate the target, since a majority of pesticides are formulated to use water (Czarnota and Thomas, 2013). An understanding of the reaction between the droplets and the surface of the target is required to increase biological activity and decrease solution loss (Gimenes et al., 2013).The addition of an adjuvant or surfactant can decrease the surface tension, allowing for

dispersion of the liquid across the surface of the leaf or target species (Czarnota and Thomas, 2013). Adjuvants can help to increase the amount of active ingredient absorbed by the target as well as by increasing the uptake ability. Surfactants, emulsifiable oils, and polymers can increase spray deposition and are commonly referred to as spreading or wetting agents (Holloway et al., 2000).

Adjuvants modify the physical and biological properties of spray mixtures, while also influencing spray atomization, formation, deposition, retention, uptake, and translocation (Gimenes et al., 2013; Nuyttens et al., 2007; Ortiz et al., 2007). Dispersion and evaporation of droplets, are two critical processes that can determine the effectiveness of a solution. Slow evaporation will allow the plant to absorb and metabolize the solution, but will stop when the droplets eventually dry out. This slow evaporation may increase the chance of growing certain pathogens within the droplets. If evaporation occurs quickly, crystals may form that reduce the absorption by plants, and may cause wind to blow the product away from the targeted plant (Gimenes et al., 2013). Smaller droplets increase retention and spread easier, while foliar uptake and efficacy is also improved (Yu et al., 2009).

Effective spray applications are needed to help reduce the operational costs with adequate spraying coverage for large acreage operations. By adding adjuvants to the spray solution, the droplet dispersion on leaf surfaces as well as the surface area of the droplets will increase, creating a larger coverage area per volume. This larger surface area will also increase the evaporation speed of the solution through the heat exchange between the environment and the plant, but the plant will have a greater opportunity to absorb the solution before evaporation. In the case of herbicide application, addition of oil adjuvants can enhance the foliar injury to the

plants (Gimenes et al., 2013; Lym and Manthey, 1996; Ortiz et al., 2007). Even though addition of an adjuvant will increase the surface area of the sprayed solution, there seems to be an optimal concentration for both economic and effective coverage (Gimenes et al., 2013).

Surfactants are adjuvants that reduce surface tension. These include anionic, cationic, amphoteric, and nonionic. Anionic surfactants are negatively charged and will typically foam which may cause issues with water flow or pumping systems. Cationic surfactants are positively charged and are considered toxic to plants, being used in cleaning products. Amphoteric surfactants with form either a positive or negative charge depending on the initial pH of the solution. Nonionic surfactants have no charge and are the most commonly used. Through proper use they remain stable, decrease water and surface tension, and they do not harm plants. Wetting agents are commonly used under hydrophobic conditions to accept water into the structure. These are like surfactants, by breaking the surface tension, but the chemistry is different. Organosilicates were developed in the 1970's for waterproofing. This allows them to withstand rain for an increased amount of time. They have been noted for extremely low surface tensions and good spreading abilities. These solutions also increased the spread area compared to other common adjuvants by reducing the contact angle. Depending on the product, application rate, and temperature, phytotoxicity or bacterial issues may occur (Czarnota and Thomas, 2013; MacIntyre-Allen et al., 2007).

Gimenes et al., (2013) found that by increasing the concentration of a surfactant from 0.01% to 1%, the foliar uptake of a solution may increase, helping to reduce crystallization. Non-ionic surfactants can lower the surface tension of a sprayed solution to 33-34 mN/m. Under some

circumstances, this decreased surface tension may allow for the droplets to coalesce and run off of the plant (MacIntyre-Allen et al., 2007).

In conclusion, there are many agronomic products on the market today for farmers to use in defense against or response to IDC symptoms within their fields. While some of these products are used regularly, the cost is very high. Research on some of these products have shown data supporting their field use, some have also shown a high variability. Experimentation with a few of these products in field trials in the Red River Valley would help gain an understanding of their ability to work this area, as well as to decide on their cost effectiveness for future growing seasons.

MATERIALS AND METHODS

Experimental overview

The effect of foliar application of Fe-chelated fertilizers and adjuvants in the management of IDC symptoms in soybeans was observed in field experiments in 2013 (FT1) and 2014 (FT2). Four different commercially produced Fe-chelated fertilizers (o-o-EDDHA, o-o-EDDHSA, HEDTA, and an amino acid), were evaluated during field experiments one and four different commercially available adjuvants (HSOC, non-ionic surfactant, an acidifier, and an organosilicone surfactant) were evaluated during field experiment two. Severity of IDC was determined through the relative chlorophyll content of the leaves throughout the study. Soil samples were taken as well, to determine the soil iron concentration.

Field Trials

Commercial fields were scouted at the beginning of the growing season for possible sites for experiments. Locations were monitored for low spots more prone to water damage, proximity to roads or trees, and distance from the headlands. Upon locating fields showing IDC symptoms and meeting the above criteria, land owners were contacted to determine if an experiment could be conducted on their land. Experiments were a randomized complete block design with four replications and five treatments per replication. Treatments were 46 m long and 11 m wide. Ten random soybean plants distributed evenly throughout the middle of each treatment were flagged for Soil and Plant Analyzer Development (SPAD) meter readings (Minolta SPAD-502, Osaka, Japan). Flags were used to help ensure that the same plant and leaflet were sampled over the course of the experiment.

Once all the treatments were soil sampled and SPAD readings taken, the solutions were applied with a bicycle wheel sprayer (FT1) and a backpack sprayer (FT2) in 93.5 liters per hectare water as carrier through 8002 flat fan nozzles (Horvick Incorporated, Fargo, ND) pressurized with CO_2 at 275.8 kpa. Sprayer was cleaned with water between treatments to ensure no treatment-to-treatment contamination. All locations were sprayed the same day to minimize forecast changes between them. A repeat application was applied to treatments across locations approximately 14 days after the first application, following the same procedures.

A Hege 125C combine (Wintersteiger Inc., Salt Lake City, UT) was used to harvest grain from the middle 1.5 m of each individual treatment. Grain was bagged and dried. Plant debris was intermingled with soybeans during harvest, and was removed using a Clipper seed cleaner (Ferrell-Ross, Bluffton, IN). Grain was weighed and analyzed for moisture using a GAC 500-XT (DICKEY-john Corporation, Minneapolis, MN).

Soils

Various soil types throughout North Dakota and Minnesota were used for both field trials (Table 1). For both FT1 and FT2, soil samples were collected from soybean fields in North Dakota and Minnesota that were displaying IDC symptoms. Soil samples were delivered to Agvise Laboratories, Northwood, ND, (FT1) and North Dakota State University soil testing laboratory, Fargo, ND (FT2) to be analyzed for various soil properties (Table 2).

Table 1. Soil series and classification of nine soybean fields in North Dakota used for testing foliar application of Fe-chelates and adjuvants during FT1 (2013) and FT2 (2014).

Year	Site	GPS	Soil Series	Classification
FT1 2013	Ada*	N 47° 18.841' W 96° 23.128'	Glyndon	Coarse-silty, mixed, superactive, frigid Aeric Calciaquolls
	Amenia	N 46° 51.483' W97° 12.843'	Wyndmere	Coarse-loamy, mixed, superactive, frigid Aeric Calciaquolls
	Prosper	N 47° 00.011' W 97° 06.685'	Kindred	Fine-silty, mixed, superactive, frigid Typic Endoaquolls
	Wheatland	N 46° 42.064' W97° 19.455'	Gardena	Coarse-silty, mixed, superactive, frigid Pachic Hapludolls
FT2 2014	Amenia H	N 47 °3.013' W97° 8.026'	Glyndon	Coarse-silty, mixed, superactive, frigid Aeric Calciaquolls
	Amenia N	N 47 °3.013' W97 °8.026'	Glyndon	Coarse-silty, mixed, superactive, frigid Aeric Calciaquolls
	Amenia S	N46°57.817' W97°13.076'	Kindred	Fine-silty, mixed, superactive, frigid Typic Endoaquolls
	Casselton	N46°48.238' W97°14.372'	Fargo	Fine, smectitic, frigid Typic Epiaquerts
	Wheatland	N46°44.020' W97°23.217'	Hecla	Sandy, mixed, frigid Oxyaquic Hapludolls

* Location is from Minnesota

Table 2. Soil properties from soils from nine locations used for testing foliar application of Fe-chelates and adjuvants.

		Location								
		2013				2014				
Soil properties	Units	Ada	Amenia	Prosper	Wheatland	Amenia H	Amenia N	Amenia S	Casselton	Wheatland
pH†		8.3	8.2	8.0	8.0	7.9	8.0	8.1	7.7	8.2
EC‡	dS m^{-1}	0.25	0.37	0.31	1.58	1.48	1.27	0.4	0.69	0.21
$CaCO_3$ Equiv.♯	g kg^{-1}	6.78	1.68	1.51	0.42	0.92	11.52	1.37	0.37	1.17
SOM″	g kg^{-1}	2.99	4.35	3.68	3.88	3.1	4.9	3.07	7.22	2.11
Olsen P††	mg kg^{-1}	30.25	15.65	25.8	15.45	30.45	9	7.6	15.45	9.25
Extract. K‡‡	mg kg^{-1}	124	310	214	250	153	340	296	537	127
Extract. Fe##	mg kg^{-1}	6.88	7.16	8.25	6.38	6.73	7.31	7.09	14.31	6.91
Nitrate-N″″	kg/ha	15.34	10.47	11.37	16.35	38.81	16.8	20	16.13	14.06
CEC●	$cmol_c$ kg^{-1}	29.63	30.79	30.59	30.13	29.01	32.08	25.47	36.61	20.22
Texture		loam	fine sandy loam	silty clay loam	silt loam	loam	loam	silty clay loam	silty clay	loamy fine sand

† Determined in 1:1 soil:water (Watson and Brown, 1998)
‡ Determined in 1:1 soil:water (Whitney, 1998b)
♯ Determined by pressure calcimeter method (Loeppert and Suarez, 1996)
″ Determined by loss on ignition (Combs and Nathan, 1998)
†† Extracted with 0.5 M $NaHCO_3$ (Frank et al., 1998)
‡‡ Extracted with 1 M NH_4OAc (Warncke and Brown, 1998)
Extracted with DTPA (Whitney, 1998a)
″″ Extracted with water (Gelderman and Beegle, 1998)
● Cation exchange capacity determined by summation (Warncke and Brown, 1998)

Solutions

Fe-chelates

Iron chelate solutions were mixed based upon the suggested rate of application by each company (Table 3). Five liters of water was placed into a clean 11 liter canister, one for each treatment. For the o-o-EDDHA and o-o-EDDHSA treatments, 55 g of dry material was added to the water and thoroughly mixed, making sure that no clumps formed. For the amino acid and HEDTA treatments, 80 mL of liquid was added to the water and thoroughly mixed. Once mixed, a non-ionic surfactant was added at the rate of 0.37 liters per acre and another five liters of water was added to each canister, taking them to the desired volume.

Table 3. Fe-chelate (FT1) and adjuvant (FT2) treatments, with the amount of product used per application.

Year	Treatments	Fe Source	Fe Rates	Adjuvant Source	Adjuvant Rate
FT1 2013	Control	Control	N/A	N/A	N/A
	Commercial-1	o-o-EDDHA	0.68 kg/A	Non-ionic surfactant	0.37 L/A
	Commercial-2	o-o-EDDHSA	0.68 kg/A	Non-ionic surfactant	0.37 L/A
	Commercial-3	Amino acid	0.95 L/A	Non-ionic surfactant	0.37 L/A
	Commercial-4	HEDTA	0.95 L/A	Non-ionic surfactant	0.37 L/A
FT2 2014	Control	N/A	N/A	Control	N/A
	Commercial-1	Fe-LS	0.57 kg/A	HSOC	1.86 L/A
	Commercial-2	Fe-LS	0.57 kg/A	Non-ionic surfactant	1.86 L/A
	Commercial-3	Fe-LS	0.57 kg/A	Acidifier	1.86 L/A
	Commercial-4	Fe-LS	0.57 kg/A	Organosilicone surfactant	0.12 L/A

Adjuvants

Adjuvant solutions were mixed per suggested application rates (Table 3). For each treatment, 50 g of Fe-lignosulfonate was added to five liters of water in an 11 liter canister. Once in solution, HSOC, non-ionic surfactant, and an acidifier were added at the rate of 1.86 liters per acre to their respective canisters. Organosilicone surfactant was added at the rate of 0.12 liters per acre. After thorough mixing, an additional five liters of water was added to each canister, taking it to the desired volume for application.

Observations

Relative chlorophyll values were collected from between the veins on the middle leaf of the second trifoliate of the marked plants and averaged across the ten plants per treatment using a Minolta SPAD-502 meter. These readings were collected before spraying, one week after the first spray, and one week after the second spray. Soil samples were collected from a depth of from 0 to 15 cm in each treatment before spraying, one week after the first spray, and one week after the second spray during FT1 and sent to Agvise Laboratories in Northwood, ND. Field trial 2 was only sampled before spraying and one week after the final spray, with the samples being sent to the North Dakota State University soil testing laboratory, Fargo, ND.

Statistical Analysis

T-tests were performed to compare the means of each treatment for the following measures: SPAD readings, Fe concentration, and overall yield to determine whether there was a statistical difference between the fertilizers in each field experiment. T-tests comparisons were conducted using SAS 9.4 for windows (SAS Inc., Cary, NC). Each parameter was then analyzed by one-way analysis of variance (ANOVA) based on a randomized complete block design.

Differences between means were determined by least significant differences (LSD) at $\alpha=0.10$ probability if the F-test values were significant.

RESULTS AND DISCUSSION

Relative Chlorophyll Content

Field Trial 1

Readings taken from a week before spraying indicated that relative chlorophyll content tended to be the same across treatments. The relative chlorophyll content was greater across treatments following the first and second foliar application of iron fertilizers (Table 4). This increase also included the four control treatments that did not receive iron chelates.

Chlorophyll content increased significantly following the first application of o-o-EDDHA at the Wheatland site from 29.3 to 40.6 and HEDTA at the Prosper location from 35.1 to 40.2. The chlorophyll readings varied across all locations, but were similar in their increase from the previous sampling. Across most of the locations, o-o-EDDHA tended to provide the smallest relative chlorophyll content increase after foliar applications, while amino acid tended to provide the greatest relative chlorophyll content increase. However, after all foliar applications were completed, the control treatments that received no foliar iron chelates tended to have a more uniform and greater increase in chlorophyll content.

Overall, all treatments caused an increase in the relative chlorophyll content of the leaves. While some of this could be attributed to the applied iron chelate treatments, chlorophyll increase could also be a natural occurrence in the plants as they mature and approach the reproductive stages, especially since at most locations, the control plots had similar chlorophyll levels as those of the treated plots.

Table 4. Relative chlorophyll (SPAD) values from a Minolta SPAD-502 meter for a soybean leaflet at each FT1 location before, during, and after spraying.

Location	Treatment	Relative chlorophyll content		
		Before Spraying	After First Spray	After Second Spray
Ada	Control	26.6	33.7	40.8
	o-o-EDDHA	27.5	33.6	41.1
	o-o-EDDHSA	28.3	32.7	41.6
	Amino acid	28.1	34.7	40.4
	HEDTA	29.6	35.4	41.7
	F-value	NS	NS	NS
Amenia	Control	35.1	39.0^{A}	43.2^{A}
	o-o-EDDHA	33.2	36.0^{B}	40.7^{B}
	o-o-EDDHSA	35.9	36.5^{AB}	41.8^{AB}
	Amino acid	32.8	37.9^{AB}	41.3^{AB}
	HEDTA	35.2	36.6^{AB}	42.1^{AB}
	F-value	NS	NS	NS
Prosper	Control	37.8	41.8^{A}	42.9
	o-o-EDDHA	35.8	37.5^{B}	42.9
	o-o-EDDHSA	35.1	41.5^{A}	42.2
	Amino acid	37.1	43.1^{A}	42.5
	HEDTA	35.1	40.2^{A}	42.7
	F-value	NS	1.6*	NS
	LSD (0.10)		3.3	
	C.V., %		6.5	
Wheatland	Control	31.3	40.3^{A}	43.3
	o-o-EDDHA	29.3	40.6^{A}	44.0
	o-o-EDDHSA	29.7	38.0^{AB}	44.9
	Amino acid	31.3	40.1^{A}	44.1
	HEDTA	29.3	37.4^{B}	43.6
	F-value	NS	NS	NS

* Means are significantly different at $p \leq 0.10$

Field Trial 2

As with the first year of field trials, readings recorded before the first foliar application indicated relative chlorophyll content was very similar across all treatments and locations. There was an increase in chlorophyll content across all treatments and locations after the first and second foliar applications, including the control treatments (Table 5).

While iron chelates applied with different surfactants gave a significant difference in relative chlorophyll content after the first and second foliar application across locations, results were variable with no single treatment providing a similar response across locations. All treatments tended to cause a similar increase in chlorophyll content after each repeat application. That stated, HSOC and the control tended to cause the greatest numeric increase in chlorophyll content following repeat applications across locations.

There was a miscalculation of applied product during the first spray of the organosilicone surfactant in field trial two. This miscalculation attributed to moderately severe leaf burn on soybean leaves that not only stunted the soybean plants, but also destroyed many of the leaves. While the plants resumed growth and produced new leaves, they were morphologically delayed compared to the other treatments. As such, the chlorophyll readings for this treatment could be skewed because of how damaged the leaves were that were being measured throughout the rest of the trial.

Table 5. Relative chlorophyll (SPAD) values from a Minolta SPAD-502 meter for a soybean leaflet at each FT2 location before, during, and after spraying.

		Relative chlorophyll content		
Location	Treatment	Before Spraying	After First Spray	After Second Spray
Amenia H	Control	26.0	30.9	41.4^{A}
	HSOC	26.6	32.0	41.2^{A}
	Non-ionic surfactant	25.4	30.2	40.8^{A}
	Acidifier	24.7	30.1	40.1^{A}
	Organosilicone surfactant	23.8	30.3	34.8^{B}
	F-value	NS	NS	6.7*
	LSD (0.10)			2.1
	C.V., %			4.1
Amenia N	Control	29.7	33.3^{A}	36.7^{AB}
	HSOC	29.1	31.4^{AB}	36.4^{AB}
	Non-ionic surfactant	28.5	30.6^{AB}	36.1^{AB}
	Acidifier	27.5	31.6^{AB}	37.0^{A}
	Organosilicone surfactant	28.2	29.2^{B}	35.2^{B}
	F-value	NS	NS	NS
Amenia S	Control	27.5	36.8^{AB}	39.5^{A}
	HSOC	28.3	36.4^{AB}	38.4^{AB}
	Non-ionic surfactant	28.3	35.1^{B}	37.0^{B}
	Acidifier	27.5	37.7^{A}	38.9^{AB}
	Organosilicone surfactant	28.2	35.1^{B}	37.1^{B}
	F-value	NS	NS	NS
Casselton	Control	30.0	39.4	42.6^{B}
	HSOC	29.4	38.9	43.1^{AB}
	Non-ionic surfactant	30.4	38.6	42.7^{B}
	Acidifier	29.9	38.1	43.3^{AB}
	Organosilicone surfactant	29.3	38.0	44.5^{A}
	F-value	NS	NS	NS
Wheatland	Control	28.3	33.9	41.9^{A}
	HSOC	26.8	34.6	41.2^{A}
	Non-ionic surfactant	26.8	34.7	39.7^{AB}
	Acidifier	26.7	34.5	40.7^{AB}
	Organosilicone surfactant	26.8	34.4	37.7^{B}
	F-value	NS	NS	NS

* Means are significantly different at $p \leq 0.10$

The relative chlorophyll content in the leaf increased after foliar applications one and two during both years of field trials. This could be because of a more direct pathway for iron to reach the chlorophyll producing organs of the leaves (Abadia et al., 2011; Horesh and Levy, 1981). An increase in chlorophyll pigments could result in a greener color throughout the upper canopy and a visual greening effect (Rodriguez-Lucena et al., 2010). While the chlorophyll content did increase throughout the trial, it did so across all of the treatments, including the control or no iron chelate and surfactant treatments. This general increase in all trials could indicate that while the plants may have been under initial stress of reduced iron, they were able to absorb iron from the soil later on at a much more sufficient rate.

Soil Iron Concentration

Field Trial 1

Results from soil iron concentration measurement were very random across treatments and locations, varying greatly during initial sample and following the first and second foliar treatment application (Table 6). Soil iron concentration was greater following the first foliar application at all locations and in all treatments. However, a decline in the iron concentration levels within the soil was observed after the second foliar application across all treatments and locations, but decline was less than the initial increase. The amino acid treatment tended to cause an increase in soil iron levels more than the other treatments, while o-o-EDDHSA tended to cause the least increase in concentration levels.

Table 6. Soil iron concentration at each location during FT1 before, during, and after spraying.

Location	Treatment	Iron concentration		
		Before Spraying	After First Spray	After Second Spray
		mg kg^{-1}		
Ada	Control	6.2^{C}	7.6	7.3
	o-o-EDDHA	6.7^{BC}	8.2	7.4
	o-o-EDDHSA	6.5^{BC}	7.5	7.0
	Amino acid	7.1^{B}	7.5	7.9
	HEDTA	8.0^{A}	8.2	7.8
	F-value	NS	NS	NS
Amenia	Control	7.9^{A}	10.0	9.6^{A}
	o-o-EDDHA	6.9^{ABC}	9.0	9.1^{AB}
	o-o-EDDHSA	6.6^{C}	8.6	8.6^{BC}
	Amino acid	7.8^{AB}	9.8	8.9^{B}
	HEDTA	6.7^{BC}	9.1	8.2^{C}
	F-value	NS	NS	NS
Prosper	Control	8.2	10.1^{B}	10.0
	o-o-EDDHA	8.1	10.6^{AB}	10.0
	o-o-EDDHSA	8.0	10.6^{AB}	9.9
	Amino acid	8.4	11.8^{A}	10.5
	HEDTA	8.6	11.0^{AB}	10.2
	F-value	NS	NS	NS
Wheatland	Control	6.3	8.3	8.1
	o-o-EDDHA	6.3	8.4	8.4
	o-o-EDDHSA	6.5	8.3	8.3
	Amino acid	6.6	8.1	7.9
	HEDTA	6.5	8.4	7.8
	F-value	NS	NS	NS

Some of the foliar-applied iron chelate treatments could have reached the soil instead of staying on the plants themselves, especially after the first foliar application. This may have

caused an initial increase in measured iron concentration levels within the soil after the first foliar application. A modest decline in iron concentration measurement after the second application may be attributed to increased plant uptake and decreased bare soil. The second iron chelate treatment application occurred when soybeans were relatively large, reducing the amount of spray solution that may have reached bare soil.

Field Trial 2

Only initial soil iron concentration and measurement after the second foliar application were recorded in the second year (Table 7). While it is difficult to draw inferences from only two sets of data, the initial iron concentration results across most of the locations tended to be similar across the various treatments. Soil iron concentration measured following the second foliar application tended to be uniformly less than initial measurements across treatments and locations.

Most of the soils in the Red River Valley have a high clay content, which can contribute to poor drainage after precipitation, inducing a waterlogged state. Under these conditions, Fe^{2+} concentration increases over Fe^{3+}, decreasing plant availability (Havlin et al., 2005). Annual precipitation was much greater during 2013 (547.9 mm) than 2012 (241.8 mm), and average temperature was less as well, averaging 7 °C during 2012 and 4 °C during 2013 (NDAWN, 2014). This increase in moisture content may have caused more Fe to be moved lower within the soil solution, or away from the plant roots. Since plant root development and nutrient absorption are also reduced under cool and wet conditions (Havlin et al., 2005), the ability to absorb the iron before it was moved away from the roots may have been decreased. A change in chelate

efficiency could have allowed other cations (Ca, Mn, Zn, or Cu) to replace Fe in the chelate, removing it from the solution (Aboulroos et al., 1983; Ylivainio, 2010).

Table 7. Soil iron concentration at each location during FT2 before and after spraying.

Location	Treatment	Iron concentration	
		Before Spraying	After Second Spray
		mg kg^{-1}	
Amenia H	Control	6.5^{B}	6.3
	HSOC	6.1^{B}	6.2
	Non-ionic surfactant	6.5^{B}	6.2
	Acidifier	7.6^{A}	6.5
	Organosilicone surfactant	7.0^{AB}	6.6
	F-value	NS	NS
Amenia N	Control	7.9	6.1^{A}
	HSOC	7.2	6.7^{A}
	Non-ionic surfactant	7.3	6.1^{A}
	Acidifier	7.3	6.3^{AB}
	Organosilicone surfactant	7.0	6.2^{AB}
	F-value	NS	NS
Amenia S	Control	7.3^{AB}	5.7
	HSOC	7.4^{A}	5.5
	Non-ionic surfactant	7.3^{AB}	5.8
	Acidifier	6.9^{AB}	5.1
	Organosilicone surfactant	6.7^{B}	5.2
	F-value	NS	NS
Casselton	Control	14.4	11.7^{AB}
	HSOC	14.1	10.9^{B}
	Non-ionic surfactant	14.2	11.0^{AB}
	Acidifier	14.8	12.5^{A}
	Organosilicone surfactant	14.0	10.4^{B}
	F-value	NS	NS
Wheatland	Control	5.9	4.9
	HSOC	7.8	7.2
	Non-ionic surfactant	6.3	12.8
	Acidifier	7.9	7.6
	Organosilicone surfactant	6.7	6.2
	F-value	NS	NS

Yield

Field Trial 1

Soybean yield were the same across most locations and treatments. While none of the treatments caused significant differences, there were some yield trends throughout each field (Table 8). Yield tended to be greater among the o-o-EDDHA and HEDTA treatments across most of the locations, and at those locations, yield from o-o-EDDHA and HEDTA treatments tended to be greater than the control. The amino acid treatment tended to reduce yield as compared to the control across the various locations, while o-o-EDDHSA gave soybean yield similar to the control treatment.

Table 8. Yield after harvest and cleaning for FT1.

Location	Control	o-o-EDDHA	o-o-EDDHSA	Amino acid	HEDTA	F-value
	----------	----------	kg ha^{-1}	----------	----------	
Ada	1914	1850	1923	1923	2014	NS
Amenia	2218	2385	2233	1853	2370	NS
Prosper	2844	2789	2670	2762	2625	NS
Wheatland	1130	1258	1258	993	1285	NS

Yield increase compared to the control could be attributed to an increase in nutrients available to the plant at critical stages, as well as nutrients that were easily accessible, allowing the plant to allocate more energy to the production of biomass and eventually reproduction rather than in the uptake of nutrients.

Field Trial 2

The control treatment tended to yield better than the other treatments in field trial two, while the organosilicone surfactant treatment tended to yield the least (Table 9). While HSOC, non-ionic surfactant, and acidifier treatments may have provided slightly greater numeric yield at some of the locations, yield tended to be variable. The Amenia N location was the only location with significant treatment differences.

Soybean yield may have been influenced by misapplication of the organosilicone surfactant treatment. That stated, soybean yield was equally variable with this treatment across locations. Soybean yield tended to be greater than the control from the organosilicone treatment at two locations where possibly the damage may have been less overall or perhaps the soybean plants may have been able to recover faster than those at the other locations.

Table 9. Yield after harvest and cleaning for FT2.

Location	Control	HSOC	Non-ionic surfactant	Acidifier	Organosilicone surfactant	F-value
	----------	----------	kg ha^{-1}	----------	----------	
Amenia H	1467	1194	1477	1467	975	NS
Amenia N	1978^{A}	1713^{ABC}	1513^{BC}	1814^{AB}	1294^{C}	NS
Amenia S	2361	2315	2388	2433	2397	NS
Casselton	1322	1413	1294	1294	1486	NS
Wheatland	2424	2406	2406	2370	2187	NS

Yield calculations are derived from the number of harvestable soybean plants per unit area and the size and weight of individual soybeans. Plants must allocate resources into the growth of the pods and beans in order to maximize yield. The soybean plant is durable and is able to overcome early season stresses. For example, while the soybean plant may be slight in stature early in the season, perhaps due to excessive moisture or herbicide injury, in some cases by harvest time, the plant may compensate and have a good overall yield by producing fewer pods but larger beans (Wiersma, 2005; Ortiz et al., 2007).

GENERAL CONCLUSIONS

Iron deficiency is a production challenge affecting many soybean fields throughout the Red River Valley, and the number of fields seems to be increasing as more fields are planted to soybeans. Farmers are investigating foliar application of an iron chelate to correct areas of deficiency symptoms in fields and use of surfactants or adjuvants to reduce application rates and costs. Presence of iron deficiency must be observed and diagnosed early in the growing season for the foliar application of these products to perform effectively. The farmer will be wasting valuable products, time, and manpower if it is not correctly diagnosed or not found early in the season. Farmers must then decide if it is financially viable to spray a portion of the field that demonstrates a mild incidence of deficiency since in some cases, sprayed fields may yield similarly to fields that have not been sprayed. While some of the products did improve yield overall in this research, they were not statistically different from the control.

There are other options available for farmers to spot treat patches within a field with a history of iron chlorosis deficiency. One option is to apply iron chelates in-furrow with the seed at planting, which will help to give the plant that extra boost upon germination and emergence. Another option is to plant a seed variety that has demonstrated resistance or has greater resilience to iron chlorosis, which may yield slightly less than a different variety, but will be able to tolerate the deficiency better and yield higher overall.

This research provided valuable insight into how future experiments might be conducted. Similar experiments could be repeated at additional locations to confirm these results during these two growing seasons. A location selection criteria might be soybean varieties with greater overall susceptibility to iron deficiency chlorosis. Greater varietal susceptibility would allow for

more severe or more uniform infestation and greater possibility of measureable differences between applied treatments. Selected locations should demonstrate even greater severity of deficiency symptoms before initial spraying, to exacerbate differences between treatments. Additional products at fewer rates, or the same products at more rates could be evaluated, which would allow more data to be collected and establish the optimal rate for deficiency symptom correction. The products that were used for this experiment are not the only commercially available products. Consideration for products that align to growing degree days for target area should also be considered in future experiments. Finally, various elements, such as Mn, have been shown to affect how iron moves within the soil and plant. Knowing this, additional soil tests could be conducted to investigate levels of Mn or other elements to determine if there is a correlation between them and iron. All of these options could possibly show a different outcome than what was observed during these field experiments.

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