LABORATORY AND GREENHOUSE EVALUATION OF FeEDDHA FERTILIZERS OF DIFFERING QUALITY

A Thesis
Submitted to The Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Sarah Elizabeth Hall Lovas

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Soil Science

August 2013

Fargo, North Dakota

North Dakota State University
Graduate School

Title

LABORATORY AND GREENHOUSE EVALUATION OF FeEDDHA FERTILIZERS OF DIFFERING QULAITY

By

Sarah Elizabeth Hall Lovas

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

SUPERVISORY COMMITTEE:

Dr. R. Jay Goos
Chair

Dr. Tom DeSutter

Dr. Dave Franzen

Dr. Rich Zollinger

Approved:

9/16/13
Date

Dr. Frank Casey
Department Chair

ABSTRACT

Commercial iron ethylene diamine di(hydroxyl phenyl acetic acid) (FeEDDHA) fertilizers containing the same Fe percent and applied at the same FeEDDHA rate control Fe deficiency chlorosis (IDC) differently due to differing ortho, ortho FeEDDHA (o,o-FeEDDHA) concentrations. This study: 1) determined the effect of o,o-FeEDDHA concentration on controlling IDC in soybeans (*Glycine max* L. Merr.); and 2) developed a soil-stability test using a simple colorimetric analysis method to determine the relative quality of soil-applied FeEDDHA fertilizers. A greenhouse experiment was conducted where nine FeEDDHA fertilizers were applied at two FeEDDHA rates. The soil-stability test compared these fertilizers with two incubation methods which utilized three soils and four incubation times, and extracts were analyzed by two methods. The results of these experiments suggest that soil-applied FeEDDHA fertilizer quality is contingent upon its o,o-FeEDDHA concentration, and the fertilizer quality can be determined by a soil-stability test with a colorimetric analysis method.

ACKNOWLEDGMENTS

I would like to express my thanks and gratitude to: Dr. Goos for his patience and guidance throughout this project; Brian Johnson, for his technical assistance; Dr. DeSutter, Dr. Franzen, and Dr. Zollinger for their guidance as my committee members; The North Dakota Soybean Council for funding this research; The North Dakota Experiment station for helping to fund my education; JAER Laboratories in Barcelona, Spain, for the HPLC analysis of the FeEDDHA fertilizers; and Curt Doetkott and Jamie Kubat of the NDSU Statistical Consulting Service for help with the statistical analysis.

DEDICATION

This is dedicated to my best friend, favorite farmer, and husband, Jason, who constantly supports me in everything I do.

This is dedicated to the farmers of North Dakota, and especially Jason, Dad, Afi, Grandpa, Kevin, Peter, and all those for whom I have scouted your crops. You have helped to cultivate my love of farming. I hope this research benefits all of you

This is dedicated to all the Professors and Soil Scientists who have opened my eyes to the world of soil science. Thank you for inspiring me and teaching me. I have learned so much from you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

LIST OF APPENDIX FIGURES

ORGANIZATION OF THESIS

The thesis includes a literature review section, a materials and methods section, and a results and discussion section. The literature review explains the distribution and severity of IDC in the Northern Plains, Fe chemistry in agricultural soils, Fe in plants, Fe deficiency chlorosis in soybean, definition of a chelate, FeEDDHA as a soil-applied fertilizer, and the research objective. The materials and methods section explains the procedures for both the greenhouse and the laboratory experiment. The results and discussion section discusses the results for both the greenhouse and the laboratory experiments. The thesis is summarized by the general conclusions section, followed by a referenced cited section. Finally, there are are two appendices with supplementary figures for the laboratory and greenhouse experiments.

LITERATURE REVIEW

Distribution and Severity of IDC in the Northern Plains

The value of the U.S. soybean production in 2010 was $37.5 billion, and the value of the North Dakota soybean production was $1.5 billion, and, thus, soybean is a crop that is significant to both U.S. and North Dakota agriculture (National Agricultural Statistics Service, 2012b; 2012c). North Dakota growers planted approximately 1.6 million hectares (4 million acres) and harvested approximately 3.75 million Mg (138 million bushels) of soybeans in the same year (National Agricultural Statistics Service, 2012b). Cass, Stutsman, Barnes, Richland and LaMoure counties planted the most acres of soybeans in North Dakota, respectively, and together account for approximately 600,000 planted hectares (1.5 million acres) (Table 1) (National Agricultural Statistics Service, 2012a).

Table 1. Counties in North Dakota in 2010 where more than 40,000 ha (100,000 acres) of soybeans were planted, average yield per hectare, and the total production (National Agricultural Statistics Service, 2012a).

County	Area planted	Yield	Production
	ha	Mg ha^{-1}	Mg
Cass	212,551	2.39	503,624
Stutsman	158,704	2.25	351,816
Barnes	134,008	2.43	323,158
Richland	126,721	2.37	298,827
LaMoure	98,785	2.29	225,889
Traill	77,328	2.55	197,476
Wells	63,968	2.17	136,513
Dickey	59,514	2.32	136,840
Sargent	59,312	2.41	142,392
Steele	58,704	2.36	138,446
Grand Forks	55,466	2.21	122,470
Benson	49,798	2.10	104,345
Foster	47,166	2.24	105,460
Ransom	42,915	2.45	103,419
Griggs	42,105	2.39	100,072

Iron deficiency chlorosis negatively impacts soybean production on calcareous, high pH, and saline soils in North Dakota and Minnesota (Franzen and Richardson, 2000; Goos and Johnson, 2000; Hansen et al., 2003, 2004; Inskeep and Bloom, 1986). Approximately 700,000 ha of soybeans were planted on soils susceptible to IDC in the North Central U.S. in 1970, and by 2002 that number had increased to be approximately 1.8 million ha (Hansen et al., 2004). A survey of western Minnesota farmers in 2004 showed that 100% of the surveyed farmers were concerned about IDC in their soybean production, and that 25% had fields with severe IDC (Hansen et al., 2004). The severity of chlorosis can be quantified using a visual chlorosis score where 1 = no chlorosis, 2 = slight chlorosis of the upper leaves, but no interveinal chlorosis, 3 = interveinal chlorosis, but no plant stunting or necrosis, 4 = interveinal chlorosis present, stunted growth, and possibly necrosis starting to occur, 5 = interveinal chlorosis, necrosis and stunting apparent, growing points are damaged, or entire plants are dead (Froehlich and Fehr, 1981; Goos and Johnson, 2010). Soybean yield loss from severe IDC with an average visual chlorosis score of 3.8 is estimated to be 0.75 Mg ha^{-1} (11 bu $acre^{-1}$) (Hansen et al., 2004). The average soybean yield in North Dakota in 2010 was 2.29 Mg ha^{-1} (34 bu $acre^{-1}$) (National Agricultural Statistics Service, 2012b) and, therefore, a yield loss of 0.75 Mg ha^{-1} would be an approximate 32% reduction in yield, if losses in North Dakota were similar to what was estimated by Hansen et al., (2004).

High soil electrical conductivity (EC) values and high soil $CaCO_3$ equivalency (CCE) have been correlated to the occurrence of IDC. Iron reaches its minimum solubility in soil in the pH range of 7.4 and 8.5 (Franzen and Richardson, 2000; Inskeep and Bloom, 1986; Lindsay, 1979; Lindsay and Schawb, 1982). An average combination of 11.4% CCE, EC values of 0.9 dS m^{-1}, and pH values of 8.0 at 60 locations in Minnesota were shown to produce chlorotic

soybeans. However, severe chlorosis has been reported on fields with CCE as low as 1% (Hansen et al., 2003, 2004; Inskeep and Bloom, 1984). Not only does the concentration of CCE contribute to the occurrence of IDC, but the particle size and surface area of $CaCO_3$ contributes to its reactivity, which influences IDC severity (Inskeep and Bloom, 1986).

Every year Agvise Laboratories of Benson, Minnesota and Northwood, North Dakota correlate agricultural soil samples by Zip Code to show the distribution of certain soil characteristics such as CCE, EC, and soil pH (Agvise Laboratories, 2012). The 2011 fall soil samples from the 0-6 in sample depth showed that approximately 25% of the soil samples in the Red River Valley of North Dakota and Minnesota had EC values of greater than 1 dS m^{-1} (1 mmho cm^{-1}) as determined with a 1:1 soil-to-water method (Fig. 1), CCE values greater than 5% (Fig. 2), and approximately 80% of the soil samples had soil pH levels greater than 7.3 (Fig. 3) (Agvise Laboratories, 2012). Comparing the geographical distribution of soybean production (Table 1) to the geographical distribution of high EC values, high CCE values, and soil pH greater than 7.3 (Fig. 1, 2, 3), suggests that IDC has a significant opportunity to negatively impact North Dakota soybean production.

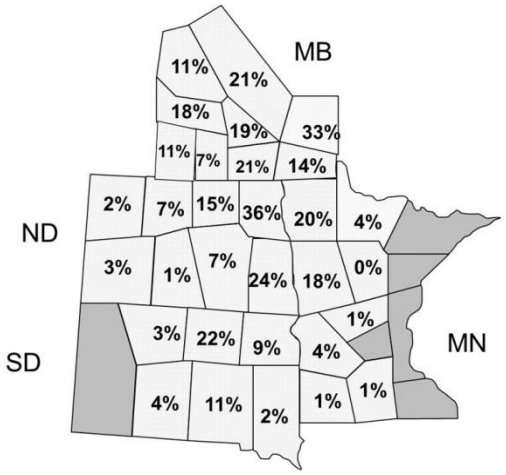

Figure 1. Percent of regional fall 2011 topsoil samples with electrical conductivity (EC) values of greater than 1 dS m^{-1} as determined with 1:1 soil-to-water method (Agvise Laboratories, 2012).

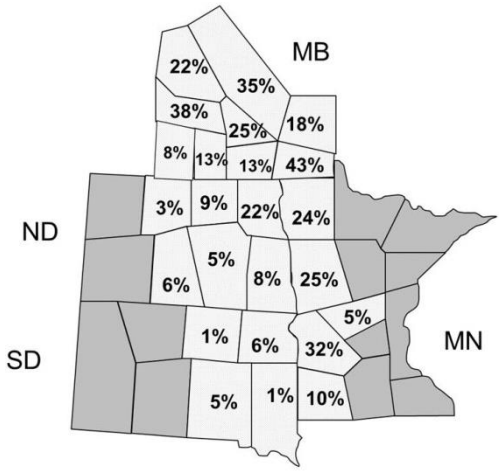

Figure 2. Percent of regional fall 2011 topsoil samples with calcium carbonate equivalency (CCE) values of greater than 5% (Agvise Laboratories 2012).

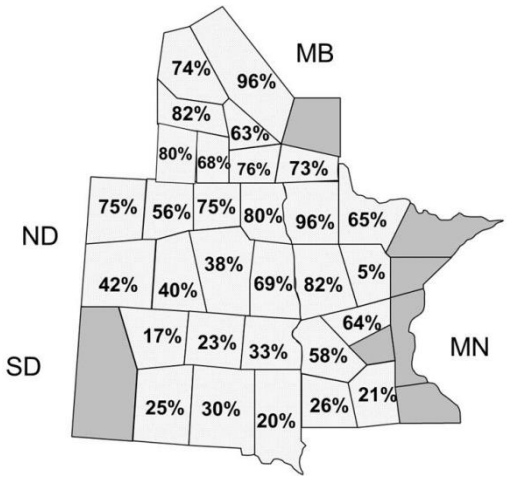

Figure 3. Percent of regional fall 2011 soil samples with soil pH of greater than 7.3 (Agvise Laboratories, 2012).

Fe Chemistry in Agricultural Soils

Fe in Rocks and Soils

Earth's crust is approximately 5% Fe (Kabata-Pendias, 2011). It is not considered a trace element in soils and rocks, and it is the fourth most abundant element in the lithosphere (Havlin et al., 1999; Kabata-Pendias, 2011; Murad and Fischer, 1988). The minerals considered to be major contributors of Fe in Earth's crust are mafic silicates, such as olivines, pyroxenes, and amphiboles, Fe sulfides, carbonates, oxides, and some clay minerals (Eggleton et al., 1988;

Murad and Fischer, 1988). Iron can also be found as a minor component of many other minerals (Murad and Fischer, 1988).

The concentration of Fe in soils averages about 3% (Murad and Fischer, 1988). Iron is found in soils as Fe-(hydr)oxides, as a structural component of layer silicates, in primary or secondary minerals, in complexes with organic matter, and in the soil solution (Loeppert and Inskeep, 1996). The term "Fe-(hydr)oxides" is used to encompass the wide range range of Fe-oxides, oxyhydroxides, and hydrated oxides in which Fe is commonly found (Allen and Hajek, 1989; Bigham et al., 2002; Schwertmann and Taylor, 1989). Iron in layer silicates tends to be plant unavailable unless very low redox conditions exist (Loeppert and Inskeep, 1996). Organic matter can form stable complexes with Fe when soil pH is less than 5.0, but above pH 6.0 complexation with organic matter becomes less important, because Fe participates in hydrolysis reactions which results in insoluble Fe-(hydr)oxide species (Loeppert and Inskeep, 1996).

Fe-(hydr)oxides

Pertaining to IDC, emphasis must be given to the Fe-(hydr)oxide minerals due to their influence on Fe solubility and plant availability in calcareous soils (Lindsay, 1979; Lindsay and Schwab, 1982; Loeppert, 1988). Calcareous soils have free $CaCO_3$ and other such carbonates which effervesce when treated with 0.1 M HCl (Soil Science Society of America, 2011). The pedogenic concentrations of Fe-(hydr)oxides in well-drained calcareous soils tend to be low due to the slow rate of dissolution of Fe(II)-containing primary and secondary minerals in equilibrium with $CaCO_3$ (Loeppert, 1988). Goethite and hematite tend to be the dominate Fe-(hydr)oxide species in calcareous soils, although ferrihydrite can be present in small quantities (Loeppert, 1988). Soils from the arid, semiarid, and tropical regions tend to have hematite as the dominant Fe-(hydr)oxide species (Kabata-Pendias, 2011). Goethite is found over an extensive

geography with climates varying from temperate to tropical (Kabata-Pendias, 2011). The relative solubilities of Fe-(hydr)oxides in order of increasing solubility are:
goethite < hematite < lepidocrocite < maghemite < soil Fe < amorphous Fe-(hydr)oxide (Lindsay, 1988).

Goethite (α-FeOOH) is a secondary ferric oxide mineral and is the most widespread Fe mineral in soils. It is found in almost every soil type and climate (Allen and Hajek, 1989; Bigham et al., 2002; Schwertmann and Taylor, 1989). Goethite tends to be more dominant in cool, temperate and cold regions compared to hematite. In calcareous soils, goethite usually dominates the Fe-(hydr)oxide mineralogy (Loeppert, 1988). Hematite (α-Fe_2O_3) is found in many soils, but tends to be more dominant in well aerated, warmer soils in climates such as arid or tropical regions (Allen and Hajek, 1989; Bigham et al., 2002; Schwertmann and Taylor, 1989).

Ferrihydrite was previously considered to be amorphous ferric oxide, however, it is now considered to be a poorly ordered ferric oxide (Bigham et al., 2002; Schwertmann and Taylor, 1989). The chemical formula of ferrihydrite has not been completely defined due to its poor crystallinity and small grain size. A formula of $5Fe_2O_3*9H_2O$ has been suggested as a typical formula (Bigham et al., 2002; Schwertmann and Taylor, 1989). Ferrihydrite is found in soils that can rapidly oxidize Fe(II) to Fe(III), especially in places where ground water may fluctuate on a seasonal basis (Allen and Hajek, 1989; Bigham et al., 2002; Schwertmann and Taylor, 1989).

Soil Solution Chemistry of Fe

The Fe in soil solution is controlled by soil pH, redox conditions, the presence of water-soluble anionic Fe-complexes, the solubility of the Fe-(hydr)oxides, and the kinetics of solubility and precipitation reactions of these products (Lindsay, 1979). However, it is the solubility of the

Fe(III)-(hydr)oxides that has the largest influence on the solubility of Fe in soils (Linsday, 1979). The solubility of both Fe(III) and Fe(II) decreases as soil pH increases (Lindsay, 1979). The concentration of Fe in the soil solution of calcareous and loamy soils ranges from 100 to 200 μg L^{-1} (Kabata-Pendias, 2011). By contrast, in acidic sandy soils with soil pH below 4.5, the Fe concentration ranges from 1000 to 2223 μg L^{-1} (Kabata-Pendias, 2011). The minimum solubility of Fe in soil solution is achieved in the soil pH range of 7.4-8.5 (Lindsay and Schwab, 1982). The pH range of 7.4-8.5 is also the normal pH range for a calcareous soil where IDC tends to be problematic (Franzen and Richardson, 2000).

Fe in Plants

Plant Uptake of Fe

The sufficiency range for Fe in plant tissues is between 50 and 250 μg g^{-1} on a dry weight basis (Havlin et al., 1999). However, based on the inorganic chemistry of Fe in "normal, agricultural" soils, the soil solution should not have enough available Fe for plants to receive the required amount of Fe for adequate plant nutrition (Havlin et al., 1999; Linsday and Schwab, 1982). Plants utilize one of two Fe uptake mechanisms to increase acquisition of Fe from soils, which can be classified as strategy I plants or strategy II plants (Hell and Stephan, 2003; Romheld and Marschner, 1986).

Strategy I plants are dicot plants, such as soybeans, which prefer to take up the Fe(II) species. This strategy utilizes a method of Fe uptake first presented by Lindsay and Schwab and later termed "the shuttle effect" by Lucena (Lindsay and Schwab, 1982; Lucena, 2003). The shuttle effect (Fig.4) functions by acidifying the root zone, chelating Fe, and reducing Fe(III) to Fe(II) at the root surface (Hell and Stephan, 2003; Lindsay and Schwab, 1982; Lucena, 2003). Roots release protons which can acidify the root zone by approximately 1 pH unit compared to

the bulk soil, which helps increase the solubility of Fe in soils (Romheld and Marschner, 1984). Organic acids play an important role in chelating Fe, which increases the solubility, mobility and plant availability of Fe (Lindsay and Schwab, 1982). Plants and other soil organisms naturally excrete organic acids such as oxalic and citric acid which can chelate soil Fe (Havlin et al., 1999). The Fe(III)-reductase enzyme is located at the plasmalemma of the root hair and reduces Fe(III) to Fe(II) which allows plant uptake of the Fe(II) species (Hell and Stephan, 2003). There is a linear correlation between Fe(II) uptake and Fe concentration in strategy I plants (Lindsay and Schwab, 1982).

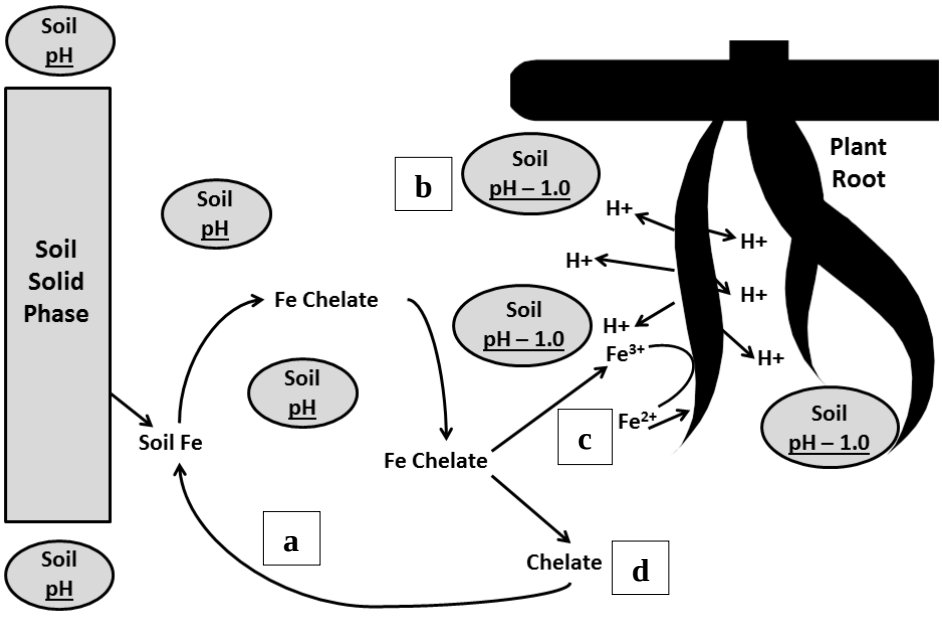

Figure 4. The shuttle effect for Fe uptake of strategy I plants as named by Lucena in 2003 and first proposed by Lindsay and Schawb (1982). a) Organic acids can participate in chelation of soil Fe. b) Roots release protons to acidify rhizosphere and increase solubility of Fe(III) c) Reduction of Fe(III) to Fe(II) occurs by Fe(III)-reductase at root and Fe(II) is taken up by plant root. d) By diffusion, the chelate moves into the rhizosphere where there is an area of lower chelate concentration and another soil Fe can be chelated.

Strategy II plants are graminaceous plants which utilize phytosiderophores (PS) to chelate Fe(III) from soil and use transporter proteins to bring the Fe(III)-PS complex across the

plasmalemma (Romheld and Marschner, 1986). Strategy II plants are similar to strategy I plants in that they also acidify the rhizosphere to increase Fe solubility (Romheld and Marschner, 1984). However strategy I plants usually have lower rhizosphere pH values than strategy II plants (Romheld and Marschner, 1984). The specific PS release mechanism is not completely understood, although release by vesicle transport through the root plasmalemma has been proposed (Negishi et al., 2002). The synthesis and release of PS is up-regulated during Fe deficiency (Romheld and Marschner, 1990). The release of PS and the uptake of Fe-PS complexes has diurnal rhythms, with the first hours of light having the most activity (Romheld and Marschner, 1986). Phytosiderophores also play an important role in the uptake of Zn, Mn, and Cu, however, the transporter proteins have a high specificity for Fe-PS complexes and will preferentially take up Fe-PS compared to other metal-PS complexes (Romheld, 1991).

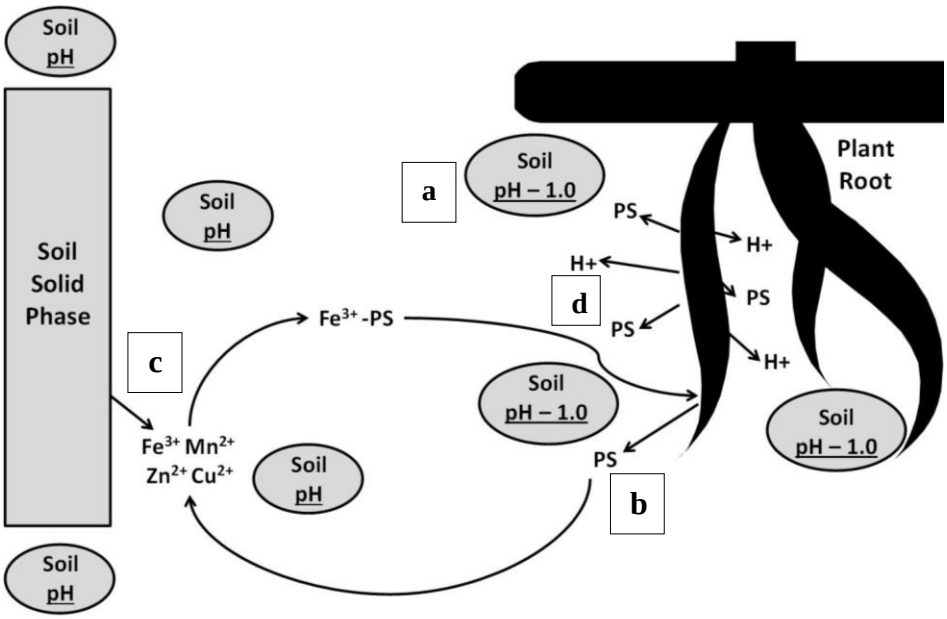

Figure 5. The Fe uptake mechanism for strategy II plants as proposed by Romheld and Marschner (1986). a) The rhizosphere is acidified to increase Fe solubility b) Phytosiderophores (PS) are synthesized and released from the plant root and c) PS complex Fe(III) or Mn(II), Zn(II), and Cu(II) d) Specific transporter proteins allow the Fe-PS to enter the root plasmalemma.

Fe Transport through Plants

Once Fe has entered the root symplast, it must be chelated to protect it from reacting with O_2, which would cause precipitation of the Fe (Hell and Stephan, 2003). Citrate and nicotianamine (NA) are important for chelating and transporting both Fe(II) and Fe(III) throughout the plant (Hell and Stephan, 2003). Also, Fe transport protein (ITP) is important for chelating Fe(III) and transporting Fe and other metals through the phloem to the sink tissues of the plant (Bauer and Hell, 2006; Hell and Stephan, 2003; Kruger et al., 2002).

Iron is transported to sink tissues by a two-step process (Bauer and Hell, 2006): 1) Fe is first transported through the xylem to the older leaf tissue, and 2) it is loaded into the phloem to be transported to the sink tissues (Bauer and Hell, 2006). The process starts when Fe(II) is chelated in the root symplast by NA until it reaches the xylem where Fe(II) is oxidized and chelated again to form a Fe(III)-citrate complex (Hell and Stephan, 2003). Fe(III)-citrate is transported through the xylem to the fully developed leaves and plant tissues (Hell and Stephan, 2003). The amount of Fe transported in the xylem of soybeans is related to the amount of citrate in the xylem sap available to chelate and transport the Fe (Brown and Chaney, 1971). The xylem unloads the Fe(III)-citrate complex at the leaf apoplast where Fe is reduced and chelated by NA prior to entering the leaf symplast where Fe is stored, used in metabolic processes, or transported to the phloem (Hell and Stephan, 2003). Iron will move symplastically through the leaf as an Fe(II)-NA complex to the sieve element where it will be oxidized and chelated by ITP and then loaded into the phloem for transport to the developing tissues of the plant (Hell and Stephan, 2003).

Metabolic Roles of Fe in Plants

Iron is important for redox reactions, electron transport, and enzymatic transformations (Broadley et al., 2012). Iron in plants is generally classified as either heme proteins or Fe-sulfur proteins, but can also be found as a constituent of other enzymes (Broadley et al., 2012). Iron must be either chelated or tightly bound into structures so that is it not allowed to react freely because free Fe(III) or Fe(II) can produce reactive oxygen species such as $OH\bullet$, $O_2\bullet^-$ (Broadley et al., 2012).

Heme proteins include cytochromes, and heme enzymes such as catalase and peroxidase (Broadley et al., 2012). Cytochromes contain a heme Fe-porphyrin complex and are a component of the redox systems in the chloroplasts and the mitochondria (Broadley et al., 1986). Heme enzymes such as catalase and peroxidase are important for detoxifying H_2O_2 (Broadley et al., 2012). Catalase detoxifies H_2O_2 produced during photorespiration and the glycolytic pathway to produce water and O_2 (Broadley et al., 2012). Peroxidases in the chloroplasts detoxify H_2O_2 to produce water (Broadley et al., 2012). Peroxidases are also located in the cell wall where they catalyze the polymerization of phenols to lignin and suberin by using phenolic compounds and H_2O_2 as substrates (Broadley et al., 2012). Catalase and peroxidase are indicators of the Fe-status in the plant (Broadley et al., 2012). Iron-deficient plants have depressed peroxidase activity and, in roots, this results in an accumulation of phenolic compounds and H_2O_2 (Broadley et al., 2012). The release of phenolic compounds into the soil can help chelate Fe and make Fe more available for plant uptake (Broadley et al., 2012).

Iron-sulfur proteins are non-heme proteins that are coordinated to either the thiol group of a cysteine or inorganic S (Broadley et al., 2012). Ferredoxin is important for many metabolic processes because it transmits electrons to plant constituents such as NADP+ in photosynthesis,

nitrite reductase, sulfite reductase, and N_2 reductase (Broadley et al., 2012). Aconitase is an Fe-S protein that catalyzes the polymerization of citrate and isocitrate in the tricarboxylic acid cycle (Broadley et al., 2012). Iron-deficient plants have reduced aconitase activity which results in accumulations of organic acids such as citrate which can be found throughout the plant (Broadley et al., 2012). Citrate is an important Fe-chelate in the plant for mobilizing Fe(III) (Hell and Stephan, 2003).

The isoenzyme of superoxide dismutase (SOD) can also be an Fe-S protein, although SOD can also utilize Cu, Zn, and Mn (Broadley et al., 2012). Iron-SOD is the primary isoenzyme of SOD in the chloroplasts, but can also be found in the mitochondria and peroxisomes. Superoxide dismutase is important for detoxifying $O_2\bullet^-$ and forms H_2O_2 (Broadley et al., 2012). Iron-deficient plants with adequate amounts of Cu and Zn SOD will produce normal concentrations of H_2O_2 (Broadley et al., 2012). Further, peroxidase and catalyze activity is reduced which should increase the amount of oxidative cell damage (Broadley et al., 2012). Iron-deficient plants have been shown to show accumulations of H_2O_2 and $O_2\bullet^-$ (Ranieri et al., 2003; Tewari et al., 2005). However, oxidative damage was not increased (Broadley et al., 2012; Tewari et al., 2005).

Iron is required for synthesis of heme coenzymes and chlorophyll, and often chlorotic leaf tissue is a symptom of Fe-deficient plants (Havlin et al., 1999; Marschner, 1986). Iron deficiency chlorosis affects the size, but not the number of chloroplasts present and also reduces protein synthesis in cells (Marschner, 1986). Iron chlorotic tissue has reduced rates of photosynthesis per unit of leaf area, but not per unit of chlorophyll (Marschner, 1986). The mechanism for photosynthesis is functioning, but there is not enough chlorophyll synthesized to support the plant needs (Marschner, 1986).

Fe Deficiency Chlorosis in Soybean

Factors that Contribute to IDC

Research has shown that soil factors such as CCE, EC, soil pH, soil moisture, DTPA extractable Fe, bicarbonate in soil solution, and soil NO_3^- are correlated to the occurrence and intensity of IDC in soybeans (Bloom et al., 2011; Franzen and Richardson, 2000; Hansen et al., 2004; Inskeep and Bloom, 1984, 1986). The HCO_3^- generated from the following reaction in Eq. [1] negatively impacts the Fe uptake of plants (Havlin et al., 1999, Lucena, 2000, Marschner, 1986).

$$CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + 2HCO_3^- \quad [1]$$

The soil air in a warm, moist, clayey, soil may contain approximately 5% CO_2 compared to the atmosphere which generally only contains 0.035% CO_2 (Schaetzl and Anderson, 2005). Soils with excessive soil moisture along with adequate CO_2 availability can increase the production of HCO_3^- as described by Eq. [1] (Havlin et al., 1999; Inskeep and Bloom, 1986). The greater the surface area of the $CaCO_3$, the more reactive it is in soil solution, and therefore clay-sized $CaCO_3$ participates more effectively in Eq. [1](Inskeep and Bloom, 1986).

Soil pH in the rhizosphere is raised and buffered by HCO_3^- which decreases the solubility of Fe in soil solution (Marschner, 1986). The plant root Fe uptake mechanism is inhibited by HCO_3^- in three ways: 1) the buffering capacity of HCO_3^- impedes the ability of the plant root to acidify the rhizosphere; 2) HCO_3^- inhibits the root's release of phenolic compounds to the rhizosphere; and 3) ferric reductase requires an acidic environment to be active (Kosegarten et al., 2004; Marschner, 1986). Excessive HCO_3^- affects Fe transport from the root to the shoot because CO_2 fixation and organic acid synthesis in the roots increases and Fe chelated by the organic acids may be sequestered in vacuoles in the roots rather than transported throughout the

plant (Marschner, 1986). Root extension is reduced in soil with high HCO_3^- levels which lowers the transpiration rate thereby impeding solute flow through the xylem, and therefore Fe transport to aerial plants parts is negatively impacted (Marschner, 1986). Bicarbonate that is transported to the aerial plant part may also affect the reduction of Fe(III) to Fe(II) in the cytoplasm and can result in uneven distribution of Fe in plant tissues as well as accumulated, unusable Fe in the tissue (Marschner, 1986).

The reason for the correlation between high EC and IDC severity is not well understood (Hansen et al., 2006). However, high EC soils result in lower osmotic potentials complicating plant uptake of water (Marschner, 1986). Lower osmotic potentials are an additional stress for soybeans already suffering from IDC, and this additional stress can enhance the IDC severity (Franzen and Richardson, 2000). The abundance of certain mobile ions in soil solution such as Mg^{2+}, Na^+, and Cl^- have been correlated specifically to chlorophyll content of Fe-deficient soybeans (Inskeep and Bloom, 1984).

The effect of excess soil NO_3^- on IDC is somewhat a point of debate (Bloom et al., 2011). One suggestion is that uptake of NO_3^- causes the roots to release HCO_3^- to maintain charge balance which thereby inhibits Fe uptake (Lucena, 2000). It is also believed that NO_3^- in the plant inhibits Fe transport by changing the leaf apoplastic pH and inhibiting the reduction of Fe(III) to Fe(II) (Kosegarten et al., 2001).

Soil temperatures in calcareous soil that are abnormally cool (12°C) or warm (26°C) give greater chlorosis levels compared to soil temperatures of 16 to 19°C (Inskeep and Bloom, 1986). Soil moisture conditions that are slightly wetter than field capacity can increase IDC severity due to greater HCO_3^- solubility (Inskeep and Bloom, 1986). Bulk density also affects IDC, however, it is debated as to whether increased bulk density enhances or reduces IDC (Hansen et al., 2006).

Areas where bulk density is increased, such as wheel tracks, have shown increased severity of IDC (Hansen et al., 2006). Areas of greater bulk density have decreased gas exchange which inhibits the diffusion of CO_2 away from the rhizosphere and resulting in accumulations of HCO_3^- in the rhizosphere due the reaction in Eq. [1] (Geiger and Loeppert, 1988). However, these same areas can also have more denitrification which reduces soil NO_3^- resulting in less chlorotic soybeans (Bloom et al., 2011). Root hair proliferation is also an important Fe stress response for strategy I plants (Hell and Stephan, 2003), and increased bulk density can restrict root growth (Brady and Weil, 2010).

Iron deficiency chlorosis may be enhanced by either soil or biochemical interactions with other nutrients or micronutrients (Hansen et al., 2006). If K is lacking in the plant, the release of Fe reductants and H^+ ions as well as reduction of Fe(III) at the root decreases which enhances IDC (Hansen et al., 2006). Excessive P in plants has also been shown to enhance IDC (Inskeep and Bloom, 1984). While not completely understood, it is thought that anionic orthophosphate binds with the cationic Fe either in soils or plants and renders the Fe unusable (Hansen et al., 2006). Micronutrient interactions that enhance IDC also exist (Hansen et al., 2006). Iron deficiency has been shown to stimulate accumulations of Mn, Zn, and Cu in shoots and, reversely, Zn and Mn deficiency have caused an accumulation of Fe in shoots (Hansen et al., 2006). Some metals, such as Ni, Cu, and Cd, have been shown to reduce the activity of Fe(III) reductase enzyme thereby impacting the Fe stress response, but other metals such as Mn, Pb, Zn, and Mo do not have an effect on Fe(III) reductase enzyme (Hansen et al., 2006).

It has been observed that Fe-deficient soybeans generally have poor nodulation (Hansen et al., 2003). The nitrogenase enzyme is essential for N_2 fixation to occur (Marschner, 1986). The Fe-stress response in soybeans, or the release of reductants and H^+ ions in Fe-deficient

situations is linked to nitrogenase activity (Terry and Jolley, 1994). Nitrogenase activity can be inhibited as well as the Fe-stress response if soybeans are inoculated with an ineffective strain of *Bradyrhiozbium japonicum* or excessive NO_3^- is applied to the soil (Terry and Jolley, 1994). However, Podrebarac (2011) observed abnormally high accumulations of ureides in field-grown soybeans suffering from IDC which suggests that Fe-deficiency inhibited overall plant growth more than nitrogen fixation.

Management Practices for IDC in Soybean

Managing IDC is complicated due to spatial variability of differing soil characteristics across a given agricultural field, and year to year climate variability (Franzen and Richardson, 2000; Hansen et al., 2006). Soil EC and CCE are more strongly correlated to the occurrence of IDC as compared to soil pH (Franzen and Richardson, 2000). Predicting where IDC may occur has been proposed by utilizing EC (1:1) and CCE soil testing data according to Table 2 (Agvise Laboratories, 2013). The information in Table 2 was derived by sampling 98 fields in MN, ND, and SD with varying levels of IDC and correlating the severity of IDC, or lack thereof, to CCE and EC (1:1) (Agvise Laboratories, 2013). It was found that this classification system would have predicted 81% of the sampled sites with high, very high, or extreme IDC symptoms and also would have predicted 73% of the sites with low to moderate levels of IDC (Agvise Laboratories, 2013).

Table 2. Risk of IDC in soybeans based on salinity (EC 1:1) and $CaCO_3$ equivalency of soil (Agvise Laboratories, 2013).

$CaCO_3$, (%)	----------EC (dS m^{-1})----------			
	<0.25	0.26-0.5	0.51-1.0	>1.0
0-2.5	Low	Low	Moderate	High
2.6-5.0	Moderate	Moderate	High	V.High
>5.1	Moderate	High	V. High	Extreme

Farmers implement IDC management strategies that involve many cultural and fertilizer practices (Hansen et al., 2006). These practices attempt to increase the solubility of Fe in soils or aid the plant in uptake of Fe (Hansen et al., 2006). These practices might include selection of Fe-efficient cultivars, increasing seeding rates, inoculating with proper strains of *Bradyrhizobium japonicum*, planting where soil NO_3^- levels are low, and utilizing either foliar applied or soil-applied Fe fertilizers (Goos and Johnson, 2000, 2001; Hansen et al., 2006; Wiersma, 2005, 2010).

Planting Fe-efficient soybean cultivars in fields where IDC is deemed to be problematic is the most effective IDC management strategy (Goos and Johnson, 2000, 2001). Field screening of soybean cultivars continues to be important for providing information to farmers about IDC cultivar tolerance (Goos and Johnson, 2010; Helms et al., 2010). Generally, a 1-5 visual chlorosis scoring method is used to determine the severity of the chlorosis (Froehlich and Fehr, 1981; Goos and Johnson, 2010). However, the visual chlorosis score is not an indicator of yield, and non-IDC-tolerant cultivars often have greater yield potential in non-IDC field conditions (Helms et al., 2010).

Increased seeding rates can help manage IDC in soybean (Goos and Johnson, 2001). Increased seeding rates either enhance root development or concentrate the Fe-stress responses (Hansen et al., 2006), or are better for removing water and improving aeration (Ferguson et al., 2006). The optimal seeding rate to manage IDC is not yet known (Goos and Johnson, 2001). However, Ferguson et al. suggested to plant 12 seeds per 30 cm of row, regardless of the row spacing (2006).

The nitrogenase enzyme is important for N_2 fixation (Marschner, 1986), and also important for inducing the Fe-stress response (Terry and Jolley, 1994). Applying N fertilizers

reduces nodulation and, even when Fe-efficient cultivars are planted, grain yields can be reduced in the presence of IDC (Wiersma, 2010). Likewise, inoculating with ineffective strains of *Bradyrhizobium japonicum* can also reduce nitrogenase activity which can also negatively impact Fe uptake mechanism (Terry and Jolley, 1994).

Greenhouse experiments where IDC was induced on soybeans have shown that foliar applications of $FeSO_4$ or an Fe-chelate have been effective for increasing chlorophyll content and recovering damaged chloroplasts (Hecht-Buchholz and Ortmann, 1986). However, chloroplasts were recovered more completely when Fe was supplied through the roots (Hecht-Buchholz and Ortmann, 1986). Foliar applications of Fe in field experiments have been met with mixed results and are not currently a recommended practice (Franzen et al., 2003; Lingenfelser et al., 2005). Even though foliar applications have not always been the most effective for controlling IDC, there have been some responses in the field suggesting further research is necessary (Goos and Johnson, 2000).

Soil-applied Fe-chelates may be effective for managing IDC in soybean (Goos et al., 2004; Goos and Germain, 2001; Lucena, 2003; Wiersma, 2005). Soil-applied Fe-chelate fertilizers must participate in Lucena's shuttle effect (Fig. 4) by: 1) being able to maintain Fe in soil solution; 2) allowing the plant to take the Fe from the chelate; and 3) being able to regenerate itself by absorbing another Fe from the solid phase (Lucena, 2003). Research shows that FeEDDHA is a Fe-chelate fertilizer that participates effectively in the shuttle effect (Lucena, 2003) and local research suggests that applications of FeEDDHA may be beneficial in managing IDC (Goos and Germain, 2001; Wiersma, 2005).

Definition of a Chelate

A chelate is an organic compound that forms complexes with metal ions which increases the solubility of the ions in soil solution thereby increasing plant availability (Havlin et al., 1999). Chelation is achieved when a metal ion shares electron pairs with a negatively charged ligand and a heterocyclic ring is formed (Lindsay, 1979; Tan, 1998). By comparison, a true complex only shares one bond between the metal ion and the anionic ligand acting as the electron donor (Lindsay, 1979). Thus, the word "chelate" is derived from the Greek word for "claw" (Havlin et al., 1999; Tan, 1998).

The ability of a chelate to maintain the metal-ligand bond is described by the formation constant (Lindsay, 1979). The higher the formation constant, or Log K value, the greater the stability of the metal-ligand bond (Lindsay, 1979). For example, Eq. [2] describes the formation constant for Fe(III)EDDHA (Lindsay, 1979).

$$Fe(III) + EDDHA \leftrightarrow Fe(III)EDDHA \qquad Log\ K=35.40 \qquad [2]$$

Where:

$$K_{Fe(III)EDDHA}=\frac{[Fe(III)EDDHA]}{[Fe(III)]\ [EDDHA]} \qquad [3]$$

Table 3 displays different formation constants for some metal chelates according to Lindsay (1979).

Table 3. Formation constants (Log K values) for some metal chelates (Lindsay, 1979).

	EDTA†	DTPA‡	EDDHA§
Reaction	---------- Log K ----------		
Fe(III) + L ↔Fe(III)L	26.50	29.19	35.40
Ca + L ↔CaL	11.61	12.02	8.20
Mg + L ↔MgL	9.83	10.61	9.00

† ethylenediaminetetraacetic acid
‡ diethylene triamine pentaacetic acid
§ ethylene diamine di(hydroxyl phenyl acetic acid)

FeEDDHA as a Soil-Applied Fertilizer

History, Synthesis, and Chemistry of FeEDDHA

FeEDDHA has shown potential as a soil-applied fertilizer for controlling IDC in soybean on soils found in the midwest states of the United States (Goos and Germain, 2001; Goos and Johnson, 2001; Lingenfelser et al., 2005; Wiersma, 2005, 2007). Low rates of FeEDDHA were found to be less effective compared to greater rates (Goos and Johnson, 2000, 2001; Wiersma, 2005, 2007). The Fe in FeEDDHA is coordinated by two bonds with phenolate groups, two bonds with amine groups, and two bonds with carboxylate groups (Lucena, 2003). The phenolate bonds in the o,o-FeEDDHA isomer is what contributes to FeEDDHA's effectiveness in soil as well as the fertilizer's dark red color (Goemz-Gallego et al., 2002). Also, EDDHA has a high affinity for Fe^{3+} in comparison to other ions such as Ca^{2+} and Mg^{2+} as described by their formation constants (Table 3). After Fe has been delivered to the plant, the EDDHA can regenerate itself by chelating more Fe from Fe-(hydr)oxides in soil (Kroll, 1957; Lucena, 2003). Research from Europe has shown that efficacy of soil-applied FeEDDHA is affected by its variable isomer concentration, which is determined by conditions during the synthesis processes (Hernandez-Apaolaza et al., 2000, 2006; Kroll et al., 1957; Lucena, 2003; Petree et al., 1978; Rojas et al., 2008; Schenkeveld, 2010).

FeEDDHA was first synthesized in 1957 in a process requiring hydrogen cyanide (Kroll et al., 1957). Later, other FeEDDHA synthesis methods were developed that used phenol as a solvent and as a reactant mixed with ethylenediamine and glyoxylic acid (Petree et al., 1978). Commercial FeEDDHA fertilizers have variable concentrations of FeEDDHA polycondensates, half products and regioisomers which include the racemic o,o-FeEDDHA, meso o,o-FeEDDHA, ortho, para FeEDDHA (o,p-FeEDDHA), and para,para FeEDDHA (p,p-FeEDDHA) due to

different synthesis conditions with the new synthesis method (Gomez-Gallego et al., 2002; Hernandez-Apaolaza et al., 1997, 2006; Petree et al., 1978; Schenkeveld, 2010; Yunta 2003a, 2003b).

The regioisomers have different formation constants which describe their efficacy as a soil-applied Fe fertilizer (Table 4) (Hernandez-Apaolaza et al., 2006; Schenkeveld, 2010; Yunta, 2003a, 2003b). The polycondensates are similar in structure to FeEDDHA but vary by isomerization and attached functional groups, which cause variation in their molecular weights and formation constants, and thus are unpredictable in their efficacy as a soil-applied Fe fertilizer (Hernandez-Apaolaza et al., 2006; Schenkeveld, 2010; Yunta 2003a, 2003b). The para position of the hydroxyl group is sterically inhibited from binding to Fe, and therefore the p,p-EDDHA isomer does not contribute to the efficacy of FeEDDHA as a soil-applied fertilizer (Hernandez-Apaolaza et al., 2006; Schenkeveld, 2010; Yunta et al., 2003a, 2003b). Previous research has categorized FeEDDHA polycondensates, half-products and p,p-FeEDDHA into one category due to the unpredictability or lack of contribution as a soil-applied Fe-chelate fertilizer (Schenkeveld, 2010).

Table 4. Formation constants of different FeEDDHA regioisomers. (Yunta et al., 2003a, 2003b).

Regioisomer	Log K
racemic o,o-FeEDDHA	35.86
meso o,o-FeEDDHA	34.15
o,p-FeEDDHA	28.72

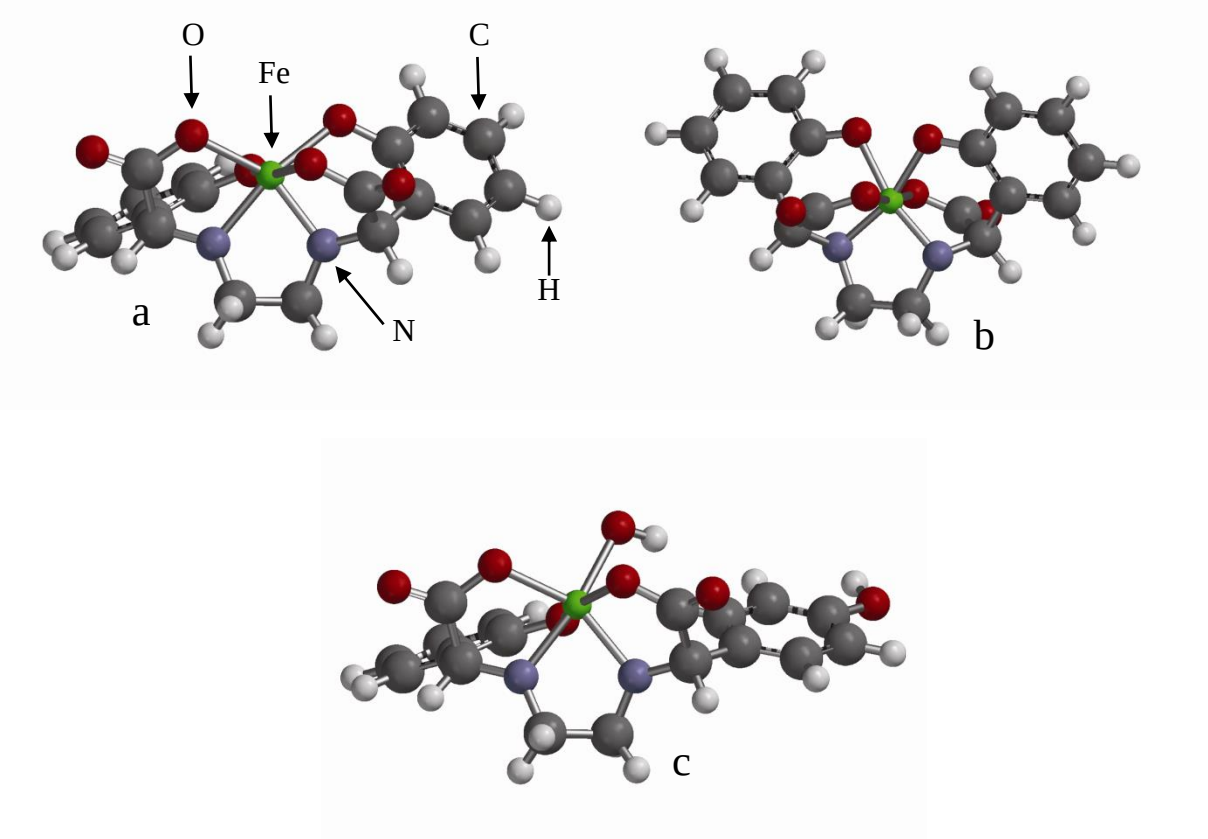

Figure 6. The molecular structure of a) meso o,o-FeEDDHA b) racemic o,o-FeEDDHA and c) o,p-FeEDDHA (Schenkeveld, 2010, reprinted with permission).

The most effective FeEDDHA components for use as a soil-applied Fe chelate are meso (Fig. 6a) and racemic (Fig. 6b) o,o-FeEDDHA diastereomers, and to a much lesser extent o,p-FeEDDHA (Fig. 6c) (Table 4) (Hernandez-Apaolaza et al., 2006; Schenkeveld, 2010; Yunta et al., 2003a, 2003b). The o,o-FeEDDHA diastereomers are generally produced in a 1:1 ratio upon chelation with Fe (Schenkeveld, 2010; Yunta 2003a).

Soil FeEDDHA Interactions

The success of FeEDDHA to participate in the shuttle effect is dependent upon overcoming such factors as excessive rainfall leading to leaching out of the root zone (Rombola and Tagliavini, 2006) and evapotranspiration causing upward movement of FeEDDHA to the soil surface (Schenkeveld, 2010). The efficacy of FeEDDHA may also be affected by the

adsorption of FeEDDHA regioisomers to various soil constituents (Alvarez-Fernandez et al., 1997, 2002; Gil-Ortiz and Bautista-Carrascosa, 2004; Hernandez-Apaolaza and Lucena, 2001; Schenkeveld, 2010) as well as Fe displacement due to competition from other ions like Cu, Zn, Mn, Ni, and Co (Bermudez et al., 1999; Juarez et al., 2001; Schenkeveld, 2010). FeEDDHA is stable over a wide range of soil pH values (Lindsay, 1979). However, FeEDDHA decomposition can occur below pH 6 and is particularly significant below pH 2 (Bermudez et al., 1999, 2002; Wallace et al., 1967). FeEDDHA is subject to photodegradation which is dependent upon the solution concentration of FeEDDHA and the length of time exposed to light (Hernandez-Apaolaza and Lucena, 2011; Wallace et al., 1967).

The efficacy of soil-applied FeEDDHA is affected by each regioisomer reacting with different soil constituents (Alverez-Fernandez et al., 1997, 2002; Cantera et al., 2002; Cerdan et al., 2007; Gil-Ortiz and Bautista-Carrascosa, 2004; Hernandez-Apaolaza and Lucena, 2001; Rojas et al., 2008; Schenkeveld, 2010; Siebner-Freibach et al., 2004). Racemic o,o-FeEDDHA is thought to be adsorbed via cationic bridging to soil organic matter, although the overall sorption is small and the kinetics are relatively slow (Alvarez-Fernandez et al., 1997, 2002; Hernandez-Apaolaza and Lucena, 2001; Schenkeveld, 2010). Meso o,o-FeEDDHA is adsorbed to reactive Fe-(hydr)oxides due to their anion exchange capacity below the point of zero charge (Alvarez-Fernandez et al., 1997, 2002; Hernandez-Apaolaza and Lucena, 2001; Schenkeveld, 2010). The adsorption of the o,p-FeEDDHA isomer is related to clay content of a soil which may be due to cationic bridging or competition from ions such as Cu^{2+} (Gil-Ortiz and Bautista-Carrascosa, 2004; Schenkeveld, 2010). Due to varying composition of the fourth category of FeEDDHA, which includes para, para FeEDDHA, FeEDDHA polycondensates and half-

products, it is complicated to predict their sorption behavior with soil constituents (Schenkeveld, 2010).

Impact of FeEDDHA Applications to Mn Uptake

Applications of FeEDDHA reduce Mn uptake and concentration in strategy I plants (Moraghan and Freeman, 1978; Moraghan, 1979; Schenkeveld, 2010; Wallace and Alexander, 1973; Wikoff and Moraghan, 1986). Applications of FeEDDHA have minimally increased the concentrations of Mn in the soil solution (Schenkveld, 2010). The addition of the o,o-EDDHA ligand to soil can increase the concentration of Mn in soil solution, but this increase generally only lasts for about 3 d due to sorption onto clays (Schenkeveld, 2010).

FeEDDHA has been used successfully for alleviating Mn toxicity in flax (Moraghan and Freeman, 1978; Moraghan, 1979). In these studies, Mn accumulations in the plant had a negative relationship with the Fe concentration in soil solution compared to a poor relationship with DTPA-extractable Mn (Moraghan, 1979). Increased Fe supply to roots has been shown to negatively impact the Mn uptake as well as the translocation of Mn throughout soybean plants (Heenan and Campbell, 1983).

Commercial FeEDDHA Fertilizers

The European Commission and the European Free Trade Association has developed a testing method to determine quality of FeEDDHA for agricultural use which has become a national standard for Europe (Technical Committee CEN/TC 260, 2011). High performance liquid chromatography (HPLC) is used to determine the concentrations of the different regioisomers contained in commercially available FeEDDHA fertilizers (Technical Committee CEN/TC 260, 2011). Consumer protection, accuracy of labeling, and environmental protection with regards to fertilizers and fertilizer use in the United States is primarily governed by the

individual states (The Fertilizer Institute, 2012). The intention of the North Dakota Fertilizer and Soil Conditioner Law is to protect consumer interests ensuring that labeling accurately communicates product composition and concentrations of essential ingredients (North Dakota Department of Agriculture, 2010). The North Dakota Fertilizer and Soil Conditioner Law can require proof of effectiveness to verify market claims (North Dakota Legislative Council, 2012). However, North Dakota fertilizer labels are only required to state the net weight of the product, the guaranteed analysis of each plant nutrient the product contains, and the name and address of the distributor (North Dakota Legislative Council, 2012). The efficacy of soil-applied FeEDDHA fertilizers are contingent upon the o,o-FeEDDHA concentration and not necessarily the percent Fe in the product (Lucena, 2003). Commercial FeEDDHA products displaying the o,o-FeEDDHA concentration in addition to the total Fe concentration may more accurately describe the quality as a soil-applied fertilizer (Hernandez-Apaolaza et al., 2000).

Alternate Testing Methods to HPLC

Implementing a simple testing method to determine the efficacy of soil-applied FeEDDHA would be beneficial to the North Dakota market since the HPLC test is difficult to conduct and no laboratory in the region offers this test commercially. Others have explored the possibility of soil stability tests for evaluating the quality of commercial Fe fertilizers (Goos and Germain, 2001; Lucena et al., 1992; Orphanos and Hadjiloucas, 1984; Alvarez-Fernandez et al., 1997).

Previous FeEDDHA soil stability testing methods have utilized incubations with soil from agricultural fields (Goos and Germain, 2001; Orphanos and Hadijloucas, 1984) as well as amorphous Fe(III) oxide, acid peat, Ca-montmorillonite, and $CaCO_3$ (Alvarez-Fernandez et al., 1997). Incubation methods with agricultural soils have utilized an approximate 30 to 50 d

incubation period (Goos and Germain, 2001; Orphanos and Hadijloucas, 1984) and results suggest that a 25-30 d reaction would be sufficient (Orphanos and Hadijloucas, 1984). However, incubation with amorphous Fe(III) oxide, acid peat, Ca-montmorillonite, $CaCO_3$ and a laboratory created soil suggested that a 3 d incubation period was sufficient (Alverez-Fernandez et al., 1997). These methods were analyzed by either atomic absorption only, or with a combination of atomic absorption and spectroscopy (Alverez-Fernandez et al., 1997).

Competition between DTPA and Fe chelates was also explored as a way to determine Fe-chelate stability (Lucena et al., 1992). This testing method expedited results since it only requires 1 h (Lucena et al, 1992). However, this testing method is dependent upon constant temperature and precise reaction times (Lucena et al., 1992). This method was analyzed by both atomic absorption as well as spectroscopy (Lucena et al., 1992).

The above incubation methods have been used to determine soil-stable Fe from other Fe chelates in addition to FeEDDHA (Alverez-Fernandez et al., 1997; Goos and Germain, 2001; Lucena et al., 1992; Orphanos and Hadijloucas, 1984). However, exploring the possibility of a soil stability test to determine differences in various FeEDDHA products was not explored and was not compared to the European Union Standard HPLC testing method to determine o,o-FeEDDHA concentration (Alverez-Fernandez et al., 1997; Goos and Germain, 2001; Lucena et al., 1992; Orphanos and Hadijloucas, 1984). These previous research results suggest that a simple soil incubation testing method could be developed to determine the differences in quality of soil-applied FeEDDHA fertilizers.

Research Objective

Iron deficiency chlorosis is an economic problem that negatively impacts soybean production in the northern plains of the United States. The dynamics of soil solution chemistry

of Fe and the plant Fe uptake and translocation mechanisms make this a complicated deficiency to manage. FeEDDHA as a soil-applied Fe fertilizer can help manage IDC in addition to other management strategies. The variable quality of commercial FeEDDHA fertilizers is not well understood in the United States and currently there is no standardized testing method in the United States to determine quality of commercial FeEDDHA products.

The objectives of this research are to: 1) determine if the quality of soil-applied FeEDDHA fertilizers is dependent on the o,o-FeEDDHA concentration; and 2) develop a simple colorimetric method to compare the quality of commercial FeEDDHA fertilizers. Two different experiments were conducted to achieve this objective. The first experiment evaluated the effect of o,o-FeEDDHA in a greenhouse pot trial. The second experiment was executed in the laboratory where different FeEDDHA fertilizers were incubated with soil and the percent soil-stable FeEDDHA and Fe were determined.

MATERIALS AND METHODS

Fe Uptake Differences Due to o,o-FeEDDHA Concentration

Experimental Overview

The effect of the o,o-FeEDDHA isomer concentration in FeEDDHA fertilizers to manage IDC in soybean was determined. A greenhouse experiment was conducted and comprised of two soybean crops where FeEDDHA fertilizers were added at the same rate of FeEDDHA per pot. The severity of IDC was quantified by determining relative chlorophyll content of leaves, as well as the dry matter yield, plant tissue Fe concentration, and Fe uptake of the plants. The impact of FeEDDHA applications on plant tissue Mn concentration and Mn uptake of plants was also determined.

FeEDDHA Treatments

Nine different FeEDDHA products were applied at two different rates and the control treatment had no FeEDDHA applied (Table 5). When the first greenhouse crop (GH1) was harvested, the soybeans did not appear chlorotic. Therefore, a second greenhouse crop (GH2) was planted into the same pots with the same soil and no additional FeEDDHA. All FeEDDHA sources contained 6% Fe (Table 5). Six of the nine fertilizers evaluated were of known o,o-FeEDDHA concentrations analyzed at JAER Laboratories (Barcelona, Spain) by ion-pair chromatography determined by HPLC according the European Standard, EN 13368-2 (Technical Committee CEN/TC 260, 2007). Three of the FeEDDHA sources were commercial fertilizers of unknown o,o-FeEDDHA concentration and were either commercially available, or were under consideration for commercial release in the U.S. fertilizer market.

Table 5. FeEDDHA treatments evaluated in two consecutive greenhouse studies. All fertilizers contained 6% Fe and were applied at 2 different rates.

FeEDDHA Treatments	Total Fe (%)	o,o-FeEDDHA (%)†	Rate applied (mg pot^{-1})
Control	-	-	-
FeEDDHA-1	6%	1.5%	10
FeEDDHA-1	6%	1.5%	20
FeEDDHA-2	6%	2.5%	10
FeEDDHA-2	6%	2.5%	20
FeEDDHA-3	6%	3.0%	10
FeEDDHA-3	6%	3.0%	20
FeEDDHA-4	6%	4.2%	10
FeEDDHA-4	6%	4.2%	20
FeEDDHA-5	6%	4.8%	10
FeEDDHA-5	6%	4.8%	20
FeEDDHA-6	6%	5.5%	10
FeEDDHA-6	6%	5.5%	20
Commercial-1	6%	Unknown	10
Commercial-1	6%	Unknown	20
Commercial-2	6%	Unknown	10
Commerical-2	6%	Unknown	20
Commercial-3	6%	Unknown	10
Commercial-3	6%	Unknown	20

† o,o-FeEDDHA concentration determined at JAER Laboratories, Barcelona, Spain.

Soils

Topsoil from the Glyndon series (coarse-silty, mixed, superactive, frigid Aeric Calciaquolls) was used for both greenhouse crops (Table 6). The soil properties were analyzed at the North Dakota State University soil testing laboratory in Fargo, ND (Table 6). The Glyndon series is calcareous (Soil Survey Staff, 2005), which is known to be problematic for soybean production due to IDC (Inskeep and Bloom, 1986).

Table 6. Soil properties for the Glyndon series used in two greenhouse experiments to evaluate FeEDDHA fertilizers. Soil tested at North Dakota State University, Fargo, ND.

Soil Properties	Units	Results
pH †		8.2
EC ‡	dS m^{-1}	0.34
$CaCO_3$ equiv. §	g kg^{-1}	61
SOM¶	g kg^{-1}	33
Olsen P#	mg kg^{-1}	20
Extract. K††	mg kg^{-1}	45
Extract. Fe‡‡	mg kg^{-1}	3.8
Nitrate-N§§	mg kg^{-1}	20
Sulfate-S¶¶	mg kg^{-1}	19.5
CEC##	$cmol_c$ kg^{-1}	24.3
Soil texture		Loam

† 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 weight 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)
¶¶ Extracted with 500 mg L^{-1} P as $Ca(H_2PO_4)_2$ (Combs et al., 1998)
Cation exchange capacity by summation (Warncke and Brown, 1998)

FeEDDHA Solution Nutrients

Basal nutrient solutions were applied equally to all pots. Approximately 1.2 g of each FeEDDHA fertilizer was dried in an oven for 24 h at 60°C. After drying, 1 g of the dried fertilizer was transferred to a 100 mL volumetric flask containing approximately 50 mL of deionized (DI) water. The fertilizer was dissolved and brought to the 100 mL volume. The 10

mg and 20 mg application rates were achieved by applying 1 mL and 2 mL, respectively, of the prepared FeEDDHA solutions per pot, as described in a later section.

Fertilizer Basal Preparation

Greenhouse Crop 1

The N basal treatment was prepared by combining 76.22 g of NH_4NO_3 in 2 L of DI water. The P basal treatment was prepared by combining 112.36 g of K_2HPO_4 in 1 L of DI water. The micronutrient basal treatment was prepared by combining 4.40 g of $ZnSO_4 \cdot 7H_2O$, 3.80 g of $MnSO_4 \cdot H_2O$, and 2.51 g of $CuSO_4$ in 1 L of DI water. All basal treatments were applied in 5 mL dose each during pot preparation and supplied 66.7 mg N, 100 mg P, 252 mg K, and 5 mg each of Zn, Mn and Cu per pot. Two subsequent applications for the N basal treatment were applied so that total N applied was 200 mg pot^{-1}.

Greenhouse Crop 2

The N basal treatment was prepared by mixing 144.4 g KNO_3 in 2 L of DI water and the P basal treatment was prepared by mixing 28.1 g K_2HPO_4 in 1 L of DI water. Two separate 5 mL applications of the N basal treatment were made to supply a total N application of 100 mg N per pot. The P basal treatment was applied in one 5 mL application, which supplied 25 mg of P per pot. The combination of the basal treatments supplied 139 mg K per pot.

Greenhouse Pot Trials

Greenhouse Crop 1

Plastic liner bags were filled with 1000 g medium white silica sand (20-40 mesh, TCC Materials, West Fargo, ND). Each fertilizer basal treatment at a rate of 5 mL as well as 1 mL or 2 mL of each FeEDDHA treatment was mixed with the sand before mixing with the soil. The control treatment had fertilizer basal treatments applied, but no FeEDDHA. The sand, fertilizer

basal treatments and FeEDDHA treatments were mixed thoroughly to achieve equal distribution of the fertilizers.

The soil was inoculated with *Bradyrhizobium joponicum* bacteria by adding 15 g of peat based inoculant (Nitrastik-S, NSS 45, EMD, Milwaukee, WI) to 1.5 kg of soil. The soil and inoculant were mixed well and 10 g of the inoculated soil were added to the sand. Then, 990 g of non-inoculated Glyndon soil were added to the sand and the bag contents were mixed thoroughly. The bags were placed in 2.5 L plastic pots (part number T60785CP, Berry Plastics, Evansville, IN).

The soybean cultivar used in this experiment was 'NuTech 0886'. The seeds were soaked for approximately 16 h in a 0.01 M $Ca(NO_3)_2$ solution prior to planting to promote even germination. Each pot was planted with 8 soybean seeds to a 0.6 cm depth, and watered with 350 mL of DI water. The pots were watered daily to a gravimetric water content of 20%, and rotated within their replicate. Replicates were also rotated daily.

The second application of 5 mL of N basal treatment was applied 4 d after planting when the soybeans emerged, and the third 5 mL N basal treatment was applied 2 d after the second N basal treatment application. Both N basal treatment applications were applied to the soil surface, and watered into the soil with DI water so that 20% gravimetric water content was maintained. The soybeans were thinned to 4 plants per pot when the growing points were visible 7 d after planting.

Greenhouse Crop 2

The second greenhouse crop was planted into the same pots and used the same soil from the first greenhouse experiment. No additional FeEDDHA was added to the pot. The pots were prepared by removing the GH1 stems and roots from the pots. The first application of the N

basal treatment and the P basal treatment were applied to the soil and mixed thoroughly into the soil. Each pot was planted with 8 soybean seeds of the same cultivar as the first experiment. The pots were watered with approximately 250 mL of DI water at planting. The pots were watered daily to a gravimetric water content of 20%, and rotated within their replicate. Replicates were also rotated daily. The second N basal treatment was applied 5 d after planting and was watered into the pots with DI water so that 20% gravimetric water content was maintained. The pots were thinned to 4 plants 8 d after planting when the growing points were clearly visible.

Evaluation

Both greenhouse studies were evaluated with a Soil and Plant Analyzer Development (SPAD) meter (Minolta SPAD-502, Osaka, Japan). The SPAD readings were taken when the unifoliate, first, second, and third trifoliolate leaves were fully open. SPAD evaluations are an accepted method to evaluate chlorophyll content for the purposes of IDC research (Goos et al., 2004; Rodriguez-Lucena et al., 2010a, 2010b). Two readings were taken on the unifoliate and three readings were obtained from the trifoliolate leaves. The readings were taken between leaf veins. The higher the SPAD values the greater the chlorophyll content of the leaf. Generally, SPAD values of greater than 30 indicate non-chlorotic plant tissue, while SPAD values less than 15 indicate severely chlorotic tissue (Goos et al., 2004).

The soybeans were clipped above the cotyledon after the third trifoliolate stage, washed in DI water (Moraghan, 1991), and placed in #6 paper bags. The bags were placed in a drying oven for 48 h at 65°C. The plant material was weighed and ground (Thomas Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ) to pass a 2 mm screen. The grinder was initially

cleaned by grinding 4 batches of 10 sheets of Whatman grade number 40 ashless filter paper. The grinder was cleaned between treatments by vacuuming.

Plant Analysis Methods

The dried and ground soybean plants were analyzed at Agvise Laboratories in Northwood, North Dakota. The plant materials were analyzed for Fe and Mn concentrations by an open vessel NO_3+H_2O_2 plant digest and concentrations determined using Inductively Coupled Plasma-Optical Emission Spectroscopy according to the procedure used at the Soil and Plant Analysis Laboratory at University of Wisconsin, Madison (University of Wisconsin, 2005).

Statistical Analysis

In order to determine if there was a statistical difference between the 10 and 20 mg pot^{-1} rates, t-tests were performed to compare the mean of the 10 mg pots to the mean of the 20 mg pots for the following measures: SPAD readings, dry matter yield, Fe concentration, Fe uptake, Mn concentration, and Mn uptake. The t-tests comparisons were conducted using SAS 9.3 for Windows (SAS Inc., Cary, NC). All means for all parameters measured were found to be significantly different, except the sum of Mn uptake for GH1 and GH2. Therefore, the 10 and 20 mg pot^{-1} rates were determined to be significantly different for all parameters except the sum of the Mn uptake for GH1 and GH2.

Each parameter for each rate, except the sum of the Mn uptake for GH1 and GH2, was then analyzed by one-way analysis of variance (ANOVA) by the GLM procedure within SAS 9.3. The means between FeEDDHA rates for the sum of the Mn uptake of GH1 and GH2 were not different as determined by t-tests, and therefore one combined ANOVA was conducted for both rates. If the F-test values were determined to be significant, differences between the means were determined by least significant differences (LSD) at α=0.05 probability.

The o,o-FeEDDHA concentration applied per pot was significantly correlated to total Fe uptake, dry matter accumulation, and Mn uptake. Correlation coeffiecients (r) and regression equations were determined by linear regression for the 10 mg and the 20 mg rate separately. The regression equations and the r values were determined using Sigma Plot 10.0 (Systat Software, Inc., Chicago, IL).

A Colorimetric Method to Compare the Quality of Commercial FeEDDHA Fertilizers

Experimental Overview

The ability of commercial FeEDDHA fertilizers to maintain Fe availability in soil was determined. Solutions of FeEDDHA were incubated with soil using two incubation methods. The two incubation methods were a shaking and a field capacity method without shaking. The experiment evaluated nine FeEDDHA fertilizers. Each incubation method used three soils and evaluated four incubation times. Two different chemical analysis methods were used to analyze the Fe content in the extracts: 1) direct spectroscopy for the FeEDDHA chromophore at 480 nm; 2) ferrozine chromophore analysis for total soluble Fe at 560 nm. The percent soil-stable Fe determined from the incubation and analysis methods were correlated to the o,o-FeEDDHA concentration determined by HPLC. The effect of different soils on the percent soil-stable Fe in the extracts was determined by correlating the percent soil-stable Fe to the o,o-FeEDDHA concentration. The proper incubation time was determined by the time it took the average percent soil-stable Fe to reach the average o,o-FeEDDHA concentration.

Soils

Topsoil from a Glyndon series, Bearden series (Fine-silty, mixed, superactive, frigid Aeric Calciaquolls), and Renshaw series (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, frigid Calcic Hapludolls) were collected from agricultural fields in North Dakota

located near Hunter, Prosper, and Streeter, respectively. The soils were collected from the 0-15 cm depth, air-dried, crushed to pass a 2 mm screen. The soil properties were analyzed at North Dakota State University Soil Testing Laboratory, Fargo, ND and are described in Table 7.

Table 7. Soil properties for three different soils used for testing quality of FeEDDHA fertilizers. Soil samples tested at North Dakota State University Soil Testing Laboratory, Fargo, ND.

		Location		
Soil properties	Units	Hunter	Prosper	Streeter
pH†		7.4	8.3	8.1
EC‡	dS m^{-1}	0.36	1.41	0.19
$CaCO_3$ Equiv.§	g kg^{-1}	72	33	0.05
SOM¶	g kg^{-1}	31	33	21
Olsen P#	mg kg^{-1}	14	33	4
Extract. K††	mg kg^{-1}	40	285	95
Extract. Fe‡‡	mg kg^{-1}	4.4	5.6	15.8
Nitrate-N§§	mg kg^{-1}	16.5	6	4
Sulfate-S¶¶	mg kg^{-1}	18	159	3
CEC##	$cmol_c$ kg^{-1}	21.3	29.1	7.6
Soil Texture		loam	silty clay loam	loam
Soil Series		Glyndon	Bearden	Renshaw

† 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)
¶¶ Extracted with 500 mg L^{-1} P as $Ca(H_2PO_4)_2$ (Combs et al., 1998)
Cation exchange capacity determined by summation (Warncke and Brown, 1998)

FeEDDHA Solutions

Nine different FeEDDHA sources and a control treatment (Table 8) were evaluated by two incubation methods. The control treatment did not include a Fe fertilizer source. All FeEDDHA sources were commercial products sold as 6% Fe products (Table 8). Six of the nine

fertilizers (FeEDDHA-1 through FeEDDHA-6) were obtained from Europe and the percent Fe as o,o-FeEDDHA determined by JAER Laboratories (Barcelona, Spain) using ion-pair chromatography detected using HPLC according to the European Standard, EN 13368-2 (Technical Committee CEN/TC 260, 2007). Three of the FeEDDHA sources are commercial fertilizers of unknown o,o-FeEDDHA concentration and were either commercially available, or being considered for commercial release in the U.S. fertilizer market.

Table 8. Six FeEDDHA treatments of different o,o-FeEDDHA concentrations and three commercial FeEDDHA fertilizers of unknown o,o-FeEDDHA concentration and their preliminary solution pH. These FeEDDHA fertilizers were tested with two incubation methods, on three soils, and analyzed with two different analysis methods.

Product	Total Fe (%)	o,o-FeEDDHA (%)†	Preliminary solution pH‡
Control	-	-	-
FeEDDHA-1	6%	1.5%	6.8
FeEDDHA-2	6%	2.5%	7.6
FeEDDHA-3	6%	3.0%	7.3
FeEDDHA-4	6%	4.2%	7.4
FeEDDHA-5	6%	4.8%	7.8
FeEDDHA-6	6%	5.5%	8.6
Commercial-1	6%	Unknown	8.5
Commercial-2	6%	Unknown	8.0
Commercial-3	6%	Unknown	7.1

† o,o-FeEDDHA concentration determined at JAER Laboratories, Barcelona, Spain.
‡ Preliminary solution pH determined in laboratory prior to fertilizer solution preparation

Fertilizer Solution Preparation

The water content of each FeEDDHA material was determined by weighing 1 g on an analytical scale (Mettler AE 160, Columbus, OH), and determining weight loss upon drying at 105°C for 24 h. A preliminary fertilizer solution pH was made to determine if any products

produced acidic solutions. The pH determinations were made by dissolving 2 g of each FeEDDHA material in a beaker containing 500 mL of DI water (Table 7).

Using an analytical scale, 2 g (undried weight) of each FeEDDHA source was weighed, transferred to a 1 L volumetric flask, dissolved in DI water, and made to 1 L volume. Only fertilizer product FeEDDHA-1 produced a solution with a pH value less than 7 (Table 8), and the pH was adjusted to pH 7 using saturated $(Na)_2CO_3$ solution before bringing to volume. The solutions were stored in a dark refrigerator at 4°C.

Reagents and Other Solutions

The color developing solution for ferrozine chromophore analysis was prepared in a 100 mL volumetric flask by dissolving 0.1 g ferrozine, 0.5 g hydroxylamine HCl in approximately 20 mL of DI water, adding 20 mL 1 M HCl, and bringing to the 100 mL volume with DI water (Stookey, 1970). The color developing solution was made fresh daily. The buffer solution for the ferrozine chromophore analysis was prepared by diluting 800 mL of 1 M ammonium acetate at pH 7 to a volume of 2 L. The buffer solution was stored in 2 L glass jar and was refrigerated at 4°C between uses. The 0.015 M $CaCl_2$ extraction solution was prepared by dissolving 4.42 g of calcium chloride dihydrate in DI water and brought to a volume of 2 L.

A set of Fe standards as FeEDDHA were prepared. A solution of FeEDDHA containing 100 mg L^{-1} of Fe was prepared according to the protocol set by European Standard EN 13368-2 (Technical Committee CEN/TC 260, 2007). The 100 mg Fe L^{-1} solution of FeEDDHA was kept in the refrigerator at 4°C. Working standards of 0, 1, 2, 5 mg L^{-1} were prepared by dilution in DI water.

Shaking Incubation Method

The shaking incubation method allowed the fertilizer solutions to react with soil using continuous agitation. This method was conducted using the three soils described in Table 7. Fifty mL screw top centrifuge tubes, 12 tubes per treatment, were prepared by adding 1 mL of fertilizer solution and 9 mL of DI water. The control treatment was prepared by using 1 mL of DI water in lieu of a fertilizer solution. Then, 10 g of soil was added to each centrifuge tube.

The centrifuge tubes were placed on a reciprocating shaker rotating at 114 rev min^{-1} in the dark. The shaker was in a laboratory with a temperature of 19 to 23°C. After 1, 2, 4, and 7 d of shaking, three tubes of each treatment were removed and 20 mL of the 0.015 M $CaCl_2$ extracting solution added. The tubes were returned to the shaker for 15 min and then centrifuged for 7 min at a relative centrifuge force of 1224×*g*. The supernatant was then filtered through #2 Whatman paper into vials. The centrifuge tubes that were not being extracted on a given sampling day were aerated by removing the caps from the centrifuge tubes and exposing to air for 30 minutes to minimize changes in redox conditions.

Field Capacity Incubation Method

The field capacity incubation method allowed the fertilizer solutions to react on the soil at a water content near field capacity moisture conditions. This method was conducted the three times as previously described. Twelve centrifuge tubes of each treatment were prepared. Each tube received 1 mL of the fertilizer solutions described in Table 8. The control treatment had 1 mL of DI water added in lieu of a fertilizer solution. Additional DI water was added depending on soil texture. The amount of additional DI water added was 1 mL, 2 mL, and 0 mL for the Glyndon, Bearden, and Renshaw, respectively. The fertilizer solution and DI water were mixed, 10 g of soil was added, and the tubes capped.

The soils were allowed to incubate in a dark chamber under a constant temperature of 22°C. Three tubes of each treatment were taken after 1, 2, 4, and 7 d of incubation. Twenty mL of the 0.015 M $CaCl_2$ solution were added, in addition to 8 mL, 7 mL, and 9 mL of DI water for the Glyndon, Bearden, and Renshaw, respectively. The centrifuge tubes were shaken, centrifuged, and the supernatants filtered as previously described. The centrifuge tube caps were periodically removed for aeration as previously described.

Analysis by Direct Spectroscopy for the FeEDDHA Chromophore

The concentration of FeEDDHA in the extracts was determined directly, using its strong adsorption at 480 nm (Kroll et al., 1957). Eight mL of extract were pipetted into optically-matched glass tubes, and percent transmittance (%T) determined at 480 nm using a Bausch and Lomb Spectronic 20 (Rochester, NY). Absorbance (A) was calculated as:

$$A = -1 \times \log (\%T \times 0.01) \quad [4]$$

The Fe concentrations in the extracts, in mg L^{-1}, were determined by developing a linear regression equation for each replicate using the known Fe concentrations of the FeEDDHA standard solutions and their absorbance values. This regression equation was applied to the absorbance values of the extracts for that specific replicate by:

$$(\text{Fe in extract}) = m \times (\text{A - blank}) + b \quad [5]$$

where *m* was the slope and *b* was the intercept of the linear regression. The percent soil-stable Fe (%Fe) in the fertilizer materials was determined by:

$$\%\text{Fe} = (\text{Fe in extract}) \times 30 \times (\text{DW})^{-1} \times 0.001 \times 100 \quad [6]$$

where Fe in extract is the concentration of Fe in the extract in mg L^{-1}, DW is the dry weight of the fertilizer analyzed, in grams, and 30, 0.001, and 100 are dilution factors.

Analysis of Total Fe by Spectroscopy for the Ferrozine Chromophore

Total Fe concentration by ferrozine chromophore analysis was determined by pipetting 2 mL of fertilizer supernatant and standards into optically matched glass tubes and mixing 1 mL of color developing reagent. The solution was allowed to react for 1 h, and then 7 mL of the buffer solution was added and mixed. The percent transmittance (%T) was determined at 560 nm. Absorbance, Fe concentration, and percent soil-stable Fe in fertilizer extracts calculated as previously described.

Statistical Analysis

The fertilizers were analyzed for each soil, reaction time, incubation method, and analysis method separately by analysis of variation (ANOVA) by GLM procedure using SAS 9.3. If the F-test values were determined to be significant at the 0.05 probability level, differences between the means were determined by Fisher's Protected LSD test at α=0.05 probability. The strength of the correlation between the fertilizers of known o,o-FeEDDHA concentration and percent soil-stable Fe were determined by linear regression using Sigma Plot 10.0. The intercept and slope of the line were determined in addition to the correlation coefficient (r).

The appropriate incubation time was determined by comparing the average percent soil-stable Fe for all soils by incubation method, analysis method, and incubation time to the average o,o-FeEDDHA concentration for FeEDDHA-1 through FeEDDHA-6. The average o,o-FeEDDHA concentration determined by HPLC for FeEDDHA-1 through FeEDDHA-6 was 3.6%. The appropriate incubation time was based on the incubation time for the average percent soil-stable Fe to approach 3.6%.

The micrograms of soil-stable Fe determined for soil, reaction time, incubation method, and analysis method were correlated to Fe uptake in Fe-chlorotic soybeans when FeEDDHA had

been applied at 10 mg pot^{-1} or 20 mg pot^{-1}. Correlation coefficients were determined to describe this relationship using Sigma Plot 10.0.

RESULTS AND DISCUSSION

Fe Uptake Differences Due to o,o-FeEDDHA Concentration

Relationship between Chlorophyll Content and o,o-FeEDDHA Concentration

The relative leaf chlorophyll content for GH1 is shown in Table 9. SPAD values of the control treatment for GH1 were 26.3 (mild chlorosis) at the unifoliate stage, 11.3 (severe chlorosis) by the first trifoliolate stage, and 8.3 by the third trifoliolate stage. The progression of chlorosis within the control treatment indicates that IDC is detrimental to the plant's ability to produce chlorophyll which is consistent with previous research (Goos et al., 2004). The addition of 10 mg of FeEDDHA (10 mg) to GH1 significantly increased the SPAD values compared to the control treatment. All FeEDDHA treatments at 10 mg produced green leaves that were non-chlorotic (SPAD > 30) at the unifoliate and first trifoliolate stages. Soybeans treated with FeEDDHA-1 became mildly chlorotic (SPAD = 24.3) by the second trifoliolate stage, and remained significantly more chlorotic than the other FeEDDHA treatments through the third trifoliolate stage. FeEDDHA-2 and FeEDDHA-3 became mildly chlorotic by the third trifoliolate stage with SPAD values of 27.3 and 27.9, respectively. FeEDDHA-4, FeEDDHA-5, FeEDDHA-6, and all commercial treatments remained non-chlorotic (SPAD > 30) through the third trifoliolate stage. Commercial-2 (29.7) and Commercial-3 (29.8) had significantly greater SPAD values than FeEDDHA-3 (27.3), and Commercial-1 (29.4) had greater SPAD values than FeEDDHA-2 (27.3) at the third trifoliolate stage.

Adding 20 mg of FeEDDHA (20 mg) to GH1 resulted in greater SPAD values than the control treatment and all 10 mg treatments, and were non-chlorotic (SPAD > 30) for all plant growth stages, except for FeEDDHA-1 (28.2) at the third trifoliolate stage (Table 9). The SPAD values for all FeEDDHA treatments applied to GH1 at 20 mg at the unifoliate stage were not

Table 9. Relative chlorophyll (SPAD) values for soybean leaflets by position for the first greenhouse crop with FeEDDHA fertilizer of varying o,o-FeEDDHA concentrations imposed.

Fertilizer	Fertilizer rate	Unifoliate	Trifoliolate		
			First	Second	Third
	mg pot^{-1}	Relative chlorophyll content †			
Control	0	26.3	11.3	10.6	8.3
FeEDDHA-1 (1.5 %)‡	10	33.6	34.4	24.6	22.0
FeEDDHA-2 (2.5 %)	10	35.0	39.0	30.3	27.3
FeEDDHA-3 (3.0 %)	10	35.7	39.9	32.3	27.9
FeEDDHA-4 (4.2 %)	10	35.4	40.8	33.1	29.0
FeEDDHA-5 (4.8 %)	10	36.3	41.3	33.9	29.7
FeEDDHA-6 (5.5 %)	10	36.2	40.6	34.3	30.0
Commercial-1	10	34.4	41.3	33.5	29.4
Commercial-2	10	36.2	40.5	33.6	29.7
Commercial-3	10	35.9	40.3	35.5	29.8
F Value		57.65*	320.72*	304.18*	123.73*
LSD (0.05)		1.2	1.4	1.2	1.7
C.V. , %		2.9	3.4	3.5	5.7
Control	0	26.3	11.3	10.6	8.3
FeEDDHA-1 (1.5 %)	20	36.0	39.5	31.9	28.2
FeEDDHA-2 (2.5 %)	20	36.3	41.8	34.5	30.6
FeEDDHA-3 (3.0 %)	20	37.0	42.3	35.4	30.9
FeEDDHA-4 (4.2 %)	20	36.5	41.9	34.9	31.2
FeEDDHA-5 (4.8%)	20	36.6	42.2	34.8	31.0
FeEDDHA-6 (5.5 %)	20	36.4	41.2	35.4	31.6
Commercial-1	20	36.7	40.9	35.8	31.6
Commercial-2	20	36.2	41.2	35.6	31.2
Commercial-3	20	36.1	40.6	34.6	31.4
F Value		68.83*	321.97*	255.87*	183.94*
LSD (0.05)		1.1	1.5	1.4	1.5
C.V., %		2.7	3.4	3.7	4.6

* Means are significantly different at $p \leq 0.05$

† Relative chlorophyll content, from a Minolta SPAD-502 meter.

‡ Numbers in parentheses are percent o,o-FeEDDHA determined by HPLC

significantly different from each other. However, FeEDDHA-1 had significantly lower SPAD values for all plant growth stages except the unifoliate stage. Legume crops rely on the redistribution of Fe from the cotyledons through the plant until those reserves are depleted and then Fe uptake is primary source of Fe for the plant (Hocking, 1980). For this reason, severely chlorotic leaf tissue was not anticipated at the unifoliate stage.

The SPAD value results for GH2 are displayed in Table 10. The control treatment for GH2 was more chlorotic at the first trifoliolate stage (5.7) than the control treatment for GH1 at the first trifoliolate stage (11.3) (Table 9), and remained severely chlorotic through the third trifoliolate stage (SPAD < 6). FeEDDHA-1 and FeEDDHA-2 applied to GH2 at 10 mg were significantly more chlorotic at the unifoliate stage than the soybeans treated with the other FeEDDHA treatments. FeEDDHA-6 was significantly less chlorotic than all other treatments applied at 10 mg for all plant growth stages, except the unifoliate stage. All GH2 treated with any FeEDDHA treatment at 10 mg were severely chlorotic (SPAD < 17) by the first trifoliolate stage, and remained severely chlorotic until the plants were harvested.

Greenhouse crop 2 treated with 20 mg had greater SPAD values than the 10 mg (Table 10). The control treatment became severely chlorotic (SPAD < 17) by the unifoliate stage, while the soybeans treated with FeEDDHA-1, FeEDDHA-2, and FeEDDHA-3 at 20 mg reached that same level of chlorosis by the first trifoliolate stage. The significantly greatest SPAD values for the unifoliate stage were attained when the soybeans were treated with FeEDDHA-5, FeEDDHA-6, and Commercial-1. By the third trifoliolate stage, FeEDDHA-5 and FeEDDHA-6 and all commercial treatments had the greatest SPAD values.

Previous research has shown that FeEDDHA treatments with o,o-FeEDDHA significantly increased SPAD values while o,p-FeEDDHA isomers did not significantly increase

Table 10. Relative chlorophyll (SPAD) values for soybean leaflets by position for the second greenhouse crop with FeEDDHA fertilizers of varying o,o-FeEDDHA concentrations imposed.

Fertilizer	Fertilizer rate	Unifoliate	Trifoliolate		
			First	Second	Third
	mg pot^{-1}	Relative chlorophyll content †			
Control	0	19.1	5.7	5.3	2.1
FeEDDHA-1 (1.5 %)‡	10	19.7	3.3	1.4	0.4
FeEDDHA-2 (2.5 %)	10	19.5	6.9	4.9	2.2
FeEDDHA-3 (3.0 %)	10	21.6	7.1	3.0	1.4
FeEDDHA-4 (4.2 %)	10	22.1	10.8	5.5	2.9
FeEDDHA-5 (4.8 %)	10	22.3	11.7	5.6	2.6
FeEDDHA-6 (5.5 %)	10	24.1	16.7	11.7	8.9
Commercial-1	10	23.6	11.3	6.0	3.7
Commercial-2	10	23.5	12.0	6.0	5.1
Commercial-3	10	24.2	10.5	7.9	5.9
F Value		6.53*	8.66*	4.27*	3.36*
LSD (0.05)		2.2	3.7	3.8	3.9
C.V., %		8.5	33.6	56.7	95.6
Control	0	19.1	5.7	5.3	2.1
FeEDDHA-1 (1.5 %)	20	21.2	6.2	4.0	1.8
FeEDDHA-2 (2.5 %)	20	24.3	12.2	8.8	5.1
FeEDDHA-3 (3.0 %)	20	24.1	17.0	11.6	7.8
FeEDDHA-4 (4.2 %)	20	29.6	26.0	18.9	17.8
FeEDDHA-5 (4.8 %)	20	32.2	30.5	26.3	22.7
FeEDDHA-6 (5.5 %)	20	33.5	34.0	29.9	24.7
Commercial-1	20	32.5	30.9	26.9	22.0
Commercial-2	20	27.7	25.4	22.1	21.8
Commercial-3	20	27.9	23.9	19.9	19.6
F Value		34.45*	46.68*	22.19*	15.24*
LSD (0.05)		2.4	4.3	5.7	6.7
C.V.,%		7.6	17.5	28.1	39.9

* Means are significantly different at $p \leq 0.05$

† Relative chlorophyll content from a Minolta SPAD-502 meter

‡ Numbers in parentheses are percent o,o-FeEDDHA determined by HPLC

SPAD values in comparison to control treatments (Rojas et al., 2008; Schenkeveld, 2010). FeEDDHA-5 with 4.8% and FeEDDHA-6 with 5.5% o,o-FeEDDHA generally had greater SPAD values than the rest of the FeEDDHA treatments. The commercial treatments may have varied somewhat in the ability to control IDC, but generally they were less chlorotic than FeEDDHA-3 but more chlorotic or similar to FeEDDHA-5. Previous greenhouse experiments have also shown that SPAD values may initially be similar, but over time the greater rates will remain greener longer (Goos et al., 2004). The results from this experiment indicate that 20 mg of FeEDDHA had greater SPAD values for a longer period of time in comparison to 10 mg. These results indicate that the rate of o,o-FeEDDHA is important for attaining greater SPAD values and long-term control of IDC.

Dry Matter Yield

Dry matter production for both greenhouse crops imposed with FeEDDHA formulations are shown in Table 11. The GH1 control treatment produced significantly less dry matter yield (1.5 g pot^{-1}) compared to the soybeans treated with any FeEDDHA at either 10 or 20 mg. The yield of the control treatment was approximately 26% less compared to the yield of the greatest FeEDDHA treatment (Commercial-1 and Commercial-2) applied at 20 mg to GH1. The GH2 control treatment (1.4 g pot^{-1}) dry matter yield was approximately 27% less than the greatest FeEDDHA treatment (FeEDDHA-6) applied at 20 mg. Applications of FeEDDHA in previous greenhouse experiments have increased plant biomass compared to the control treatment, and control treatment soybeans have yielded between 20 to 40% of those treated with FeEDDHA (Goos et al., 2004; Rojas et al., 2008; Schenkeveld, 2010).

The addition of 10 mg of FeEDDHA to GH1 resulted in FeEDDHA-5 and FeEDDHA-6 and all commercial treatments yielding the greatest dry matter, and FeEDDHA-1 yielding the least dry matter (Table 11). The greatest dry matter yield for GH2 with 10 mg FeEDDHA applied was achieved with FeEDDHA-6. The commercial treatments applied at 10 mg to GH2 performed similarly to FeEDDHA-4 and FeEDDHA-5. The results for the dry matter yield sum at 10 mg FeEDDHA showed that FeEDDHA-6 yielded the greatest dry matter and FeEDDHA-1 yielded the least dry matter. The results for the sum of the dry matter yield also showed that the Commercial-1 and Commercial-2 yielded similarly compared to FeEDDHA-4 and FeEDDHA-5, while Commercial-3 yielded similarly compared to FeEDDHA-5 and FeEDDHA-6.

Greenhouse crop 1 and GH2 treated with 20 mg produced numerically more dry matter than those treated with 10 mg (Table 11). FeEDDHA-1 applied to either GH1 or GH2 at 20 mg yielded significantly less dry matter compared to all other FeEDDHA treatments. When applied

Table 11. Above-ground dry matter production of soybean in the greenhouse as influenced by Fe fertilization with FeEDDHA fertilizers of varying o,o-FeEDDHA imposed.

Fertilizer	Fertilizer rate	Greenhouse crop		
		First	Second	Sum
	mg pot^{-1}	g pot^{-1}		
Control	0	1.5	1.4	2.9
FeEDDHA-1 (1.5 %†)	10	3.5	1.2	4.7
FeEDDHA-2 (2.5 %)	10	4.3	1.4	5.6
FeEDDHA-3 (3.0 %)	10	4.5	1.5	5.9
FeEDDHA-4 (4.2 %)	10	4.6	1.7	6.3
FeEDDHA-5 (4.8 %)	10	4.9	1.7	6.7
FeEDDHA-6 (5.5%)	10	4.9	2.4	7.3
Commercial-1	10	4.6	1.9	6.5
Commercial-2	10	4.8	1.9	6.7
Commercial-3	10	5.0	1.9	6.9
F Value		50.34*	7.64*	60.93*
LSD (0.05)		0.4	0.4	0.5
C.V., %		8.7	19.1	7.0
Control	0	1.5	1.4	2.9
FeEDDHA-1 (1.5 %)	20	4.6	1.5	6.0
FeEDDHA-2 (2.5 %)	20	5.0	2.2	7.2
FeEDDHA-3 (3.0 %)	20	4.9	2.6	7.5
FeEDDHA-4 (4.2 %)	20	4.9	2.8	8.8
FeEDDHA-5 (4.8 %)	20	5.1	4.6	9.7
FeEDDHA-6 (5.5 %)	20	5.3	5.3	10.6
Commercial-1	20	5.4	4.7	10.1
Commercial-2	20	5.4	4.3	9.6
Commercial-3	20	5.3	3.6	8.9
F Value		54.57*	32.47*	81.74*
LSD (0.05)		0.5	0.7	0.7
C.V., %		8.3	17.8	7.8

* Means are significantly different at $p \leq 0.05$.

† Numbers in parentheses are the percent o,o-FeEDDHA concentration determined by HPLC

to either GH1 or GH2, FeEDDHA-5 and FeEDDHA-6 yielded the most dry matter compared to the treatments of known o,o-FeEDDHA concentration. All commercial treatments applied to GH1 yielded similarly to FeEDDHA-5 and FeEDDHA-6. Commercial-1 applied to GH2 yielded similarly to FeEDDHA-5 and FeEDDHA-6, while Commercial-2 and Commercial-3 yielded more dry matter than FeEDDHA-3. The sum of the dry matter yield showed that FeEDDHA-6 and Commercial-1 yielded the most dry matter, FeEDDHA-1 yielded the least, and Commercial-2 and Commercial-3 yielded more dry matter compared to FeEDDHA-3.

There was a strong linear correlation between the application rate of Fe as o,o-FeEDDHA and the dry matter accumulation for FeEDDHA-1 through FeEDDHA-6 (Fig. 7). The r value for the 10 mg rate was 0.98 and the r value for the 20 mg rate was 1.00. The control treatment was not included in the linear regression because the chlorosis was so severe at the conclusion of the GH2 some of the growing points had died.

Previous greenhouse experiments have shown that Fe treatments with o,o-FeEDDHA produced plants with more biomass than those treated with o,p-FeEDDHA (Rojas et al., 2008), that greater rates of FeEDDHA produced more dry matter (Goos et al., 2004), and those treated with greater concentrations of o,o-FeEDDHA produced more plant biomass (Schekeveld, 2010). Greater rates of FeEDDHA applied in field research have increased plant height, the number of seeds per sq m^{-1}, and grain yield (Wiersma, 2005).

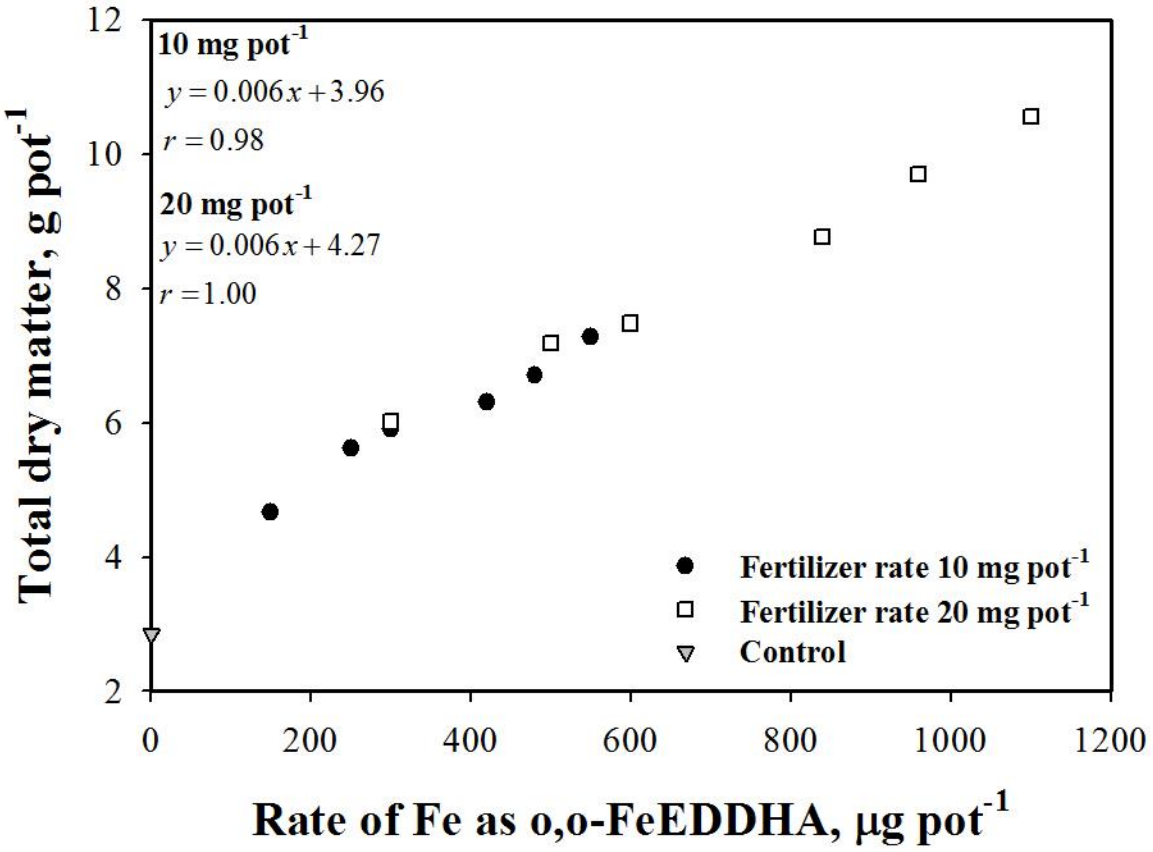

Figure 7. Total dry matter production for greenhouse crops 1 and 2 as related to the micrograms of Fe as o,o-FeEDDHA applied per pot. The control treatment was not included in the linear regression.

Fe Concentration and Fe Uptake Due to o,o-FeEDDHA Applications

Previous research has shown that plants treated with o,o-FeEDDHA had greater concentrations of Fe than plants treated with o,p-FeEDDHA (Rojas et al., 2008). Other soybean greenhouse experiment results have shown that Fe treatments with more o,o-FeEDDHA concentration had more Fe concentration and Fe uptake (Schenkeveld, 2010). The Fe concentration in soybean seeds has been increased when greater rates of FeEDDHA were applied to soybeans in field research (Wiersma, 2005).

Table 12 shows the Fe concentration and Fe uptake results for both greenhouse crops. All GH1 soybeans treated with FeEDDHA had significantly greater Fe concentrations than the control treatment. FeEDDHA-1 applied to GH1 at 10 mg had significantly less Fe concentration than all other FeEDDHA treatments. There were few significant differences between FeEDDHA treatments applied to GH1 at 20 mg. The Fe concentrations for GH2 soybeans at 10 mg and 20 mg were significantly lower than GH1 soybeans (Table 12). FeEDDHA-6 applied to GH2 at 10 mg had greater Fe concentration than the other treatments with known o,o-FeEDDHA concentrations. The Fe concentration for GH2 at 20 mg had few significant differences.

Iron uptake is a function of dry matter production and Fe concentration in the plant. The soybeans treated with greater o,o-FeEDDHA concentrations tended to have more dry matter production (Table 11) and more Fe concentration (Table 12). Thus, Fe uptake tended to be greater in soybeans treated with greater o,o-FeEDDHA concentrations as well (Table 12). The Fe uptake for the GH1 control treatment (36.1 µg pot^{-1}) was significantly lower than any FeEDDHA treatment, and was only 13% of the treatment with the most Fe uptake (Commercial-3 at 20 mg). FeEDDHA-1 applied to GH1 at 10 mg had significantly lower Fe uptake (131.4 µg pot^{-1}) than the other FeEDDHA treatments. FeEDDHA-6 (219.2 µg pot^{-1}) applied to GH1 at 10 mg had significantly more Fe uptake than FeEDDHA-3 (184.2 µg pot^{-1}). Greenhouse crop 1 at 10 mg treated with Commercial-1 had significantly greater Fe uptake (188.4 µg pot^{-1}) than FeEDDHA-1 (131.4 µg pot^{-1}). Commercial-2 (212.8 µg pot^{-1}) had significantly greater Fe uptake than FeEDDHA-3 (184.2 µg pot^{-1}), and Commercial-3 (239.3 µg pot^{-1}) was not significantly different to FeEDDHA-6 (219.2 µg pot^{-1}).

Greenhouse crop 2 had numerically less Fe uptake than GH1 (Table 12). The GH2 treated with FeEDDHA-6 (55.8 µg pot^{-1}) at 10 mg had significantly more Fe uptake than all

Table 12. Iron concentration and above-ground Fe uptake for two greenhouse crops with FeEDDHA fertilizers of varying o,o-FeEDDHA concentration imposed.

Fertilizer	Fertilizer rate	-Fe concentration-		----------Fe uptake----------		
		First†	Second†	First†	Second†	Sum‡
	mg pot^{-1}	-------µg g^{-1}-------		----------µg pot^{-1}----------		
Control	0	24.7	18.3	36.1	25.4	61.5
FeEDDHA-1 (1.5 %)§	10	37.5	17.5	131.4	20.5	151.9
FeEDDHA-2 (2.5 %)	10	41.4	19.3	175.4	27.0	203.2
FeEDDHA-3 (3.0 %)	10	41.8	19.2	184.2	28.0	212.9
FeEDDHA-4 (4.2 %)	10	42.0	19.7	192.2	34.1	226.3
FeEDDHA-5 (4.8 %)	10	41.7	19.2	204.7	33.2	237.9
FeEDDHA-6 (5.5 %)	10	45.2	22.8	219.2	55.8	275.0
Commercial-1	10	41.5	20.7	188.4	39.8	228.1
Commercial-2	10	44.7	20.0	212.8	38.6	251.4
Commercial-3	10	47.8	20.0	239.3	39.3	278.6
F Value		11.49*	2.17*	36.46*	6.99*	41.91*
LSD (0.05)		5.4	2.8	27.8	10.7	28.9
C.V.,%		11.2	12.0	13.1	27.1	11.5
Control	0	24.7	18.3	36.1	25.4	61.5
FeEDDHA-1 (1.5 %)	20	46.0	18.8	210.0	27.8	237.8
FeEDDHA-2 (2.5 %)	20	44.8	20.7	225.6	46.0	271.6
FeEDDHA-3 (3.0 %)	20	45.3	20.5	220.7	53.6	274.3
FeEDDHA-4 (4.2 %)	20	44.5	22.0	219.5	83.6	303.1
FeEDDHA-5 (4.8 %)	20	43.2	22.0	219.9	100.2	320.1
FeEDDHA-6 (5.5 %)	20	45.3	22.3	240.0	117.4	357.4
Commercial-1	20	47.2	23.2	253.5	109.3	362.8
Commercial-2	20	43.3	24.5	230.1	102.8	332.9
Commercial-3	20	49.8	22.8	265.1	83.2	348.2
F Value		12.18*	2.23*	36.79*	22.25*	59.20*
LSD (0.05)		5.6	3.7	30.0	20.6	32.9
C.V.,%		11.1	14.7	12.2	23.7	9.9

* Means are significantly different at $p \leq 0.05$.

† First or second greenhouse crop.

‡ The sum of the Fe uptake for the first and second greenhouse crops.

§ Numbers in parentheses are percent o,o-FeEDDHA determined by HPLC

other FeEDDHA treatments. Greenhouse crop 2 treated with any commercial fertilizer at 10 mg had similar Fe uptake compared to FeEDDHA-3, FeEDDHA-4 and FeEDDHA-5. Greenhouse crop 2 treated with FeEDDHA-4, FeEDDHA-5, and FeEDDHA-6 at 20 mg had significantly greater Fe uptake compared to FeEDDHA-3 (53.6 µg pot^{-1}). The Fe uptake from Commercial-1 (109.3 µg pot^{-1}), Commercial-2 (102.8 µg pot^{-1}), and Commercial-3 (83.6 µg pot^{-1}) applied at 20 mg had more Fe uptake than FeEDDHA-3 (53.6 µg pot^{-1}).

The control treatment Fe uptake sum (61.6 µg pot^{-1}) for both greenhouse crops had less Fe uptake in comparison to all FeEDDHA treatments at either application rate (Table 12). The control treatment Fe uptake was only 17% compared to the FeEDDHA treatment with the most Fe uptake, which was Commercial-1 applied at 20 mg (362.8 µg pot^{-1}). The Fe uptake sum for both FeEDDHA rates had a trend of increasing Fe uptake when increasing rates of o,o-FeEDDHA were applied. The results for Fe uptake sum at 10 mg showed that FeEDDHA-6 (275.0 µg pot^{-1}), Commercial-2 (251.4 µg pot^{-1}), and Commercial-3 (278.6 µg pot^{-1}) had significantly more Fe uptake, and that FeEDDHA-1 (151.9 µg pot^{-1}) had significantly less Fe uptake compared to all other FeEDDHA treatments. The results for the sum of the Fe uptake at the 20 mg showed that FeEDDHA-6 (257.4 µg pot^{-1}), Commercial-1 (362.8 µg pot^{-1}), Commercial-2 (332.9 µg pot^{-1}), and Commercial-3 (348.2 µg pot^{-1}) had the greatest Fe uptake, and that FeEDDHA-1 (237.8 µg pot^{-1}) had the least Fe uptake compared to the other FeEDDHA treatments.

There was a strong linear correlation between the application rate of o,o-FeEDDHA and Fe uptake (Fig. 8). The r value for 10 mg was 0.96 and r value for 20 mg was 0.98 (Fig. 8). The control treatment was not included in the linear regression because the chlorosis had become so severe that some of the growing points had died.

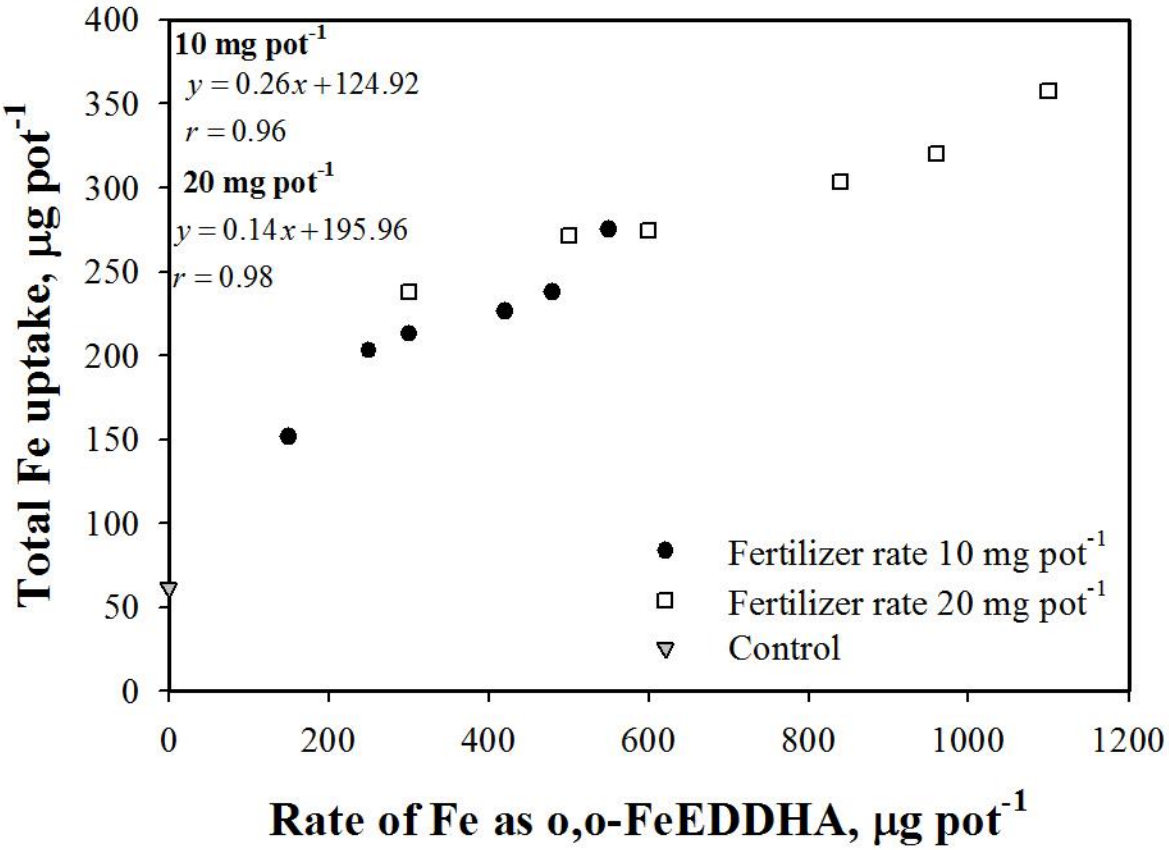

Figure 8. Total above-ground Fe uptake as related to the micrograms of Fe as o,o-FeEDDHA applied per pot. The control treatment was not included in the linear regression.

Mn Concentration and Mn Uptake Due to o,o-FeEDDHA Applications

Wallace and Alexander (1973) suggested that applications of FeEDDHA may antagonize Mn uptake and that Mn deficiency could result. Other studies have shown that FeEDDHA can reduce Mn uptake in strategy I crops (Heitholt et al., 2003; Moraghan, 1979; Moraghan and Freeman, 1978; Wikoff and Moraghan, 1986). Further, soybeans treated with increasing o,o-FeEDDHA concentrations greatly decreased Mn content (Schenkeveld, 2010). Thus, it was important for this greenhouse experiment to consider Mn concentration and Mn uptake.

The Mn concentration and uptake are shown in Table 13. Greenhouse crop 1 treated at both 10 mg and 20 mg had decreasing Mn concentrations as the o,o-FeEDDHA concentration

increased. The Mn concentration for GH1 treated with FeEDDHA-1 at 10 mg was 192.8 µg g^{-1} and significantly decreased to 110.0 µg $g^{-1}$for FeEDDHA-6. Greenhouse crop 1 at 20 mg had an even more dramatic decrease in Mn concentration. The Mn concentration for GH1 treated with FeEDDHA-1 at 20 mg was 164.2 µg g^{-1} and significantly decreased to 44.5 µg g^{-1} for FeEDDHA-6. The Mn concentration results for GH1 at 10 mg or 20 mg showed that Commercial-1, Commercial-2, and Commercial-3 were not significantly different than FeEDDHA-4, FeEDDHA-5, and FeEDDHA-6.

The results for the Mn uptake are shown on Table 13. Manganese uptake is a function of dry matter production and Mn concentration. FeEDDHA-1 (675.7 µg pot^{-1}), FeEDDHA-2 (764.2 µg pot^{-1}), and FeEDDHA-3 (714.1 µg pot^{-1}) applied to GH1 at 10 mg had significantly more Mn uptake compared to all other treatments. The rest of the FeEDDHA treatments applied to GH1 at 10 mg had decreasing Mn uptake, and FeEDDHA-6 (535.0 µg pot^{-1}) and Commercial-1 (482.1 µg pot^{-1}) had the least Mn uptake. The Mn uptake for soybeans treated with Commercial-2 (621.0 µg pot^{-1}) was similar compared to FeEDDHA-4 (585.5 µg pot^{-1}) and FeEDDHA-5 (565.5 µg pot^{-1}). Commercial-3 (714.1 µg pot^{-1}) was similar compared to FeEDDHA-1 (675.7 µg pot^{-1}), FeEDDHA-2 (764.2 µg pot^{-1}), and FeEDDHA-3 (714.1 µg pot^{-1}).

FeEDDHA-1 applied to GH1 at 20 mg had the greatest Mn uptake (750.7 µg pot^{-1}) in comparison to the other FeEDDHA treatments. Mn uptake for GH1 at 20 mg decreased sharply as more o,o-FeEDDHA was applied, and FeEDDHA-6 had the least Mn uptake (234.6 µg pot^{-1}). Commercial-1 (249.2 µg pot^{-1}) was not significantly different compared to FeEDDHA-4 (293.9 µg pot^{-1}), FeEDDHA-5 (271.6 µg pot^{-1}), and FeEDDHA-6 (234.6 µg pot^{-1}). Commercial-2 (314.9 µg pot^{-1}) was not significantly different compared to FeEDDHA-4 (293.9 µg pot^{-1}) and

Table 13. Manganese concentration and above-ground manganese uptake for two greenhouse crops with FeEDDHA fertilizers of varying o,o-FeEDDHA concentrations imposed.

Fertilizer	Fertilizer rate	--Mn concentration--		----------Mn uptake----------		
		First†	Second†	First†	Second†	Sum‡
	mg pot^{-1}	---------µg g^{-1}--------		----------µg pot^{-1}----------		
Control	0	146.8	84.2	215.0	116.9	331.9
FeEDDHA-1 (1.5 %)§	10	192.8	72.5	675.7	85.6	761.3
FeEDDHA-2 (2.5 %)	10	179.7	91.2	764.2	127.0	891.2
FeEDDHA-3 (3.0 %)	10	160.2	86.0	714.1	125.2	839.3
FeEDDHA-4 (4.2 %)	10	127.8	98.8	585.5	172.0	757.4
FeEDDHA-5 (4.8 %)	10	115.0	101.0	565.5	176.0	740.8
FeEDDHA-6 (5.5 %)	10	110.0	109.7	535.0	267.0	801.5
Commercial-1	10	105.7	103.0	482.1	196.9	679.1
Commercial-2	10	130.0	112.2	621.0	218.8	839.7
Commercial-3	10	137.0	106.8	684.8	206.5	891.4
F Value		27.60*	7.35*	28.73*	9.62*	¶
LSD (0.05)		15.9	13.3	82.6	50.7	
C.V.,%		9.8	11.9	12.2	25.9	
Control	0	146.8	84.2	215.0	116.9	331.9
FeEDDHA-1 (1.5 %)	20	164.2	90.7	750.7	131.6	882.3
FeEDDHA-2 (2.5 %)	20	122.7	103.2	617.3	227.0	844.4
FeEDDHA-3 (3.0 %)	20	88.3	112.2	431.0	293.3	724.2
FeEDDHA-4 (4.2 %)	20	59.5	121.8	293.9	462.7	756.6
FeEDDHA-5 (4.8 %)	20	53.2	119.2	271.6	549.0	820.6
FeEDDHA-6 (5.5 %)	20	44.5	123.5	234.6	645.1	879.7
Commercial-1	20	46.2	127.8	249.2	597.4	846.6
Commercial-2	20	59.3	123.7	314.9	523.7	838.6
Commercial-3	20	66.2	120.0	351.8	439.8	791.6
F Value		115.24*	6.80*	101.45*	30.45*	13.73*
LSD (0.05)		11.6	16.4	50.2	99.4	95.0
C.V.,%		11.8	12.6	11.6	21.5	10.6

* Means are significantly different at $p \leq 0.05$.

† First or second greenhouse crop.

‡ The sum of the Mn uptake for the first and second greenhouse crops.

§ Numbers in parentheses are percent o,o-FeEDDHA determined by HPLC

¶ The means between FeEDDHA rates for combined Mn uptake were not different as determined by t-tests. Therefore, one combined ANOVA was conducted for the rates.

FeEDDHA-5 (271.6 µg pot^{-1}), and Commercial-3 (271.6 µg pot^{-1}) was significantly less than FeEDDHA-3 (351.8 µg pot^{-1}).

The antagonism of Mn uptake due to FeEDDHA applications is well documented in previous research (Moraghan and Freeman, 1978; Moraghan, 1979; Wikoff and Moraghan, 1986). The expected negative relationship between Fe uptake and Mn uptake in GH1 is probably not due to lack of Mn in soil solution (Schenkeveld, 2010). It is more likely that Fe and Mn are cometing for uptake sites at the root surface (Hell and Stephan, 2003).

The Mn concentration for GH2 was lower than GH1 (Table 13). In sharp contrast to GH1, the Mn concentration for GH2 at 10 mg and 20 mg increased with increasing o,o-FeEDDHA concentration. The Mn uptake for both 10 mg and 20 mg applied to GH2 also increased with increasing rates of o,o-FeEDDHA (Table 13). The results for GH2 at 10 mg showed that FeEDDHA-6 (267.0 µg pot^{-1}) and Commercial-2 (218.8 µg pot^{-1}) had the greatest Mn uptake, and FeEDDHA-1 (131.6 µg pot^{-1}) had the least Mn uptake. The results for GH2 treated with 20 mg of Fe showed that FeEDDHA-5 (549.0 µg pot^{-1}), FeEDDHA-6 (645.1 µg pot^{-1}), and Commercial-1 (587.4 µg pot^{-1}) had the greatest Mn uptake and FeEDDHA-1 (131.6 µg pot^{-1}), FeEDDHA-2 (227.0 µg pot^{-1}), and FeEDDHA-3 (293.3 µg pot^{-1}) had the least Mn uptake. This uptake response was opposite of what was observed for GH1. Previous research has not observed a positive relationship between applied o,o-FeEDDHA concentration and Mn uptake, but rather, the opposite and occasionally no effect to Mn uptake (Heitholt et al., 2003; Moraghan, 1979; Moraghan and Freeman, 1978; Schenkeveld, 2010; Wikoff and Moraghan, 1986).

The sum of the Mn uptake for GH1 and GH2 had less Mn uptake for the control treatment than the FeEDDHA treatments (Table 13). However, the Mn uptake sum had no apparent trend for the FeEDDHA treatments due to the inverse uptake behaviors between GH1

and GH2. Figure 9 shows the positive correlation between Fe uptake and o,o-FeEDDHA concentration (r =0.77), as well as the negative correlation between Mn uptake and o,o-FeEDDHA concentration (r=0.99) in GH1. Figure 10 shows a positive correlation between Fe uptake and o,o-FeEDDHA concentration (r =0.98) and the unexpected positive correlation between Mn uptake and o,o-FeEDDHA (r =0.99) for GH2. The control treatment was not included in the linear regression because the chlorosis had become so severe that some of the growing points had died.

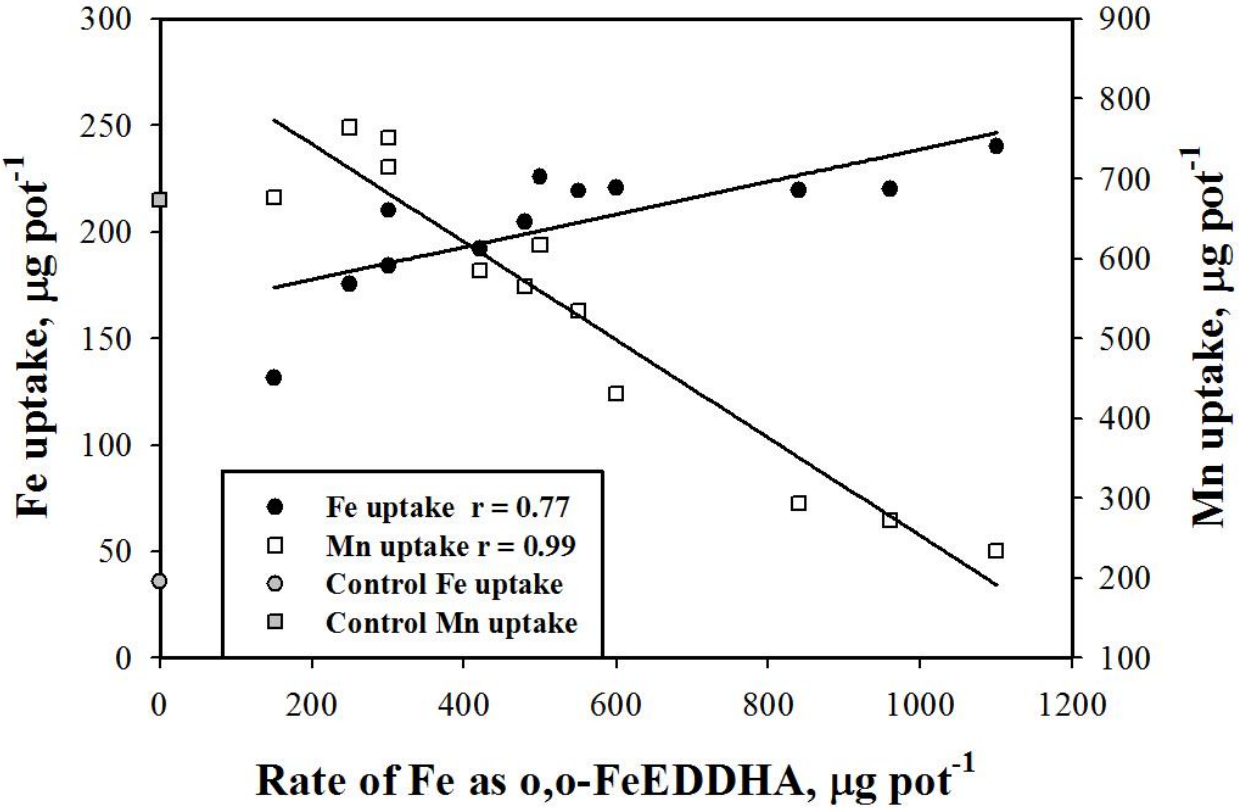

Figure 9. Greenhouse crop 1 Fe uptake and Mn uptake compared to Fe applied as o,o-FeEDDHA in µg pot^{-1}. The control treatment was not included in the linear regression.

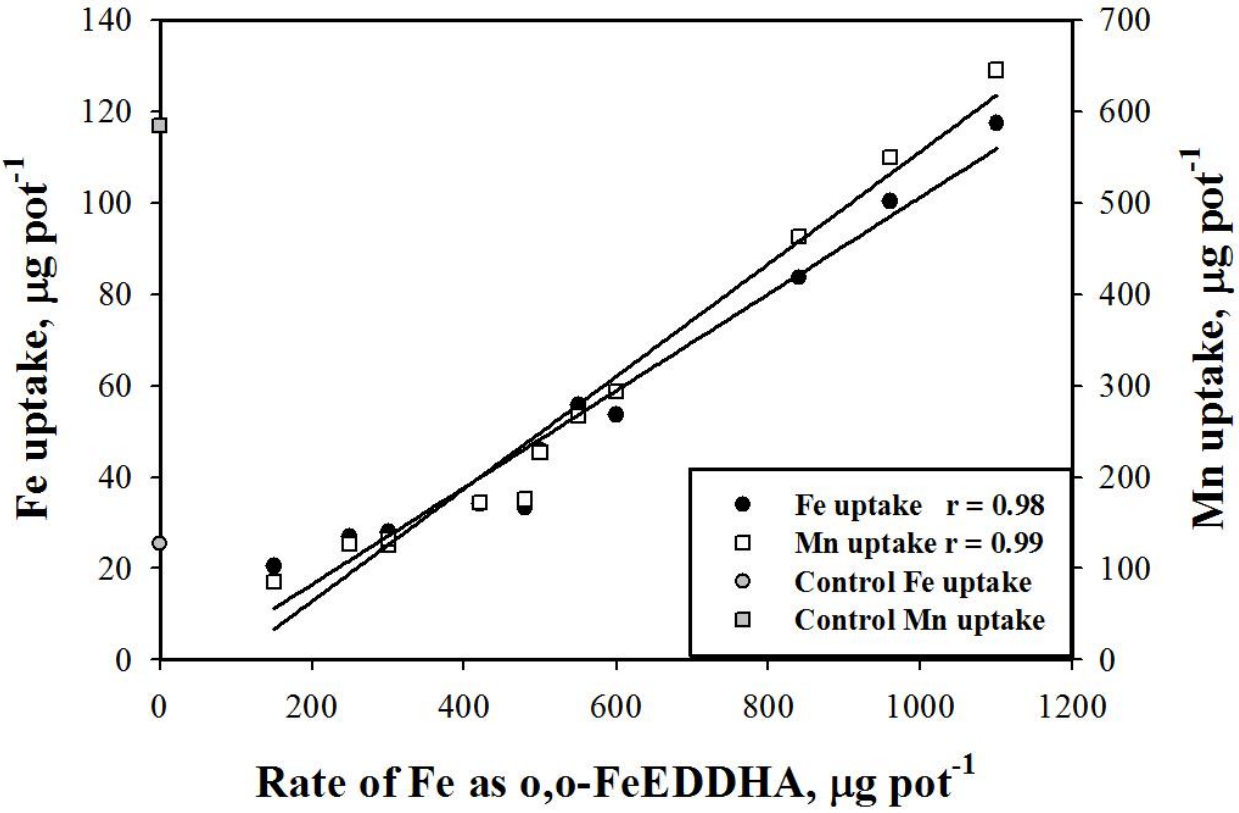

Figure 10. Greenhouse crop 2 Fe uptake and Mn uptake compared to Fe applied as o,o-FeEDDHA in µg pot^{-1}. The control treatment was not included in the linear regression.

Previous research is not able to explain the reason for the positive relationship between Fe uptake and Mn uptake as seen in GH2. Small percentages of applied racemic o,o-EDDHA and even smaller percentages of meso o,o-EDDHA and o,p-EDDHA can chelate Mn^{2+}, which is usually oxidized by the ligand to form Mn(III)EDDHA complexes (Schenkeveld, 2010). However, Mn(III)EDDHA concentrations decrease significantly after approximately 3 d due to sorption onto reactive soil surfaces (Schenkeveld, 2010). Currently, there is no existing research about MnEDDHA desorption rates from reactive soil surfaces (W.D.C. Schenkeveld, 2013, personal communication). Perhaps the MnEDDHA desorbed from the soil surfaces and was able

to successfully deliver Mn to the plant. Further research is needed to understand MnEDDHA desorption behavior reactive soil surfaces.

Summary of Greenhouse Experiment

All FeEDDHA treatments used in this greenhouse experiment contained 6% Fe and were applied at the same Fe rates. However, they controlled IDC in soybean differently. The soybeans treated with greater o,o-FeEDDHA concentrations also had more chlorophyll content as determined by a SPAD meter. The soybeans treated with greater o,o-FeEDDHA concentrations had more dry matter yield and more Fe uptake. The 20 mg rate produced greater SPAD values, dry matter yield, and Fe uptake indicating that higher rates may be necessary for controlling IDC in soybean throughout a growing season in the field.

Greater concentrations of o,o-FeEDDHA antagonized Mn uptake for GH1. This antagonism was more pronounced at 20 mg compared to 10 mg. These results indicate that o,o-FeEDDHA concentration can have a negative effect on the uptake of other nutrients. The antagonism of Mn uptake by o,o-FeEDDHA should be considered if controlling IDC in geographical areas where Mn deficiencies might be of concern.

The Mn uptake from GH2 had a positive relationship to the application of o,o-FeEDDHA. Although previous research has shown instances where Mn uptake was not affected by applications of FeEDDHA, positive correlations between Mn uptake and o,o-FeEDDHA applications have not been observed. The results from this experiment are contradictory to previous research. However, more research is needed to understand MnEDDHA desorption rates from reactive soil surfaces and the possibility of increasing Mn for plant uptake.

The commercial FeEDDHA fertilizers generally produced greater SPAD values, Fe concentrations, and Fe uptake compared to FeEDDHA-1, FeEDDHA-2 and FeEDDHA-3 and

were generally similar to FeEDDHA-4 and FeEDDHA-5. This trend was also true for Mn concentration and Mn uptake results for both greenhouse crops whether the FeEDDHA application has antagonized Mn concentration and Mn uptake or not. These results suggest that the commercial fertilizers probably had more o,o-FeEDDHA than FeEDDHA-1, FeEDDHA-2, and FeEDDHA-3 and similar o,o-FeEDDHA concentrations compared to FeEDDHA-4 and FeEDDHA-5.

The results of this greenhouse experiment indicate that it is the o,o-FeEDDHA concentration that determines the quality of soil-applied FeEDDHA to manage IDC in soybean. Currently, only the percent Fe must be stated on the fertilizer label to be in compliance with North Dakota law. However, these results would suggest that the percent Fe chelated to the o,o-FeEDDHA isomer should be included. This information would provide the user needed information to determine the correct rate to apply various FeEDDHA fertilizers for adequate IDC control.

A Colorimetric Method to Compare the Quality of Commercial FeEDDHA Fertilizers

Shaking and Field Capacity Incubation Methods Analyzed by Direct Spectroscopy for the FeEDDHA Chromophore and the Ferrozine Chromophore

The purpose of developing this soil stability test is to provide a simple alternative to the HPLC method used to determine o,o-FeEDDHA concentration utilized by the European Union. The results of these soil stability tests were correlated to the o,o-FeEDDHA concentration determined by HPLC (o,o-FeEDDHA). An ideal soil stability test would have the percent soil-stable Fe in a 1:1 relationship to o,o-FeEDDHA, which means the correlation coefficient (r) would be 1, the slope would be 1, and the intercept would be 0. The shaking incubation method allowed the fertilizer to react with soil in a 1:1 suspension, which allowed the fertilizer to react

with more of the soil's surface area. The field capacity incubation method utilized no shaking and allowed the fertilizer to react with soil in a simulated field capacity moisture situation. The extracts were analyzed by direct spectroscopy for the FeEDDHA chromophore at 480 nm to determine the percent soil-stable Fe as FeEDDHA, and also by ferrozine chromophore analysis at 560 nm to determine percent soil-stable total Fe.

Table 14 shows the results for soil-stable Fe from the shaking incubation method, analyzed by direct spectroscopy for the Glyndon soil for nine FeEDDHA fertilizers. The FeEDDHA fertilizers with greater o,o-FeEDDHA had greater percent soil-stable Fe. Commercial-1 had similar percent soil-stable Fe compared to FeEDDHA-5 and FeEDDHA-6 across all incubation times. After a 7 d incubation, Commercial-2 had similar percent soil-stable Fe compared to FeEDDHA-4 and Commercial-3 had similar percent soil-stable Fe compared to FeEDDHA-3. The percent soil-stable Fe for all FeEDDHA fertilizers were greater for 1 and 2 d incubation times, but decreased with longer incubation times. The percent soil-stable Fe was well correlated to the o,o-FeEDDHA for all reaction times (1 d $r = 0.99$; 2 d $r = 1.00$; 4 d $r = 0.99$, 7 d $r = 0.98$). The relationship between percent soil-stable Fe and o,o-FeEDDHA approached a 1:1 relationship as the intercept was 0.18 after a 7 d incubation, and the slope was 0.99 after a 4 d incubation.

Table 15 and Table 16 shows the results for the Bearden and Renshaw soils, respectively, for the shaking incubation method analyzed by direct spectroscopy. The FeEDDHA fertilizers with greater o,o-FeEDDHA had greater percent soil-stable Fe. Generally, Commercial-1 had similar percent soil-stable Fe compared to FeEDDHA-5 or FeEDDHA-6, and Commercial-2 and Commercial-3 was either similar or had significantly more percent soil-stable Fe compared to FeEDDHA-3. The percent soil-stable Fe was well correlated to the o,o-FeEDDHA, and the

correlation coefficient (r) values were between 0.99 and 1.00 for all reaction times and both soils. The correlation relationship was almost a 1:1 relationship. The slope for the Bearden soil was 0.98 and the intercept was 0.00 after a 7 d incubation (Table 15). The slope for the Renshaw soil was 1.04 and the intercept was 0.28 after 7 d incubation (Table 16).

The results for the field capacity incubation method analyzed by direct spectroscopy for the Glyndon, Bearden and Renshaw soils are shown on Tables 17, 18 and 19, respectively. The field capacity incubation method for all soils had a similar trend to the shaking incubation method when analyzed by direct spectroscopy. Commercial-1 had similar percent soil-stable Fe compared to Fe-EDDHA-5, Commercial-2 and Commercial-3 were either similar or had significantly more percent soil-stable Fe compared to FeEDDHA-3. The percent soil-stable Fe was correlated to the o,o-FeEDDHA. The r values were 1.00 for all incubation times for the Glyndon soil (Table 17). The r values for the Bearden soil were between 0.98 and 0.99 (Table 18). The r values for the Renshaw soil were between 0.99 and 1.00 (Table 19). The correlation for all soils approached a 1:1 relationship. The slope for the Glyndon soil was 1.04 with a 1 d incubation, and the intercept was 0.22 with a 7 d incubation (Table 17). The slope for the Bearden soil was 1.00 after a 7 d incubation and the intercept was 0.29 after a 4 d incubation (Table 18). The slope for the Renshaw soil after a 7 d incubation was 0.96 and the intercept was -0.06 (Table 19).

Table 14. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Glyndon soil, shaking incubation method, Fe analyzed by direct spectroscopy for the FeEDDHA chromophore at 480 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation 1	2	4	7
	% Fe				
FeEDDHA-1	1.5	2.0	2.2	1.8	1.4
FeEDDHA-2	2.5	3.0	3.1	2.8	2.4
FeEDDHA-3	3.0	4.0	4.0	3.7	3.1
FeEDDHA-4	4.2	5.3	5.4	4.6	4.6
FeEDDHA-5	4.8	6.1	6.0	5.5	4.5
FeEDDHA-6	5.5	6.4	6.4	5.7	5.1
Commercial-1		6.1	6.0	5.6	5.2
Commercial-2		4.8	4.9	4.5	4.1
Commercial-3		4.8	5.1	4.3	4.0
Value of F		484.00*	149.36*	76.05*	14.26*
LSD (0.05)		0.2	0.3	0.4	0.9
C.V., %		2.2	3.8	5.5	13.7
Correlation†					
Intercept		0.34	0.52	0.43	0.18
Slope		1.15	1.11	1.01	0.94
r		0.99	1.00	0.99	0.98

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Table 15. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Bearden soil, shaking incubation method, Fe analyzed by direct spectroscopy for the FeEDDHA chromophore at 480 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation 1	2	4	7
	% Fe				
FeEDDHA-1	1.5	1.8	1.8	1.5	1.4
FeEDDHA-2	2.5	2.5	2.7	2.4	2.3
FeEDDHA-3	3.0	3.6	3.6	3.3	3.1
FeEDDHA-4	4.2	5.0	4.8	4.5	4.3
FeEDDHA-5	4.8	5.7	5.5	5.4	4.8
FeEDDHA-6	5.5	6.0	6.0	5.8	5.2
Commercial-1		5.5	5.7	5.1	4.8
Commercial-2		4.4	4.5	4.0	3.7
Commercial-3		4.4	4.5	3.6	3.4
Value of F		221.07*	155.53*	63.73*	48.10*
LSD (0.05)		0.2	0.3	0.5	0.5
C.V., %		3.5	4.1	6.9	7.6
Correlation†					
Intercept		0.04	0.15	-0.14	0.00
Slope		1.13	1.09	1.11	0.98
r		0.99	1.00	1.00	0.99

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Table 16. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Renshaw soil, shaking incubation method, Fe analyzed by direct spectroscopy for the FeEDDHA chromophore at 480 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation 1	2	4	7
	% Fe				
FeEDDHA-1	1.5	2.0	2.2	2.0	1.8
FeEDDHA-2	2.5	3.0	3.3	3.0	2.6
FeEDDHA-3	3.0	4.0	4.2	3.8	3.6
FeEDDHA-4	4.2	5.4	5.6	5.2	4.9
FeEDDHA-5	4.8	6.1	6.3	5.8	5.3
FeEDDHA-6	5.5	6.5	6.7	6.1	5.8
Commercial-1		6.1	6.3	5.8	5.6
Commercial-2		5.1	5.1	4.9	4.4
Commercial-3		4.9	5.0	4.7	4.3
Value of F		1373.6*	165.05*	86.90*	124.89*
LSD (0.05)		0.1	0.3	0.4	0.3
C.V., %		1.3	3.7	5.1	4.4
Correlation†					
Intercept		0.24	0.49	0.41	0.28
Slope		1.19	1.18	1.08	1.04
r		0.99	0.99	0.99	0.99

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Table 17. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Glyndon soil, field capacity incubation method, Fe analyzed by direct spectroscopy for the FeEDDHA chromophore at 480 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation			
		1	2	4	7
	% Fe				
FeEDDHA-1	1.5	1.9	1.8	1.7	1.9
FeEDDHA-2	2.5	2.8	2.6	2.6	2.6
FeEDDHA-3	3.0	3.6	3.5	3.4	3.6
FeEDDHA-4	4.2	4.8	4.7	4.6	4.8
FeEDDHA-5	4.8	5.4	5.4	5.2	5.4
FeEDDHA-6	5.5	5.9	5.9	5.9	6.0
Commercial-1		5.5	5.4	5.2	5.4
Commercial-2		4.4	4.3	4.1	4.3
Commercial-3		4.3	4.2	4.0	4.1
Value of F		545.69*	1092.35*	791.79*	1313.51*
LSD (0.05)		0.2	0.1	0.1	0.1
C.V., %		2.1	1.5	1.8	1.4
Correlation†					
Intercept		0.35	0.12	0.09	0.22
Slope		1.04	1.07	1.07	1.07
r		1.00	1.00	1.00	1.00

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Table 18. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Bearden soil, field capacity incubation method, Fe analyzed by direct spectroscopy for the FeEDDHA chromophore at 480 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation			
		1	2	4	7
	% Fe				
FeEDDHA-1	1.5	1.9	2.0	1.8	1.8
FeEDDHA-2	2.5	2.8	2.8	2.7	2.5
FeEDDHA-3	3.0	3.8	3.8	3.7	3.9
FeEDDHA-4	4.2	5.1	5.2	4.9	4.7
FeEDDHA-5	4.8	5.9	5.7	5.5	5.2
FeEDDHA-6	5.5	6.2	6.1	5.9	5.7
Commercial-1		5.6	5.5	5.3	5.0
Commercial-2		4.6	4.5	4.3	4.1
Commercial-3		4.5	4.4	4.1	4.0
Value of F		657.56*	1391.68*	821.56*	68.23*
LSD (0.05)		0.2	0.1	0.1	0.4
C.V., %		1.9	1.3	1.7	5.8
Correlation†					
Intercept		0.19	0.37	0.29	0.37
Slope		1.14	1.09	1.06	1.00
r		0.99	0.99	0.99	0.98

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Table 19. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Renshaw soil, field capacity incubation method, Fe analyzed by direct spectroscopy for the FeEDDHA chromophore at 480 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation 1	2	4	7
	% Fe				
FeEDDHA-1	1.5	1.9	1.9	1.7	1.4
FeEDDHA-2	2.5	2.7	2.7	2.5	2.1
FeEDDHA-3	3.0	3.7	3.7	3.4	2.9
FeEDDHA-4	4.2	5.1	5.1	4.7	4.2
FeEDDHA-5	4.8	5.6	5.6	5.3	4.6
FeEDDHA-6	5.5	6.0	6.0	5.8	5.0
Commercial-1		5.5	5.5	5.3	4.6
Commercial-2		4.7	4.5	4.4	3.8
Commercial-3		4.4	4.4	4.2	3.6
Value of F		305.31*	1309.79*	2040.82*	996.48*
LSD (0.05)		0.2	0.1	0.1	0.1
C.V., %		2.8	1.4	1.1	1.7
Correlation†					
Intercept		0.24	0.24	0.10	-0.06
Slope		1.09	1.11	1.07	0.96
r		0.99	1.00	0.99	0.99

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Implementing the ferrozine chromophore analysis with these incubation methods may serve as a way to predict total Fe in solution. This analysis method may be necessary to evaluate the effectiveness of other soil-applied Fe-chelates that have a different chromophore than FeEDDHA. The ferrozine chromophore analysis method utilizes an acidic color-developing solution which reduces Fe(III) to Fe(II), dissociates the Fe from the EDDHA ligand, and then chelates the Fe(II) with ferrozine. The ferrozine chromophore emits a purple color and can be analyzed with light wavelengths at 560 nm (Stookey, 1970).

The results for the shaking incubation method analyzed by ferrozine chromophore analysis for the Glyndon, Bearden, and Renshaw soils are shown in Tables 20, 21, and 22, respectively. The trends for the shaking incubation method analyzed by ferrozine chromophore analysis have similar trends to the shaking incubation method by direct spectroscopy. The FeEDDHA fertilizers with greater o,o-FeEDDHA had greater percent soil-stable Fe. The percent soil-stable Fe from Commercial-1 was similar to FeEDDHA-5 and FeEDDHA-6, and Commercial-2 and Commercial-3 were generally similar or greater than FeEDDHA-3. The percent soil-stable Fe was well correlated to the o,o-FeEDDHA, and the r values for all the soils were between 0.98 and 1.00. The relationship between percent soil-stable Fe and o,o-FeEDDHA approached a 1:1 relationship. The slope for the Glyndon soil was 1.01 after a 2 d incubation, and the intercept was 0.38 after a 4 d incubation (Table 20). The slope for the Bearden soil was 1.01 after a 7 d incubation, and the intercept was -0.03 after a 4 d incubation (Table 21). The slope for the Renshaw soil was 1.03 and the intercept was 0.26 after a 4 d incubation (Table 22).

The results for the field capacity incubation method determined by ferrozine chromophore analysis for the Glyndon, Bearden, and Renshaw soils are shown in Tables 23, 24, and 25, respectively. The trends for the field capacity incubation method analyzed by ferrozine

chromophore analysis were similar to the field capacity incubation method analyzed by direct spectroscopy. The FeEDDHA fertilizers with greater o,o-FeEDDHA had greater percent soil-stable Fe. Commercial-1 had similar percent soil-stable Fe compared to FeEDDHA-4 and FeEDDHA-5, and Commercial-2 and Commercial-3 were similar or greater than FeEDDHA-3. The percent soil-stable Fe was correlated to the o,o-FeEDDHA, and the r values across all soils and reactions times ranged from 0.98 to 0.99. The correlation was almost a 1:1 relationship. The slope was 1.00 and the intercept was 0.11 for the Glyndon soil after a 7 incubation (Table 23). The slope was 0.98 and the intercept was 0.23 after a 7 d incubation for the Bearden soil (Table 24). The slope was 1.00 and the intercept was 0.16 for the Renshaw soil after a 4 d incubation (Table 25).

Table 20. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Glyndon soil, shaking incubation method, total soluble Fe analyzed for the ferrozine chromophore at 560 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation 1	2	4	7
	% Fe				
FeEDDHA-1	1.5	2.0	2.0	1.7	1.6
FeEDDHA-2	2.5	3.1	2.8	2.7	2.4
FeEDDHA-3	3.0	3.9	3.8	3.6	3.3
FeEDDHA-4	4.2	5.3	5.0	4.4	4.4
FeEDDHA-5	4.8	6.0	5.5	5.2	4.4
FeEDDHA-6	5.5	6.0	5.8	5.5	5.0
Commercial-1		6.0	5.7	5.5	4.7
Commercial-2		4.7	4.5	4.4	4.0
Commercial-3		4.7	4.4	4.5	3.7
Value of F		85.00*	126.16*	72.24*	18.06*
LSD (0.05)		0.4	0.3	0.4	0.7
C.V., %		5.3	4.2	5.7	11.0
Correlation†					
Intercept		0.50	0.55	0.38	0.44
Slope		1.08	1.01	0.97	0.86
r		0.99	0.99	0.99	0.98

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Table 21. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Bearden soil, shaking incubation method, total soluble Fe for the ferrozine chromophore at 560 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation 1	2	4	7
	% Fe				
FeEDDHA-1	1.5	1.6	1.7	1.5	1.3
FeEDDHA-2	2.5	2.4	2.4	2.4	2.1
FeEDDHA-3	3.0	3.5	3.4	3.5	3.0
FeEDDHA-4	4.2	4.8	4.5	4.4	4.3
FeEDDHA-5	4.8	5.2	5.2	5.3	4.8
FeEDDHA-6	5.5	5.6	5.8	5.5	5.1
Commercial-1		5.3	5.4	5.0	4.7
Commercial-2		4.0	4.4	4.1	3.5
Commercial-3		4.0	4.3	3.5	3.3
Value of F		74.16*	214.50*	90.83*	73.72*
LSD (0.05)		0.4	0.3	0.4	0.4
C.V., %		6.2	3.5	5.6	6.5
Correlation†					
Intercept		0.03	0.04	-0.03	-0.16
Slope		1.07	1.06	1.06	1.01
r		0.99	1.00	0.99	0.99

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Table 22. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Renshaw soil, shaking incubation method, total soluble Fe for the ferrozine chromophore at 560 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation 1	2	4	7
	% Fe				
FeEDDHA-1	1.5	2.0	1.9	1.8	1.7
FeEDDHA-2	2.5	3.0	3.0	2.7	2.5
FeEDDHA-3	3.0	4.2	4.0	3.4	3.4
FeEDDHA-4	4.2	5.6	5.4	4.9	4.7
FeEDDHA-5	4.8	6.1	5.7	5.2	4.9
FeEDDHA-6	5.5	6.3	6.3	5.8	5.3
Commercial-1		6.0	5.7	5.1	5.2
Commercial-2		5.3	4.7	4.4	4.1
Commercial-3		4.9	4.6	4.4	4.2
Value of F		267.45*	203.43*	41.53*	107.80*
LSD (0.05)		0.2	0.3	0.5	0.3
C.V., %		2.9	3.4	7.5	4.6
Correlation†					
Intercept		0.43	0.35	0.26	0.39
Slope		1.14	1.12	1.03	0.93
r		0.98	0.99	0.99	0.99

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Table 23. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Glyndon soil, field capacity incubation method, total soluble Fe for the ferrozine chromophore at 560 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation 1	2	4	7
	% Fe				
FeEDDHA-1	1.5	1.7	1.6	1.6	1.6
FeEDDHA-2	2.5	2.6	2.5	2.5	2.3
FeEDDHA-3	3.0	3.3	3.3	3.3	3.4
FeEDDHA-4	4.2	4.8	4.1	4.6	4.4
FeEDDHA-5	4.8	5.1	5.1	4.8	5.1
FeEDDHA-6	5.5	5.3	5.4	5.3	5.4
Commercial-1		5.2	4.7	5.0	5.6
Commercial-2		4.2	4.0	4.1	3.8
Commercial-3		4.3	4.0	3.9	3.5
Value of F		105.74*	120.77*	108.81*	55.78*
LSD (0.05)		0.3	0.3	0.3	0.5
C.V., %		4.8	4.4	4.7	6.8
Correlation†					
Intercept		0.34	0.20	0.27	0.11
Slope		0.97	0.97	0.95	1.00
r		0.98	0.99	0.99	0.99

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Table 24. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Bearden soil, field capacity incubation method, total soluble Fe for the ferrozine chromophore at 560 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation 1	2	4	7
	% Fe				
FeEDDHA-1	1.5	1.8	1.9	1.7	1.7
FeEDDHA-2	2.5	2.8	2.7	2.5	2.4
FeEDDHA-3	3.0	3.9	3.8	3.5	3.4
FeEDDHA-4	4.2	5.2	5.0	4.7	4.5
FeEDDHA-5	4.8	5.7	5.5	5.1	4.9
FeEDDHA-6	5.5	5.9	6.0	5.5	5.5
Commercial-1		5.6	5.2	5.1	5.8
Commercial-2		5.6	4.4	4.1	4.0
Commercial-3		4.5	4.2	4.0	3.8
Value of F		143.23*	421.99*	354.30*	300.88*
LSD (0.05)		0.3	0.2	0.2	0.2
C.V., %		4.1	2.4	2.6	2.8
Correlation†					
Intercept		0.36	0.27	0.32	0.23
Slope		1.08	1.09	0.98	0.98
r		0.98	0.99	0.99	0.99

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Table 25. Apparent percentage of soil-stable Fe in nine Fe fertilizers. Renshaw soil, field capacity incubation method, total soluble Fe for the ferrozine chromophore at 560 nm.

Fertilizer	Fe content as o,o-FeEDDHA	Days of incubation 1	2	4	7
	% Fe				
FeEDDHA-1	1.5	1.9	1.9	1.6	1.5
FeEDDHA-2	2.5	2.7	2.6	2.4	2.1
FeEDDHA-3	3.0	3.7	3.7	3.2	3.0
FeEDDHA-4	4.2	5.0	5.1	4.7	4.1
FeEDDHA-5	4.8	5.6	5.6	5.2	4.4
FeEDDHA-6	5.5	5.9	6.0	5.2	4.6
Commercial-1		5.3	5.4	5.0	4.4
Commercial-2		4.6	4.6	4.3	3.6
Commercial-3		4.5	4.4	4.0	3.7
Value of F		202.82*	224.69*	114.87*	108.28*
LSD (0.05)		0.3	0.3	0.3	0.3
C.V., %		3.3	3.3	4.7	4.7
Correlation†					
Intercept		0.36	0.26	0.16	0.21
Slope		1.05	1.08	1.00	0.85
r		0.99	0.99	0.98	0.98

* Means are significantly different at $p \leq 0.05$.

†Correlation between the % Fe as o,o-FeEDDHA and the apparent % Fe stable upon reaction with soil for samples FeEDDHA-1 through FeEDDHA-6.

Previous research has used both shaking and motionless incubation methods to evaluate the soil stability of Fe-chelates and both methods have been found effective for that purpose (Alvarez-Fernandez et al., 1997; Goos and Germain, 2001; Orphanos and Hadjilloucas, 1984). Research comparing stationary and shaking incubations to determine soil-stable Fe from Fe-chelates was not found. The commercial fertilizers of unknown o,o-FeEDDHA as well as the fertilizers of known o,o-FeEDDHA generally had similar soil-stable Fe values across all soils with both incubation methods. Results indicate that both the shaking and the field capacity incubations methods are reliable and consistent for predicting the soil-stable Fe due to FeEDDHA applications.

Schenkeveld (2010) utilized a shaking incubation method with various soils to determine the stability of various FeEDDHA components and found that, over time, the o,o-FeEDDHA diastereomers were able to maintain Fe in soil solution. The o,p-FeEDDHA did not maintain any Fe in solution after a one week incubation with soil, and the polycondensates remained Fe in solution only slightly more than the o,p-FeEDDHA (Schenkeveld, 2010). The results of this experiment also showed that FeEDDHA sources with greater o,o-FeEDDHA concentration maintained more soil-stable Fe.

Previous Fe-chelate soil stability tests have utilized various soils from around the world including Aridsols, Enitsols, Spodosols, and Mollisols, some were calcareous and some were not calcareous (Goos and Germain, 2001; Orphanos and Hadjiloucas, 1984; Schenkeveld, 2010). Soil stability tests for Fe-chelates on various soils are reliable, which is similar to the results from this experiment (Goos and Germain, 2001; Orphanos and Hadjiloucas, 1984; Schenkeveld, 2010). However, Fe-chelate stability testing with individual laboratory-produced soil constituents, such as peat and amorphous Fe(III) oxides, reduced soil-stable Fe and especially

soil-stable Fe from FeEDDHA (Alvarez-Fernandez et al., 1997, 2002, Schenkeveld, 2010), while reaction with $CaCO_3$ and Ca-montmorillonite has only minimally reduced soil-stable Fe from FeEDDHA (Alvarez-Fernandez et al., 1997, 2002). The meso o,o-FeEDDHA is adsorbed by Fe(hyr)oxides and the racemic o,o-FeEDDHA is adsorbed by organic matter (Alvarez-Fernandez et al., 2002). The EDDHA ligand has a high affinity for trivalent ions as opposed to divalent ions, such as Ca^{2+} or Mn^{2+} (Schenkeveld, 2010). Therefore, utilizing any "normal" agricultural soil for a given geography would likely be appropriate for implementing this Fe-chelate soil stability testing method.

Previous research has utilized atomic absorption, ICP-AES, and spectroscopy methods for analysis of the soil-stable Fe (Alvarez-Fernandez et al., 1997; Goos and Germain, 2001; Orphanos and Hadjiloucas, 1984; Schenkeveld, 2010). This laboratory experiment utilized spectroscopy to determine the amount of soil-stable Fe in attempt to implement a simple analysis method. Direct spectroscopy determined the amount of Fe-chelated to the EDDHA ligand after soil incubation. The ferrozine chromophore analysis determined total Fe in solution after soil incubation, whether it was chelated to the EDDHA ligand or not. Previous Fe-chelate soil stability research found that non-chelated Fe exists in soil solution in addition to chelated Fe (Alverez-Fernandez et al., 2002). Other research has found that, after FeEDDHA applications, most of the Fe found in soil solution after 7 d was due to o,o-FeEDDHA exclusively (Schenkeveld, 2010). The results from this experiment found less Fe when analyzed by ferrozine chromophore analysis compared to the analysis of the direct spectroscopy, which indicates that the concentration of chelated Fe was more than the total Fe in solution. This may indicate that further research is needed to determine if a longer reaction time is needed for the color-

developing solution to react with the extract so that full chelation of Fe to the ferrozine occurs for the ferrozine chromophore analysis.

Reaction Kinetics for Shaking and Field Capacity Incubation Methods

Previous soil incubation methods have utilized a variety of reaction times ranging from a 3 to 50 d (Alvarez-Fernandez et al., 1997, 2002; Goos and Germain, 2001; Orphanos and Hadjiloucas, 1984). Orphanos and Hadjiloucas (1984) suggested that a 25-30 d incubation period was sufficient, while Alvarez-Fernandez et al. (1997) suggested that 3 d was sufficient. Other research has shown that, when FeEDDHA is applied, soil-stable Fe doesn't significantly change after a 7 d incubation (Schenkeveld, 2010). This experiment attempted to determine the possibility of a shorter incubation time than 7 d to accurately determine soil-stable Fe due to applied o,o-FeEDDHA.

Table 26 shows the averages of the percent soil-stable Fe for FeEDDHA-1 through FeEDDHA-6 for each reaction time, soil, incubation method, and analysis method. The shaking incubation method overestimated the soil-stable Fe with 1 and 2 d incubation times. However, the percent soil-stable Fe with the shaking incubation method decreased and, after a 7 d incubation, the percent soil-stable Fe was 3.7 and 3.6% determined by direct spectroscopy and ferrozine chromophore analysis, respectively. The field capacity incubation method also overestimated the percent soil-stable Fe with shorter reaction times for both analysis methods, and saw a gradual decrease in percent soil-stable Fe over time. The averages of the percent soil-stable Fe for the field capacity incubation method were 3.8 and 3.6%, determined by direct spectroscopy and ferrozine chromophore analysis, respectively.

Table 26. Apparent percentage of soil-stable Fe in six FeEDDHA fertilizers of known o,o-FeEDDHA concentrations determined by HPLC, averaged across FeEDDHA-1 through FeEDDHA-6. The average value by HPLC was 3.6%.

Soil	Incubation method	Analysis method	Reaction days 1	2	4	7
			% soil-stable Fe			
Glyndon	Shaking	FeEDDHA†	4.5	4.5	4.0	3.5
Bearden			4.1	4.1	3.8	3.5
Renshaw			4.5	4.7	4.3	4.0
		Average	4.4	4.4	4.0	3.7
Glyndon	Field Capacity	FeEDDHA	4.1	4.0	3.9	4.1
Bearden			4.3	4.3	4.1	4.0
Renshaw			4.2	4.2	3.9	3.4
		Average	4.2	4.2	4.0	3.8
Glyndon	Shaking	Ferrozine‡	4.4	4.2	3.9	3.5
Bearden			3.9	3.8	3.8	3.4
Renshaw			4.5	4.4	4.0	3.8
		Average	4.3	4.1	3.9	3.6
Glyndon	Field Capacity	Ferrozine	3.8	3.7	3.7	3.7
Bearden			4.2	4.2	3.8	3.7
Renshaw			4.1	4.2	3.7	3.3
		Average	4.0	4.0	3.7	3.6

† FeEDDHA determined by direct spectroscopy at 480 nm.
‡ Total soluble Fe by ferrozine determined by spectroscopy at 560 nm.

Figure 11 shows the percent soil-stable Fe averages for both incubation methods and both analysis methods in comparison to the reaction times. The average o,o-FeEDDHA is also plotted as a horizontal line on Figure 11. The shorter reaction times for both the shaking incubation method and field capacity incubation method overestimated the amount of soil-stable Fe. The shaking incubation method initially overestimated the percent soil-stable Fe to a greater extent than the field capacity incubation method. However, after a 7 d incubation, the percent soil-stable Fe for all incubation methods and analysis methods approached the average o,o-FeEDDHA concentration of 3.6%. The shaking incubation method and field capacity incubation method with a 7 d incubation time, analyzed by ferrozine chromophore analysis both reached the 3.6% soil-stable Fe.

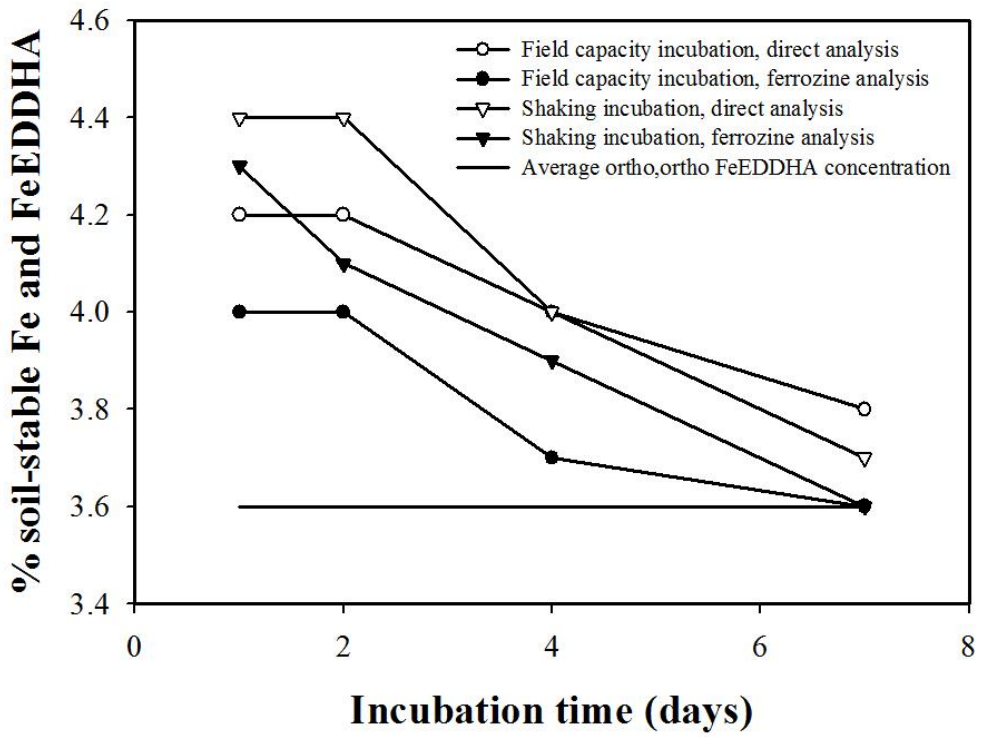

Figure 11. Average of apparent soil-stable Fe and FeEDDHA for each incubation method and analysis method across all soils and FeEDDHA fertilizers of known o,o-FeEDDHA concentrations plotted against the reaction time in days. The horizontal line indicates the average percent Fe for the o,o-FeEDDHA concentrations determined by HPLC of 3.6%.

The average of all percent soil-stable Fe values approached the average o,o-FeEDDHA concentration with a 7 d incubation time and the shorter incubations overestimated the o,o-FeEDDHA concentration. Previous research has found that the percent soil-stable Fe due to o,p-FeEDDHA and the polycondensate FeEDDHA concentrations are negligible after a 7 d incubation (Schenkeveld, 2010). Therefore, it was determined that a 7 d incubation period is appropriate for this soil stability test.

Correlation to Fe Uptake of Fe Chlorotic Soybeans

The micrograms of soil-stable Fe for each soil, incubation method, analysis method, and reaction time was correlated to the Fe uptake of Fe chlorotic soybeans and these results are shown on Table 27. The same Fe fertilizers were used in both the laboratory experiment and the greenhouse experiment. These correlations include the commercial FeEDDHA fertilizers. Both of the incubation methods were well correlated ($r = 0.88$ to 0.93) to the Fe uptake of Fe chlorotic soybeans. Both analysis methods were also well correlated ($r = 0.88$ to 0.93) to the Fe uptake in Fe chlorotic soybeans.

The results of the greenhouse experiment showed that soil applications of FeEDDHA with these fertilizers controlled IDC differently. The FeEDDHA fertilizers with greater o,o-FeEDDHA concentration had greater SPAD values (Tables 9, 10), dry matter yield (Table 11), and Fe uptake (Table 12). The results of the laboratory experiment showed that FeEDDHA fertilizers of greater o,o-FeEDDHA incubated with soil had greater percent soil-stable Fe. Previous research has found that the concentration of Fe in soil solution after a FeEDDHA application was largely attributed to the applied o,o-FeEDDHA concentration, and that the Fe uptake by soybeans was determined by the amount of Fe in soil solution (Schenkeveld, 2010). The correlation between Fe uptake and percent soil-stable Fe is further validation that this soil

incubation method can be effective for predicting the quality of soil-applied FeEDDHA fertilizers. The results in this experiment show that soil-stable Fe is correlated to Fe uptake as well.

Table 27. Correlation coefficients (r) between the micrograms of soil-stable Fe applied to soil and the Fe uptake by two crops of soybeans in the greenhouse applied at two Fe rates. Three soils, two incubation methods and analysis of total soluble Fe and total soluble FeEDDHA used for estimation of soil-stable Fe. Correlations did not include the control treatment.

Soil	Incubation method	Analysis method	Reaction days 1	2	4	7
			r			
Glyndon	Shaking	FeEDDHA†	0.91	0.93	0.92	0.92
	Shaking	Ferrozine‡	0.91	0.91	0.93	0.92
	Field Capacity	FeEDDHA	0.91	0.91	0.91	0.91
	Field Capacity	Ferrozine	0.91	0.92	0.91	0.88
Bearden	Shaking	FeEDDHA	0.91	0.92	0.88	0.89
	Shaking	Ferrozine	0.89	0.93	0.88	0.88
	Field Capacity	FeEDDHA	0.91	0.90	0.90	0.90
	Field Capacity	Ferrozine	0.91	0.90	0.90	0.90
Renshaw	Shaking	FeEDDHA	0.92	0.91	0.92	0.91
	Shaking	Ferrozine	0.92	0.91	0.92	0.92
	Field Capacity	FeEDDHA	0.91	0.91	0.92	0.91
	Field Capacity	Ferrozine	0.92	0.91	0.90	0.92

† FeEDDHA determined by direct spectroscopy at 480 nm.
‡ Total soluble Fe by ferrozine determined by spectroscopy at 560 nm.

Summary of Laboratory Experiment

The shaking incubation method allowed for the FeEDDHA to react with more soil surface area while the field capacity incubation method simulated field capacity moisture conditions and utilized no shaking. Both the shaking incubation method and the field capacity incubation method have a strong linear correlation to the o,o-FeEDDHA concentration ($r = 0.98$ to 1.00) and to Fe uptake of Fe chlorotic soybeans ($r = 0.88$ to 0.93). These data suggest that both incubation methods could be a predictor of quality for soil-applied FeEDDHA fertilizers.

The strong linear correlations were persistent across different soils ($r = 0.98$ to 1.00) and this observation was found to be consistent with previous research. This suggests that the choice of soil for a soil stability test is not critical. The soil used with these incubation methods should be a typical representation of a soil where FeEDDHA applications are likely to occur. The percent soil-stable Fe approached the average o,o-FeEDDHA concentration after 7 d of incubation for both the field capacity and shaking incubation methods when analyzed by ferrozine chromophore analysis. Thus, a 7 d incubation time was determined to be appropriate.

Both direct spectroscopy and ferrozine chromophore analysis methods, used with either shaking or field capacity incubation methods, were well correlated ($r = 0.98$ to 1.00) to o,o-FeEDDHA. Both direct spectroscopy and ferrozine chromophore analysis, used with either the shaking or the field capacity incubation methods, were also well correlated ($r = 0.88$ to 0.93) to the Fe uptake of Fe chlorotic soybeans treated with FeEDDHA fertilizers. Direct spectroscopy determined only the amount of percent soil-stable Fe chelated as FeEDDHA or related compounds. Ferrozine chromophore analysis may be able to be utilized as an analysis method for the effectiveness of other soil-applied Fe-chelate fertilizers. However, further testing with

Fe-chelates other than FeEDDHA should be conducted with the ferrozine analysis method to verify its validity.

GENERAL CONCLUSIONS

The effectiveness of soil-applied FeEDDHA to control IDC in soybean is contingent upon the concentration of o,o-FeEDDHA. Greater concentrations of o,o-FeEDDHA increased chlorophyll content, dry matter yield, Fe concentration, and Fe uptake. The rate of o,o-FeEDDHA is more important for controlling IDC in soybean than the rate of Fe. Currently, fertilizer labels in North Dakota only list the percentage of Fe contained in an Fe fertilizer. It would be beneficial to list the percent Fe chelated to o,o-FeEDDHA isomer in addition to listing the percent Fe contained in the fertilizer.

Applications of o,o-FeEDDHA antagonized Mn concentration and Mn uptake in the first greenhouse crop soybeans, but increased Mn concentration and Mn uptake in the second greenhouse crop soybeans. Care should be taken to supply adequate Mn when utilizing FeEDDHA to control IDC in geographies where Mn deficiencies occur simultaneously with IDC. The positive relationship between o,o-FeEDDHA and Mn concentration and Mn uptake from the second greenhouse crop was not anticipated and previous research has not seen such results. More research is needed to understand if o,o-FeEDDHA can increase Mn concentration and uptake under certain conditions.

An HPLC testing method is currently used in Europe to determine the o,o-FeEDDHA concentration in FeEDDHA fertilizers. However, this test is expensive, complicated, and is currently not commercially available in the USA. A simple soil stability test, analyzed colorimetrically, can successfully determine relative differences between FeEDDHA fertilizers.

The shaking and the field capacity incubation methods were well-correlated to o,o-FeEDDHA concentration determined by HPLC. These incubation methods could be used with any that is deemed to be representative soil for a given geographical area. The percent soil-stable

Fe approached the average o,o-FeEDDHA concentration determined by HPLC after a 7 d incubation. The direct spectroscopy analysis method determined the amount of FeEDDHA left in solution after incubation with soil. The ferrozine analysis method determined the total Fe in soil solution. The soil-stable Fe analyzed by both methods were well-correlated to the o,o-FeEDDHA concentration determined by HPLC. More research is needed, but the ferrozine method could be implemented as a potential analysis method to determine soil-stable Fe from other Fe-chelates with a different chromophore than FeEDDHA.

REFERENCES

Agvise Laboratories. 2012. Soil test summaries. Available at: http://www.agvise.com/soil-test-summaries/ (accessed 14 Jul. 2012). Agvise Laboratories, Northwood, North Dakota and Benson, Minnesota.

Agvise Laboratories. 2013. Carbonate testing and iron chlorosis. Available at: http://www.agvise.com/educational-articles/carbonate-testing-and-iron-chlorosis/ (accessed 3 Mar. 2013). Agvise Laboratories, Northwood, North Dakota and Benson, Minnesota.

Allen, B.L. and B.F. Hajek. 1989. Mineral occurrence in soil environments. In: J. B. Dixon and S.B. Weed, editors, Minerals in soil environments. 2nd ed. SSSA book series 1. SSSA, Madison, WI. p. 199-278.

Alvarez-Fernandez, A., A. Garate, and J.J. Lucena. 1997. Interaction of iron chelates with several soil materials and with a soil standard. J. Plant Nutr. 20:559-572.

Alvarez-Fernandez, A., M. A. Sierra, and J.J. Lucena. 2002. Reactivity of synthetic Fe chelates with soils and soil components. Plant Soil 241:129-137.

Bermudez, M.D., M. Juarez, J.Jorda, J. Sanchez-Andreu, and J. J. Lucena. 1999. Kinetics fo reactions of chelates FeEDDHA and FeEDDHMA as affected by pH and competing ions. Commun. Soil Sci. Plant Anal. 30:2769-2784.

Bermudez, D., M. Juarez, J.D. Jorda, J.J. Sandchez-Andreu, J.J. Lucena, and A. Sandchez-Sanchez. 2002. Effect of pH on the stability of the chelates FeEDDHA, FeEDDHMA and their isomers. Agrochimica 46:203-211.

Bigham, J.M., R.W. Fitzpatrick, and D.G. Schulze. 2002. Iron oxides. In: J.B. Dixon and D.G. Schulze, editors, Soil mineralogy with environmental applications. SSSA book series 7. SSSA, Madison, WI. p. 323-366.

Bloom, P.R., G.W. Rehm, J.A. Lamb, and A.J. Scobbie. 2011. Soil nitrate is a causative factor in iron deficiency chlorosis in soybeans. Soil Sci. Soc. Am. J. 75:2233-2241.

Bauer, P. and R. Hell. 2006. Translocation of iron in plant tissues. In: L.L. Barton and J. Abadia, editors, Iron nutrition in plants and rhizospheric microorganisms. Springer, Dordrecht, The Netherlands. p. 279-288.

Brady, N.C. and R.R. Weil. 2010. Elements of the nature and properties of soils. 3rd ed. Prentice Hall, Upper Saddle River, NJ.

Broadley, M., P. Brown, I. Cakmak, Z. Rengel, and F. Zhao. 2012. Function of nutrients: micronutrients. In: P. Marschner, editor, Mineral nutrition of higher plants. Academic Press, London, U.K. p. 191-248.

Brown, J.C. and R. L. Chaney. 1971. Effect of iron on the transport of citrate into the xylem of soybeans and tomato. Plant Physiol. 47:836-840.

Cantera, R. G., A.M. Zamarreno, and J. M. Garcia-Mina. 2002. Characterization of commercial iron chelates and their behavior in and alkaline and calcareous soil. J. Agric. Food Chem. 50:7609-7615.

Cerdan, M., S. Alcaniz, M. Juarez, J. D. Jorda, and D. Bermudez. 2007. Kinetic behavior of Fe(o,o-EDDHA)-humic substance mixtures in several soil components and in calcareous soils. J. Agric. Food Chem. 55:9159-9169.

Combs, S.M., J. Denning, and K.D. Frank. 1998. Sulfate-sulfur. In: J.R. Brown et al., editors, Recommended chemical soil test procedures for the north central region. North Central Reg. Res. Pub. No 221 (Revised). Missouri Agric. Exp. Sta. Bul. SB 1001, Columbia, MO. p. 35-40.

Combs, S.M. and M.V. Nathan. 1998. Soil organic matter. In: J.R. Brown et al., editors, Recommended chemical soil test procedures for the north central region. North Central Reg. Res. Pub. No 221 (Revised). Missouri Agric. Exp. Sta. Bul. SB 1001, Columbia, MO. p. 53-58.

Eggleton, R.A., D. G. Schulze, and J.W. Stucki. 1988.Introduction to crystal structures of iron-containing minerals. In: J.W. Stucki et al., editors, Iron in soils and clay minerals. Reidel, Dordrecht, The Netherlands. p. 141-164.

Ferguson, R.B., C.A. Shapiro, A.R. Dobermann, and C.S. Wortmann. 2006. Fertilizer recommendations for soybeans, NebGuide G859. Available at: http://www.ianrpubs.unl.edu/pages/publicationD.jsp?publicationId=146 (accessed 5 Feb 2013). University of Nebraska, Lincoln, Nebraska.

The Fertilizer Institute. 2012. Fertilizer regulations. Available at: http://www.tfi.org/introduction- fertilizer/environmental-stewardship/fertilizer-regulations (accessed 17 Dec 2012). The Fertilizer Institute, Nourish, Replenish, Grow, Washinton, D.C.

Frank, K., D. Beegle, and J. Denning. 1998. Phosphorus. In: J.R. Brown et al., editors, Recommended chemical soil test procedures for the north central region. North Central Reg. Res. Pub. No 221 (Revised). Missouri Agric. Exp. Sta. Bul. SB 1001, Columbia, MO. p. 21-26.

Franzen, D.W. and J.L Richardson. 2000. Soil factors affecting iron chlorosis of soybean in the Red River Valley of North Dakota and Minnesota. J. Plant Nutr. 23:67-78.

Franzen, D.W., J.H. O'Barr, and R.K. Zollinger. 2003. Interaction of a foliar application of iron HEDTA and three postemergence broadleaf herbicides with soybeans stressed from chlorosis. J. Plant Nutr. 12:2365-2374.

Froehlich, D.M. and W.R. Fehr. 1981. Agronomic performance of soybeans with differing levels of iron deficiency chlorosis on calcareous soil. Crop Sci. 21:438-440.

Gelderman, R.H. and D. Beegle. 1998. Nitrate-nitrogen. In: J.R. Brown et al., editors, Recommended chemical soil test procedures for the north central region. North Central Reg. Res. Pub. No 221 (Revised). Missouri Agric. Exp. Sta. Bul. SB 1001, Columbia, MO. p. 17-20.

Geiger, S.C. and Loeppert, R.H. 1988. The effect of soil gas-phase CO2 concentration on Fe-deficiency chlorosis of soybean and sorghum grown in calcareous soils. J. Plant Nutr. 11:1493-1502

Gil-Ortiz, R. and I. Bautista-Carrascosa. 2004. Effect of Fe-EDDHA chelate application on evolution of soil extractable iron, copper, manganese, and zinc. Commun. Soil. Sci. Plant Anal. 35:559-570.

Gomez-Gallego, M., MA. Sierra, R. Alcaza, P. Ramirez, C. Pinar, M.J. Mancheno, S. Garcia-Marco, F. Yunta, and J.J. Lucena. 2002. Synthesis of o,p-EDDHA and its detection as the main impurity in o,o-EDDHA commercial iron chelates. J. Agric. Food Chem. 50:6395-6399.

Goos, R.J. and S. Germain. 2001. Solubility of twelve iron fertilizer products in alkaline soils. Commun. Soil. Sci. Plant Anal. 32:2317-2323.

Goos, R.J. and B. Johnson. 2000. A comparison of three methods for reducing iron-deficiency chlorosis in soybean. Agron. J. 92:1135-1139.

Goos R.J. and B. Johnson. 2001. Seed treatment, seeding rate, and cultivar effects on iron deficiency chlorosis of soybean. J. Plant Nutr. 24:1255-1268.

Goos, R.J. and B.E. Johnson. 2010. Evaluation of soybean cultivars for resistance to iron deficiency chlorosis in rows versus hills. J. Plant Nutr. 33:105-114.

Goos, R.J., B. Johnson, G. Jackson, and G. Hargrove. 2004. Greenhouse evaluation of controlled-release iron fertilizers for soybean. J. Plant Nutr. 27:43-55.

Hansen, N.C., M.A. Schmitt, J.E. Anderson, and J.S. Strock. 2003. Iron deficiency of soybean in the upper Midwest and associated soil properties. Agron. J. 95:1595-1601.

Hansen, N.C., V.D. Jolley, S.L. Naeve, and R.J. Goos. 2004. Iron deficiency of soybean in the north central U.S. and associated soil properties. Soil Sci. Plant Nutr. 50:983-987.

Hansen, N.C., B.G. Hopkins, J.W. Ellsworth, and V.D. Jolley. 2006. Iron nutrition in field crops. In: L.L. Barton and J. Abadia, editors, Iron nutrition in plants and rhizospheric microorganisms. Springer, Dordrecht, The Netherlands. p. 23-59.

Havlin, J.L, J.D. Beaton, S.L. Tisdale, and W.L. Nelson. 1999. Soil fertility and fertilizers an introduction to nutrient management. 6th ed. Prentice Hall, Upper Saddle River, NJ.

Hecht-Buchholz and U. Ortmann. 1986. Effect of foliar iron application on regreening and chloroplast development in iron-chlorotic soybean. J. Plant Nutr. 9:647-659.

Heenan, D.P. and L.C. Campbell. 1983. Manganese and iron interactions on their uptake and distribution. Plant Soil 70:317-326.

Heitholt, J.J., J.J. Sloan, C.T. MacKown, and R.I. Cabrera. 2003. Soybean growth on calcareous soil as affected by three iron sources. J. Plant Nutr. 26:935-948.

Hell, R. and U.W. Stephan. 2003. Iron uptake, trafficking and homeostasis in plants. Planta 216: 541-551.

Helms, T.C., R.A. Scott, W.T. Schapaugh, R.J. Goos, D.W. Franzen, and A.J. Schlegel. 2010. soybean iron-deficiency chlorosis tolerance and yield decrease on calcareous soils. Agron. J. 102:492-498.

Hernandez-Apaolaza, L., A. Alvarez-Fernandez, and J.J. Lucena. 2000. Chromatographic determination of commercial Fe(III)-chelates. J. Plant Nutr. 23:2035-2045.

Hernandez-Apaolaza, L., P. Barak, J.J. Lucena. 1997. Chormatographic determination of commercial Fe(III) chelates of ethylenediaminetetraactic acid, ethylenediaminedi(o-hydroxyphenylacetic) acid and ethylenediaminedi(o-hydroxy-p-hydroxyphenylacetic) acid and ethylenediaminedi(o-hydroxy-p-methylphenylacetic) acid. J. Chromatogr. 789:453-460.

Hernandez-Apaolaza, L., S. Garcia-Marco., P. Nadal, and J.J. Lucena. 2006. Structure and fertilizer properties of byproducts formed in the synthesis of EDDHA. J. Agric. Food Chem. 54:4355-4363.

Hernandez-Apaolaza, L. and J.J. Lucena. 2001. Fe(III)-EDDHA and –EDDHMA sorption on Ca-montmorillonite, ferrihydrite, and peat. J. Agric. Food Chem. 49:5258-5264.

Hernandez-Apaolaza, L. and J.J. Lucena. 2011. Influence of irradiation time and solution concentration on the photochemical degradation of EDDHA/Fe^{3+}: effect of its photodecomposition products on soybean growth. J. Sci Food Agric. 91:2024-2030.

Hocking, P.J. 1980. Redistribution of nutrient elements from cotyledons of two species of annual legumes during germination and seedling growth. Ann. Bot. 45:383-396.

Inskeep, W.P. and P.R. Bloom. 1984. A comparative study of soil solution chemistry associated with chlorotic and nonchlorotic soybeans in western Minnesota. J. Plant Nutr. 7:513-531.

Inskeep, W.P. and P.R. Bloom. 1986. Effects of soil moisture on soil pCO_2, soil solution bicarbonate, and iron chlorosis in soybeans. Soil Sci. Soc. Am. J. 50:946-952.

Juarez, M., D. Bermudez, J.D. Jorda, J. Sanchez-Andreu, and M.M. Cerdan. 2001. Effect of copper, nickel, zinc, and phosphorus on reactions of FeEDDHA and FeEDDHMA isomers under variable pH. Commun. Soil. Sci. Plant Anal. 32:509-519.

Kabata-Pendias, Alina. 2011. Trace elements in soils and plants. 4th ed. CRC Press, Taylor & Francis Group. Boca Raton, FL.

Kosegarten, H., B. Hoffmann, and K. Mengel. 2001. The paramount influence of nitrate in increasing apoplastic pH of young sunflower leaves to induce Fe deficiency chlorosis, and the re-greening effect brought about by acidic foliar sprays. J. Plant Nutr. Soil Sci. 164: 155-163.

Kosegarten, H., B Hoffmann, E. Rroco, F. Grolig, K.-H. Glusenkamp, and K. Mengel. 2004. Apoplastic pH and Fe^{III} reduction in youn sunflower (Helianthus annuus) roots. Physiol. Plant. 122:95-106.

Kroll, H., M. Knell, J. Powers, and J. Simonian. 1957. A phenolic analog of ethyenediamine-tetraacetic acid. J. Am. Chem. Soc. 79: 2023-2025.

Kruger, C., O. Berkowitz, U.W. Stephan, and R. Hell. 2002. A metal-binding member of the late embryogenesis abundant protein family transports iron in the phloem of *Ricinus communis* L. J. Biol. Chem. 277:25062-25069.

Lingenfelser, J.E., W.T. Schapaugh, Jr., and J.P. Schmidt. 2005. Comparison of genotype and cultural practices to control iron deficiency chlorosis in soybean. Commun. Soil. Sci. and Plant Anal. 36:1047-1062.

Lindsay, W.L. 1979. Chemical equilibria in soils. The Blackburn Press, Caldwell, New Jersey.

Lindsay, W.L. 1988. Solubility and redox equilibria of iron compounds in soils. In: J.W. Stucki et al., editors, Iron in soils and clay minerals. Reidel, Dordrecht, The Netherlands. p. 37-62.

Lindsay, W.L. and A.P. Schwab. 1982. The chemistry of iron in soils and its availability to plants. J. Plant Nutr. 5:821-840.

Loeppert, R.H. 1988. Chemistry of iron in calcareous systems. In: J.W. Stucki et al., editors, Iron in soils and clay minerals. Reidel, Dordrecht, The Netherlands. p. 689-713.

Loeppert, R.H. and D.L. Suarez. 1996. Carbonate and gypsum. In: D.L. Sparks, editor, Methods of soil analysis. SSSA book series, No. 5. SSSA, Madison, WI. p. 437-474.

Loeppert, R.H. and W.P Inskeep. 1996. Iron. In: D.L. Sparks, editor, Methods of soil analysis. SSSA book series, No. 5. SSSA, Madison, WI. p. 639-664.

Lucena, J.J. 2000. Effects of bicarbonate, nitrate and other environmental factors on iron deficiency chlorosis. A review. J. Plant Nutr. 23:1591-1606.

Lucena, J.J. 2003. Fe chelates for remediation of Fe chlorosis in strategy I plants. J. Plant Nutr. 26: 1969-1984.

Lucena, J.J., M. Manzanares, and A. Garate. 1992. A test to evaluate the efficacy of commercial Fe-chelates. J. Plant Nutr.15:1553-1566.

Marschner, H. 1986. Mineral nutrition in higher plants. Academic Press Inc., London, U.K.

Moraghan, J.T. 1979. Manganese toxicity in flax growing on certain calcareous soils low in available iron. Soil Sci. Soc. Am. J. 43:1177-1180.

Moraghan, J.T. 1991. Removal of endogenous iron, manganese and zinc during plant washing. Commun. Soil Sci. Plant Anal. 22:323-330.

Moraghan, J.T. and T. J. Freeman. 1978. Influence of FeEDDHA on growth and manganese accumulation in flax. Soil Sci. Soc. Am. J. 42:455-460.

Murad, E. and W.R. Fischer. 1988. The geobiochemical cycle of iron. In: J.W. Stucki et al., editors, Iron in soils and clay minerals. Reidel, Dordrecht, The Netherlands. p. 1-18.

National Agricultural Statistics Service-USDA. 2012a. Ranking North Dakota Agriculture. Available at: http://www.nass.usda.gov/Statistics_by_State/North_Dakota/Publications/Top_Commodities/pub/rank11.pdf (accessed 10 May 2012). USDA-NASS, North Dakota field Office, Fargo, ND.

National Agricultural Statistics Service-USDA. 2012b. Statistics by state, North Dakota. Available at http://www.nass.usda.gov/Statistics_by_State/North_Dakota/Search/index.asp (accessed 4 April 2012). USDA-NASS, North Dakota Field Office, Fargo, ND.

National Agricultural Statistics Service- USDA. 2012c. Statistics by subject. Available at http://www.nass.usda.gov/Statistics_by_Subject/index.php (accessed 4 April 2012). USDA-NASS, Washington, D.C.

Negishi, T., H. Nakanishi, J. Yazaki, N. Kishimoto, F. Fujii, K. Shimbo, K. Yamamoto, K. Sakata, T. Sasaki, S. Kikuchi, S. Mori, and N. K. Nishizawa. 2002. cDNA microarray analysis of gene expression during Fe-deficiency stress in barley suggests that polar transport of vesicles is implicated in phytosiderophore secretion in Fe-deficient barley roots. Plant J. 30:83-94.

North Dakota Department of Agriculture. 2010. NDDA Fertilizer Registration and Licensing Guidelines. Available at: http://www.nd.gov/ndda/general-resource/north-dakota-state-government/ndda-fertilizer-registration-and-licensing-guidelines (accessed 17 Dec 2012). North Dakota Department of Agriculture. Bismarck, North Dakota.

North Dakota Legislative Council. 2012. Chapter 19-20.1 Fertilizer and Soil Conditioner Law. Available at: http://www.legis.nd.gov/cencode/t19c20-1.pdf (accessed 17 Dec 2012). North Dakota Legislative Branch. Bismarck, North Dakota.

Orphanos, P.I. and C. Hadjiloucas. 1984. A laboratory test for screening iron chelates for use in alkaline soils. Plant Soil 77:401-404.

Petree, H.E., L. Myatt, and A.M. Jelenevsky. 1978. Preparation of phenolic ethylenediaminepolycarboxylic acids. U.S. Pantent 4,139,582. Date issued: 19 January.

Podrebarac, F.A. 2011. The relative nitrogen fixation rate and colonization of arbuscular mycorrhizal fungi of iron deficient soybeans. M.S. thesis. North Dakota State Univ., Fargo, North Dakota.

Ranieri, A., A. Castagna, B. Baldan, L., Sebastiani, and G.F. Soldatini. 2003. H_2O_2 accumulation in sunflower leaves as a consequence of iron deprivation. J. Plant Nutr. 26: 2187-2196.

Rodriguez-Lucena, P., L.Hernandez-Apaolaza, J.J. Lucena. 2010a. Comparison of iron chelates and complexes supplied as foliar sprays and in nutrient solution to correct iron chlorosis of soybean. J. Plant Nutr. Soil Sci. 173: 120-126.

Rodriguez-Lucena, P., E. Ropero, L. Hernandez-Apaolaza, J.J. Lucena. 2010b. Iron supply to soybean plants through the foliar application of IDHA/Fe^{3+}: effect of plant nutritional status and adjuvants. J. Sci. Food Agric. 90:2633-2640.

Rombola, A.D., and M. Tagliavini. 2006. Iron nutrition of fruit tree crops. In: L.L Barton and J. Abadia, editors, Iron nutrition in plants and rhizospheric microorganisms. Springer, Dordrecht, The Netherlands. p. 61-83.

Romheld, V. 1991. The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: An ecological approach. Plant Soil 130:127-134.

Romheld, V. and H. Marschner. 1984. Plant-induced pH changes in the rhizosphere of "Fe-efficient" and "Fe-inefficient" soybean and corn cultivars. J. Plant Nutr. 7:623-630.

Romheld, V. and H. Marschner, 1986. Evidence for specific uptake system for iron phytosiderophores in roots of grasses. Plant Physiol. 80:175-180.

Romheld, V. and H. Marschner. 1990. Genotypical differences among graminaceous species in release of phytosiderophores and uptake of iron phytosiderophores. Plant Soil 123:147-153.

Rojas, C. L., F. J. Romera, E. Alcantara, R. Perez-Vicente, C. Sariego, J. I. Garcia-Alonso, J. Boned, G. Marti. 2008. Efficacy of Fe(o,o-EDDHA) and Fe(o,p-EDDHA) isomers in supplying Fe to strategy I plants differs in nutrient solution and calcareous soil. J. Agric. Food Chem. 56:10774-10778.

Schaetzl, R. and S. Anderson. 2005. Soils: genesis and geomorphology. Cambridge University Press, New York.

Schenkeveld, W.D.C. 2010. Iron fertilization with FeEDDHA the fate and effectiveness of FeEDDHA chelates in soil-plant systems. Ph.D. diss. Wageningen Univ., Wageningen, Netherlands.

Schwertmann, U. and R.M. Taylor. 1989. Iron oxides. In: J.B. Dixon and S.B Weed, editors, Minerals in soil environments. 2nd edition. SSSA book series No. 1. SSSA, Madison, WI. p. 379-438.

Siebner-Freibach, H., Y. Hadar, and Y. Chen. 2004. Interaction of iron chelating agents with clay minerals. Soil Sci. Soc. Am J. 68:470-480.

Soil Science Society of America. 2011. Glossary of soil science terms 2008. SSSA, Madison, WI.

Soil Survey Staff. 2005. Web soil survey: official series description. USDA-NRCS. https://soilseries.sc.egov.usda.gov/OSD_Docs/G/GLYNDON.html (accessed 22 Jan. 2013).

Stookey, L.L. 1970. Ferrozine – A new spectrophotometric reagent for iron. Anal. Chem. 42:779-781.

Tan, K.H.1998. Principles of soil chemistry. 3rd ed. Marcel Dekker, Inc., New York, NY.

Technical Committee CEN/TC 260. 2007. Fertilizers – determination of chelating agents in fertilizers by chromatography – part 2: Determination of Fe chelated by o,o-EDDHA and o,o-EDDHMA by ion pair chromatography. prEN 13368-2:2007. European Commission and the European Free Trade Association, Brussels, Belguim.

Technical Committee CEN/TC 260. 2011. Fertilizers - determination of chelating agents in fertilizers by chromatography - Part 2: Determination of Fe chelated by o,o-EDDHA, o,o-EDDHMA and HBED by ion pair chromatography. prEN 13368-2:2011. European Commission and the European Free Trade Association, Brussels, Belguim.

Terry, R.E. and V.D. Jolley. 1994. Nitrongenase activity is required for the activation of iron-stress response in iron-inefficient T203 soybean. J. Plant Nutr. 17:1417-1428.

Tewari, R.K., P. Kumar, and N.P.N. Sharma. 2005. Signs of oxidative stress in the chlorotic leaves of iron starved plants. Plant Sci. 169:1037-1045.

University of Wisconsin, Soil and Plant Analysis Laboratory. 2005. Standard Operation Procedure: Analysis of major, minor, and trace elements in plant tissue samples with ICP-OES and ICP-MS. University of Wisconsin. Madison, WI.

Wallace, A. and G.V. Alexander. 1973. Manganese in plants as influenced by manganese and iron chelates. Commun. Soil Sci. Plant Anal. 4:51-56.

Wallace, A., E. Frolich, and R.T. Mueller. 1967. Effect of solution pH on sunlight stability of FeEDDHA. Agron. J. 59:86.

Warncke, D. and J.R. Brown. 1998. Potassium and other basic cations. In: J.R. Brown et al., editors, Recommended chemical soil test procedures for the north central region. North Central Reg. Res. Pub. No 221 (Revised). Missouri Agric. Exp. Sta. Bul. SB 1001, Columbia, MO. p. 31-33.

Watson, M.E. and J.R. Brown. 1998. pH and lime requirement. In: J.R. Brown et al., editors, Recommended chemical soil test procedures for the north central region. North Central Reg. Res. Pub. No 221 (Revised). Missouri Agric. Exp. Sta. Bul. SB 1001, Columbia, MO. p. 13-16.

Whitney, D.A. 1998a. Micronutrients: zinc, iron, manganese, and copper. In: J.R. Brown et al., editors, Recommended chemical soil test procedures for the north central region. North Central Reg. Res. Pub. No 221 (Revised). Missouri Agric. Exp. Sta. Bul. SB 1001, Columbia, MO. p. 41-44.

Whitney, D.A. 1998b. Soil salinity. In: J.R. Brown et al., editors, Recommended chemical soil test procedures for the north central region. North Central Reg. Res. Pub. No 221 (Revised). Missouri Agric. Exp. Sta. Bul. SB 1001, Columbia, MO. p. 59-60.

Wiersma, J.V. 2005. High rates of Fe-EDDHA and seed iron concentration suggest partial solutions to iron deficiency in soybean. Agron. J. 97:924-934.

Wiersma, J.V. 2007. Iron acquisition of three soybean varieties grown at five seeding densities and five rates of Fe-EDDHA. Agron. J. 99:1018-1028.

Wiersma, J.V. 2010. Nitrate-induced iron deficiency in soybean varieties with varying iron-stress responses. Agron. J. 102:1738-1744.

Wikoff, L. and J.T. Moraghan. 1986. Different iron-manganese relationships in two flax cultivars. J. Plant Nutr. 9:839-849.

Yunta, F., S. Garcia-Marco, J.J. Lucena, M. Gomez-Gallego, R. Alcazar, and M.A. Sierra. 2003a. Chelating agents related to ethylenediamine Bis(2-hydroxyphenyl)acetic acid (EDDHA): synthesis, characterization, and equilibrium studies of the free ligands and their Mg^{2+}, Ca^{2+}, Cu^{2+}, Fe^{3+} chelates. Inorg. Chem. 42:5412-5421.

Yunta, F., S. Garcia-Marco, and J.J. Lucena. 2003b. Theoretical speciation of ethylenediamine-N-(o-hydroxyphenylacetic)-N-(p-hydroxyphenylacetic) acid (o,p-EDDHA) in agronomic conditions. J. Agric. Food Chem. 51:5391-5399.

APPENDIX A. LINEAR CORRELATIONS FOR SOIL STABILITY TEST

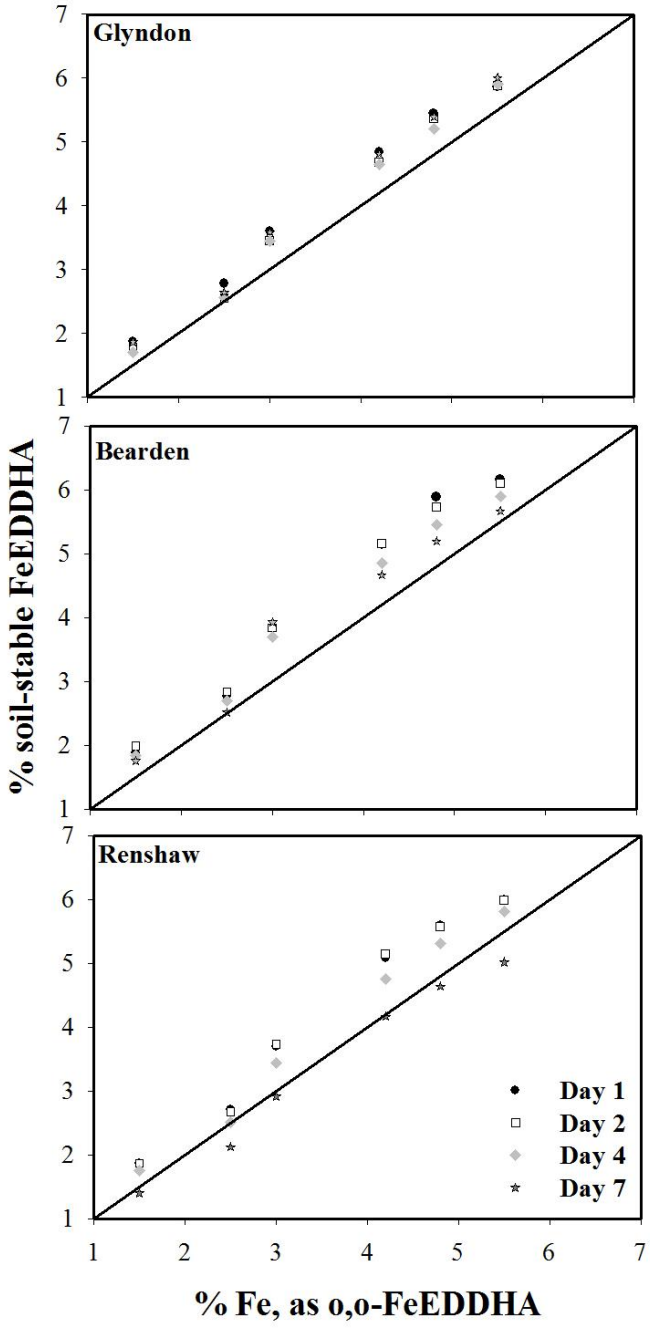

Figure A1. Apparent percentage of "soil-stable" Fe in six fertilizers for three different soils, evaluated by the field capacity incubation method, and analyzed by direct spectroscopy compared to their o,o-FeEDDHA concentrations determined by HPLC.

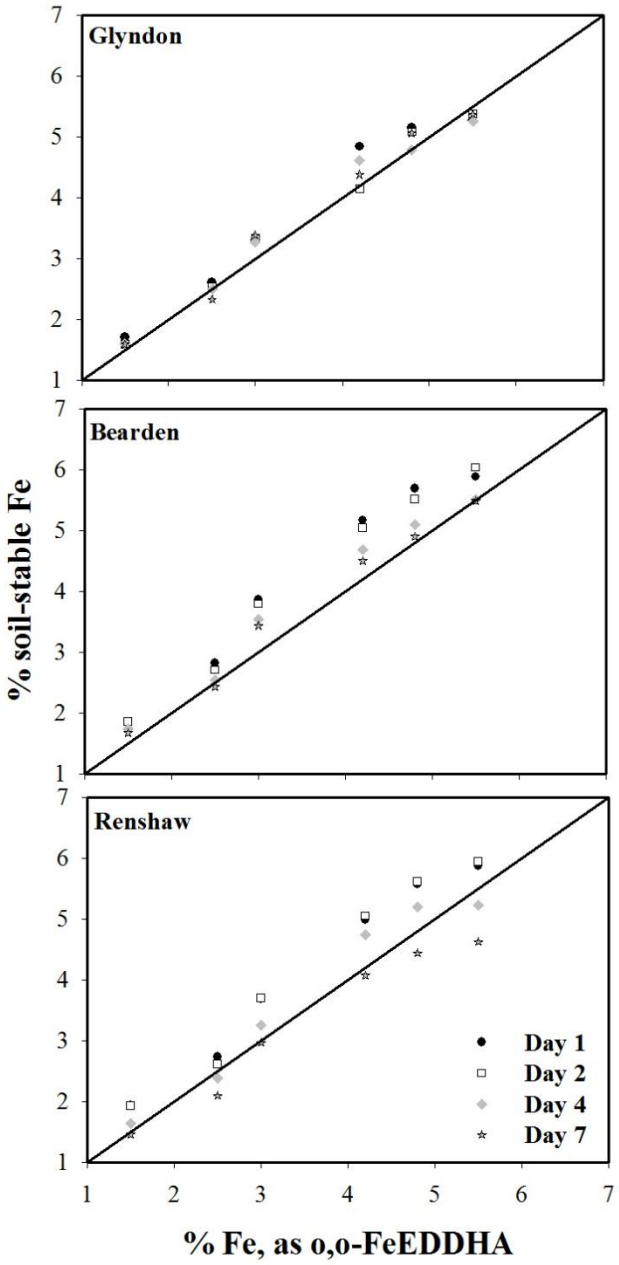

Figure A2. Apparent percentage of "soil-stable" Fe in six fertilizers for three different soils, evaluated with the field capacity incubation method, and analyzed for the ferrozine chromophore by spectroscopy compared to their o,o-FeEDDHA concentrations determined by HPLC.

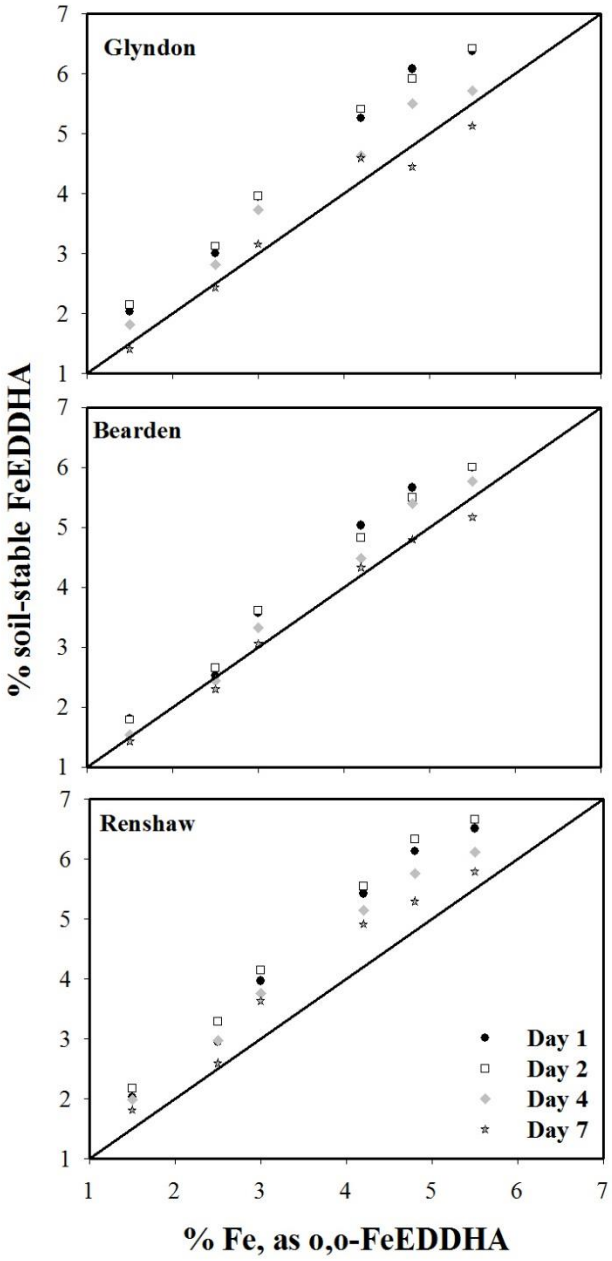

Figure A3. Apparent percentage of "soil-stable" Fe in six fertilizers for three different soils, evaluated with the shaking incubation method, and analyzed by direct spectroscopy compared to their o,o-FeEDDHA concentrations determined by HPLC.

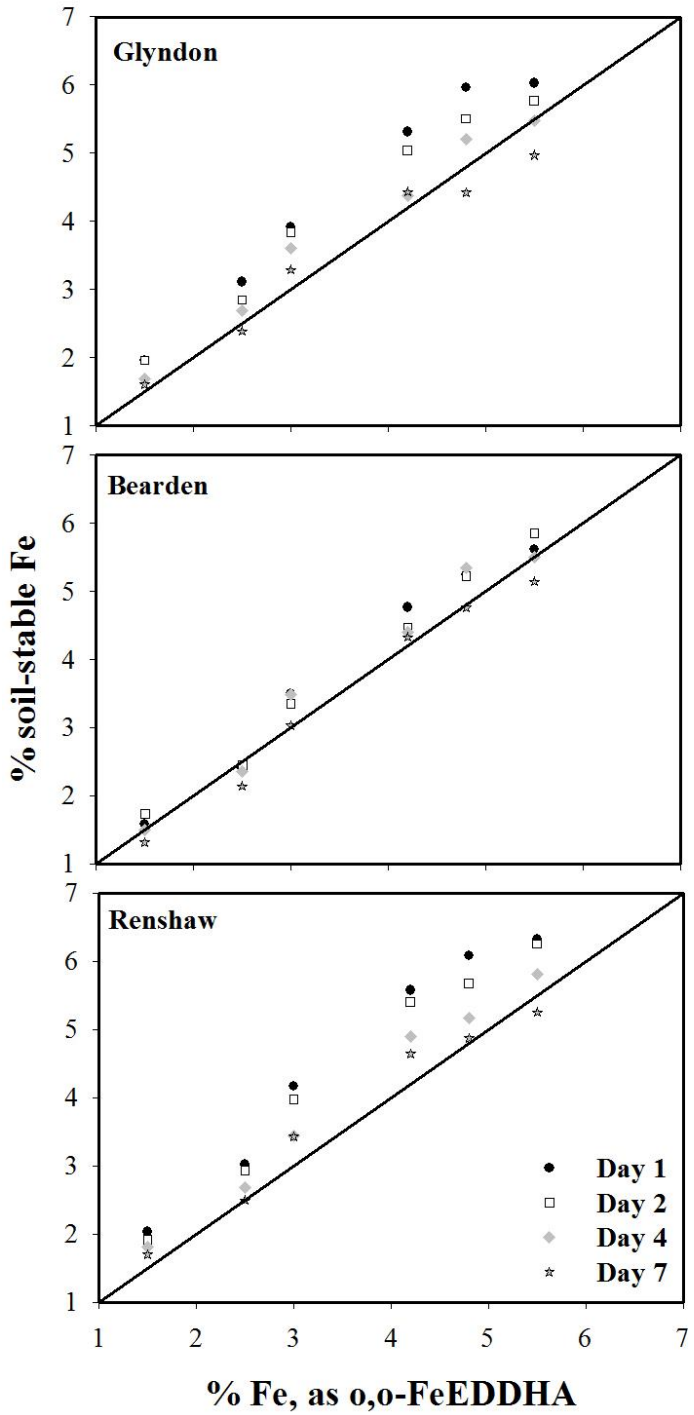

Figure A4. Apparent percentage of "soil-stable" Fe in six fertilizers for three different soils, evaluated with the shaking incubation method, and analyzed by spectroscopy for the ferrozine chromophore compared to their o,o-FeEDDHA concentrations determined by HPLC.

APPENDIX B. ILLUSTRATION OF SOYBEANS TREATED WITH DIFFERENT RATES OF o,o-FeEDDHA

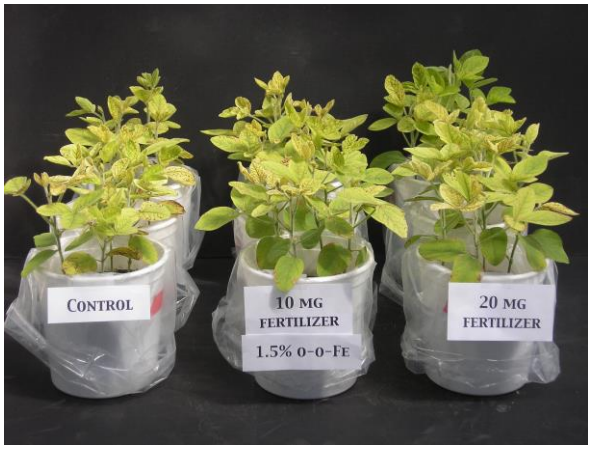

Figure B1. Greenhouse crop 1 control treatment and FeEDDHA-1 treatment applied at 10 mg and 20 mg of FeEDDHA.

Figure B2. Greenhouse crop 1 control treatment and FeEDDHA-2 treatment applied at 10 mg and 20 mg of FeEDDHA.

Figure B3. Greenhouse crop 1 control treatment and FeEDDHA-3 treatment applied at 10 mg and 20 mg of FeEDDHA.

Figure B4. Greenhouse crop 1 control treatment and FeEDDHA-4 treatment applied at 10 mg and 20 mg of FeEDDHA.

Figure B5. Greenhouse crop 1 control treatment and FeEDDHA-5 treatment applied at 10 mg and 20 mg of FeEDDHA.

Figure B6. Greenhouse crop 1 control treatment and FeEDDHA-6 treatment applied at 10 mg and 20 mg of FeEDDHA.

Figure B7. Greenhouse crop 2 control treatment and FeEDDHA-1 treatment applied at 10 mg and 20 mg of FeEDDHA.

Figure B8. Greenhouse crop 2 control treatment and FeEDDHA-2 treatment applied at 10 mg and 20 mg of FeEDDHA.

Figure B9. Greenhouse crop 2 control treatment and FeEDDHA-3 treatment applied at 10 mg and 20 mg of FeEDDHA.

Figure B10. Greenhouse crop 2 control treatment and FeEDDHA-4 treatment applied at 10 mg and 20 mg of FeEDDHA.

Figure B11. Greenhouse crop 2 control treatment and FeEDDHA-5 treatment applied at 10 mg and 20 mg of FeEDDHA.

Figure B12. Greenhouse crop 2 control treatment and FeEDDHA-6 treatment applied at 10 mg and 20 mg of FeEDDHA.