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Iron Chelates Alleviate Iron Chlorosis in Soybean on High pH Soils

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ABSTRACT

High pH soils frequently lead to iron deficiency chlorosis (IDC) in soybean [*Glycine max* (L.) Merr.]. As a result, yields for soybean are often reduced. Studies in the north-central United States have shown improvements in grain yield after applying Fe chelates on calcareous soils, but this practice has not been evaluated in a southern climate. Two sites within the Blackbelt region of Alabama were evaluated for response to Fe-EDDHA, Fe-Citrate, and $FeSO_4$ for their effect on yield and chlorosis in 2010, 2011, and 2012. Treatments were applied in-furrow at planting, as a foliar spray at the V3 growth stage, or as a split-application. Remote sensing, relative chlorophyll meter readings, and visual chlorosis scores (VCS) were assessed as methods for identifying degree of Fe chlorosis. At the location where IDC was most pronounced, all treatments of Fe-EDDHA were effective at reducing VCS ratings when applied in-furrow at planting; however, chlorosis evaluation through remote sensing and relative chlorophyll readings was not able to detect improvements in chlorosis measured with VCS. Treatments of Fe-EDDHA at 4.5 kg product ha^{-1} increased yield whether applied as an in-furrow at planting treatment or as a split application at planting and at V3 growth stage as a foliar spray. Treatments of Fe-Citrate and $FeSO_4$ did not improve yield as applied in this study. Results suggest that Fe-EDDHA can be used in southern climates as a strategy to overcome IDC yield limitations.

Iron deficiency chlorosis is a problem in high pH, calcareous soils that often leads to yield reduction. Solubility of Fe in these soils is very low, and high levels of native bicarbonate provide a difficult environment for plant-mediated Fe reduction mechanisms (Inskeep and Bloom, 1987; Hansen et al., 2003). A continuous supply of Fe is needed by the plant for chlorophyll production, nodulation, and N fixation for optimal plant growth. There are two major strategies that improve plant acquisition of Fe when Fe is limiting for growth (Marschner et al., 1986). Strategy I plants (e.g., dicots, non-graminaceous monocots) acidify the rhizosphere by releasing protons and enhancing Fe(III) reduction to Fe(II) (Marschner and Römheld, 1994). Some Strategy I plants also release chelating agents to increase the solubility of Fe. Strategy II plants (e.g., graminaceous monocots) release phytosiderophores to complex Fe(III) and take-up the phytosiderophore-iron chelate through a specific uptake system. Due to the high selectivity of the phytosiderophore for Fe, Strategy I plants are more greatly affected by reduced Fe availability caused by high pH and calcareous soils than Strategy II plants.

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Yield losses in soybean, a Strategy I plant, are often reported in the north-central region of the United States as a result of IDC (Goos and Johnson, 2000; Hansen et al., 2003; Helms et al., 2010). Producers in western Minnesota estimate an annual yield loss of 0.8 Mg ha^{-1} for chlorotic soybean grown on calcareous soils (Hansen et al., 2003). Producers in the Southeast also suffer yield losses from IDC in regions where high pH, calcareous soils are prevalent, such as the Blackbelt region of Alabama (Campbell and Seymour, 2011). Iron deficiency is particularly challenging to treat due to the variability of pH and carbonates within a location (Hansen et al., 2003; Helms et al., 2010). In addition, soil NO_3^- concentration may affect IDC (Wiersma, 2007). During NO_3^- acquisition, OH^- or HCO_3^- ions are often released in exchange for NO_3^-, which can reduce solubility of Fe(III) and limit its reduction (Aktas and van Egmond, 1979).

Selection of Fe-efficient cultivars is often the most practical way to combat IDC (Niebur and Fehr, 1981; Goos and Johnson, 2000; Wiersma, 2005). Cultivars that are less susceptible to IDC have a better ability to reduce Fe^{3+} to Fe^{2+} (O'Rourke et al., 2007). However, variety trials to identify IDC-resistant cultivars often produce inconsistent results (Naeve and Rehm, 2006). Furthermore, breeding programs are often focused on issues associated with production and on varieties grown in major soybean-producing areas, rather than in the southeastern United States. Without breeding efforts, other strategies are needed to improve Fe acquisition, such as use of synthetic chelate fertilizers.

Abbreviations: IDC, iron deficiency chlorosis; NDVI, normalized difference vegetation index; VSC, visual chlorosis score.

Synthetic chelates are often used to increase solubility of metal cations to make them more plant available. Chelates such as EDTA, DTPA, and EDDHA have a relatively high specificity for Fe^{3+} and are often used for improving IDC in Strategy I plants (Lucena et al., 1992), but are considered much less effective for Strategy II plants (Römheld and Marschner, 1986). Iron-EDDHA is commonly considered the most stable of these chelates in calcareous soils due to its stronger affinity for Fe^{3+} compared to other cations such as Ca^{2+} (Norvell, 1972; Reed et al., 1988). Studies in north-central United States have shown that seed, soil, or foliar application of Fe chelates can improve chlorophyll content and grain yield of soybean grown on calcareous soils (Karkosh et al., 1988; Goos and Johnson, 2000; Wiersma, 2007), particularly when applied before the onset of chlorosis (Schenkeveld et al., 2008). Wiersma (2007) obtained increases in grain yield of 15% with seed-applied Fe-EDDHA. Soil-applied Fe-Citrate (Aly and Soliman, 1998) and foliar-applied Fe-EDTA (Goos and Johnson, 2000) have also shown some efficacy to ameliorate IDC. Much of the research on Fe chelates has shown small or inconsistent increases in productivity for Fe-deficient soybean (Aly and Soliman, 1998; Wiersma, 2005). Often improvements are more pronounced in cultivars that are susceptible to IDC (Goos and Johnson, 2000; Wiersma, 2007). Research is lacking to determine the efficacy of Fe chelates in the southeastern United States, where higher soil temperatures are likely to enhance degradation of chelates resulting in the release and immobilization of Fe.

Iron deficiency chlorosis symptoms most often appear as interveinal chlorosis at the first trifoliate stage. When IDC is severe, it can result in necrosis of the leaves or death of the plant (Franzen et al., 2004). Visual chlorosis scores are often used to assess degree of chlorosis and are correlated with yield reduction of soybean. Froechlich and Fehr (1981) observed a 20% decrease in grain yield of soybean for every unit increase in visual chlorosis score on a 1 to 5 scale in Iowa. Chlorophyll meters (Varvel et al., 1997; Solari et al., 2008; Kitchen et al., 2010) and canopy sensors (Teal et al., 2006; Barker and Sawyer, 2010; Scharf et al., 2011) are other potentially more objective methods for measuring chlorosis that are currently used to predict crop N needs. Chlorophyll meters measure red and near-infrared transmittance through the leaf, which is highly correlated with chlorophyll content of plant material (Markwell et al., 1995). Canopy sensors measure the red and near-infrared reflectance above the crop canopy and have been used to predict crop yield potential (Teal et al., 2006). Because Fe is required for chlorophyll synthesis, IDC also impacts chlorophyll contents and causes stunting of the plant. Chlorophyll meters and canopy sensors may be useful for predicting yield potential of soybean through their assessment of chlorophyll content and canopy cover.

The primary objective of this study was to evaluate the effectiveness of Fe chelates and supplements to alleviate IDC in soybean grown in high pH soils of Alabama. In addition, the use of remote sensing and chlorophyll meters were compared with visual ratings for determining degree of chlorosis in soybean.

MATERIALS AND METHODS

Two sites within the Blackbelt region of Alabama were selected for a 3-yr evaluation from 2010 to 2012 at Blackbelt Research and Extension Center (BBREC) in Marion Junction, AL (32°27′ N, 87°14′ W), and Givhan Farms (GIV) in Safford, AL (32°19′ N, 87°20′ W). Both locations were characterized by high pH soils that have been previously affected by IDC in soybean. Soils at the BBREC are classified as Sumter fine-silty, carbonatic, thermic Rendollic Eutrudepts. Soils at the GIV site are classified as Leeper fine, smectitic, nonacid, thermic Vertic Epiaquepts. Soil chemical properties are outlined in Table 1.

Nine treatments were organized in randomized complete block design and replicated four times. Each replication consisted of four-row plots, 6.1 m long with 0.76 m row spacing with the exception of BBREC in 2010 and 2011 in which a 0.91 m row spacing was used. A moderately IDC-sensitive variety was used, Pioneer 95M50 (maturity group 5.5). Seeds were pretreated with Pioneer FST/IST/Moly (Dupont, Wilmington, DE) (fungicide, insecticide, and molybdenum treatment) per manufacturer's instructions and planted at a rate of 450,000 seed ha^{-1} based on Alabama Cooperative Extension System recommendations (Glass et al., 2012). Both sites were managed with conventional tillage (e.g., disk, harrow). At BBREC, trials were planted on 14 May, 18 May, and 21 May for 2010, 2011, and 2012, respectively. At GIV, trials were planted on 17 May, 18 May, and 21 May for 2010, 2011, and 2012, respectively. Fertilizer and pesticide were applied according to Alabama Cooperative Extension System recommendations (Batchelor, 2013; Everest et al., 2014).

Five Fe-EDDHA treatments, two Fe-Citrate treatments, and one $FeSO_4$ treatment were used in this study in addition to an untreated control. The five treatments of Fe-EDDHA included: three in-furrow treatments of 2.2, 3.4, 4.5 kg product ha^{-1} (FeEDDHA-AP_1, FeEDDHA-AP_2, FeEDDHA-AP_3), a foliar spray of 2.2 kg product ha^{-1} (FeEDDHA-FS), and a split-application of 4.5 kg product ha^{-1} divided equally between in-furrow treatment and a foliar spray (FeEDDHA-SP). The product was labeled as 6% Fe in the form of *ortho-ortho* FeEDDHA. Two treatments of Fe-Citrate were applied at a rate of 2.2 kg product ha^{-1}, one in-furrow (FeCitrate-AP) and one as a split-application divided equally between in-furrow treatment and a foliar spray (FeCitrate-SP). The product was labeled as 20% Fe in the form of Fe citrate salt. One treatment of $FeSO_4$ at 4.5 kg product ha^{-1} ($FeSO_4$–FS) was applied as a foliar spray. The product was labeled as 20% Fe in the form of ferrous sulfate. All in-furrow treatments were applied at planting, and all foliar sprays were applied at the second trifoliate (V3) growth stage (Fehr and Caviness, 1977). Foliar sprays were applied using a backpack CO_2 boom sprayer equipped with 4 TeeJet (Spraying Systems Co., Wheaton, IL) XR 11002-VS nozzles that were approximately 50 cm apart. The sprayer was held approximately 45 cm above the crop canopy of the two interior rows of each plot.

SOIL AND PLANT MATERIAL COLLECTION

Before planting each year, 12 soil cores were taken in each plot to a 15-cm depth using a hand-held 4.0 cm diam. soil probe. These samples were mixed to form one composite sample for each plot. Soil samples were oven-dried at 60°C until constant mass, ground, and saved for laboratory analysis.

The uppermost fully developed leaf was collected from 10 randomly chosen soybean plants in the two interior rows of each four-row plot at the V3 stage and 15 d later at the V5 stage (Fehr and Caviness, 1977). These 10 leaves were mixed to form

Table 1. Soil properties including pH, DTPA-extractable Fe, Mississippi-extractable Fe, and calcium carbonate equivalence (CCE) from study sites located at Blackbelt Research and Extension Center (BBREC) and Givhan Farm (GIV) in western Alabama for three sampling years.

Location	Year	pH	DTPA-extractable Fe	Mississippi-extractable Fe	CCE
			mg kg^{-1}		g kg^{-1}
BBREC	2010	8.2	6.7	38.0	684.7
	2011	8.2	4.8	42.4	656.9
	2012	8.2	11.9	64.8	651.9
GIV	2010	7.9	14.6	152.4	9.3
	2011	7.9	11.0	143.0	7.3
	2012	7.9	32.6	234.7	8.6

one composite sample for each plot. Leaf samples taken at the V3 growth stage were collected directly before applying foliar spray treatments. Samples were washed three times with deionized water, dried at 60°C, and saved for leaf tissue analysis.

Grain yield was determined by machine harvesting seed from the entire length of the two interior rows for each plot and adjusting to 13% moisture content. At BBREC, plots were harvested on 29 September, 17 October, and 17 October for 2010, 2011, and 2012, respectively. At GIV, plots were harvested on 22 September, 4 October, and 17 October for 2010, 2011, and 2012, respectively. Seed mass was determined by weighing 100 randomly selected oven-dried (40°C) seed from each plot. Seed were saved for seed tissue analysis.

Soil Analysis

Soil pH was evaluated for a 1:1 (v/w) mixture of deionized water and soil. Soils were extracted using diethylene triamine pentaacetic acid (DTPA) and Landcaster (Mississippi) soil test methods for soil Fe. Measurement of DTPA-extractable Fe has been identified as a useful tool for predicting IDC (Hansen et al., 2003; Wiersma, 2007). The Mississippi soil extraction method is commonly used for calcareous soils in Alabama to determine plant-available nutrient concentrations. The DTPA-Fe was extracted using a 2:1 extractant to soil (v/w) mixture, following a procedure by Loeppert and Inskeep (1996). Extracts with DTPA were analyzed using an atomic adsorption spectrophotometer (Video 12 AA/AE spectrophotometer, Instrumentation Laboratory, Lexington, MA) to determine Fe content. Mississippi-Fe was extracted using a 2:1 extractant to soil (v/w) mixture, following a procedure by Hue and Evans (1979). Mississippi solutions were analyzed on inductively coupled plasma–optical emission spectrometry (ICP–OES) (Spectro Ciros ICP, SPECTRO Analytical Instruments, Kleve, Germany) to determine Fe content. Soil calcium carbonate equivalent (CCE) was determined using a method by Allison and Moodie (1965). Nitrate was extracted in a 4:1 water to soil (v/w) mixture according to a procedure by Teem (1986), followed by analysis on IC (Dionex ICS-3000, Dionex Corporation, Sunnyvale, CA).

Plant Material Analysis

Leaves collected from V3 and V5 growth stages and seed were ground and microwave digested in concentrated nitric acid. Microwave digestion of plant material followed USEPA Method 3051A (USEPA, 2007). After digestion, samples were analyzed on ICP–OES to determine leaf and seed Fe content.

Chlorosis Evaluation

Relative leaf chlorophyll content was measured using a Chlorophyll Meter SPAD-502 (Konica Minolta, Osaka, Japan). Three chlorophyll meter readings were taken and averaged from the uppermost fully developed leaf of 10 randomly selected soybean plants in each plot. Normalized difference vegetation index (NDVI) was used as an additional method for detecting IDC by measuring by reflectance above the crop canopy. A Greenseeker canopy sensor (NTech Industries, Inc., Ukiah, CA) was used to determine NDVI by hand-pulling a bicycle-mounted sensor down the entire length of the two interior rows of each plot at approximately 3 km h^{-1}. The sensor was held at a constant 80 cm directly above the crop canopy. Relative leaf chlorophyll content and NDVI measurements were taken twice during the growing season, at the V3 and V5 growth stages.

Visual chlorosis scores were also performed at the V3 and V5 growth stages, without knowledge of the treatment that was being rated, and followed a method used by Auburn University Department of Crop, Soil and Environmental Science for variety trial screening (Glass et al., 2012). This method uses a scale of 1 through 10, where 1 = no chlorosis and 10 = severe chlorosis with stunted growth and necrosis or death of entire plant. The same person was responsible for all VCS scoring at both locations for this 3-yr study. The individual rated the two interior rows of each plot.

Data Analysis

Data were subject to ANOVA using mixed models methodology as implemented in the MIXED procedure of SAS (SAS version 9.3; SAS Institute, 2011). Year and block (location × year) were treated as random effects, whereas treatment and location as well as their interaction were treated as fixed effects. From the ANOVA, variables and their interactions were examined at $\alpha = 0.05$. Treatments were compared to the untreated control using Dunnett's test, and significance was determined at $\alpha = 0.05$. A simple pairwise comparison was used to compare locations. The normality assumption was observed to be valid based on the StudentPanel output in the above mentioned procedure. Similarly, using the group option to model the residual variance structure, the homogeneity of variances hypotheses could not be rejected for treatments or locations.

The effect of rate of FeEDDHA applied at planting in-furrow at V3 and V5 growth stages was also modeled within a mixed models environment using the linear model: response = location + rate(location). Simple Pearson product moment correlations were calculated from raw data across all locations and years. The reader should be aware that *P* values for correlation coefficients are highly influenced by the number of data pairs. With >200 pairs even a small correlation ($r < 0.15$) will be declared significant. Correlation was not discussed if $r < 0.5$.

RESULTS AND DISCUSSION

Soil Properties

Average soil pH was 8.2 at BBREC and 7.9 at GIV (Table 1). At BBREC, soil DTPA-extractable Fe averaged at 7.8 mg kg^{-1}, while GIV averaged 19.3 mg kg^{-1}. These values are similar to those obtained by Inskeep and Bloom (1987), who observed DTPA-extractable Fe concentrations of 10 to 20 mg kg^{-1} on

Table 2. Summary of ANOVA in response to treatment (i.e.,Fe-EDDHA, Fe-citrate, or $FeSO_4$), location, and their interaction for visual chlorosis score (VCS), normalized difference vegetation index (NDVI), chlorophyll meter readings (SPAD), grain yield, seed mass, leaf Fe concentration (leaf Fe), and seed Fe concentration (seed Fe). Analysis for VCS, NDVI, SPAD, and leaf Fe are given for V3 and V5 growth stages (Fehr and Caviness, 1977).

		ANOVA, $P > F$										
Source of variance	df	VCS (V3)	VCS (V5)	NDVI (V3)	NDVI (V5)	SPAD (V3)	SPAD (V5)	Grain yield	Seed mass	Leaf Fe (V3)	Leaf Fe (V5)	Seed Fe
Location (L)	1	0.003	0.020	<0.001	0.005	<0.001	0.537	0.001	<0.001	0.024	0.386	0.663
Treatment (T)	8	<0.001	0.001	0.651	0.009	0.359	0.492	0.045	0.208	0.120	0.030	0.981
L × T	8	0.004	0.033	0.395	0.620	0.525	0.490	0.343	0.654	0.437	0.057	0.903

calcareous soils affected by IDC. Similar results were observed using the Mississippi-extractable Fe test, where extractable Fe was lower ($P = 0.010$) at the BBREC site compared to GIV (48.4 mg kg^{-1} at BBREC compared to 176.7 mg kg^{-1} at GIV). Although both locations were comprised of high-pH soils, plant-available Fe using both DTPA and Mississippi methods was lower at BBREC. Inskeep and Bloom (1987) determined that measurements of pH and DTPA-extractable Fe alone were insufficient to predict whether IDC would occur; concentrations of HCO_3^- and $CaCO_3$ were better predictors. Calcium carbonate equivalence was greater in soils at BBREC (657 g kg^{-1}) than GIV (9.17 g kg^{-1}; $P < 0.0001$). Inskeep and Bloom (1987) observed a CCE ranging between 70 and 130 g kg^{-1} in soils associated with IDC and 6 to 30 g kg^{-1} for adjacent soils not associated with IDC. Thus, all four predictors of plant-available Fe that were used (i.e., pH, DTPA-extractable Fe, Mississippi-extractable Fe, and CCE) indicated that Fe availability would be more limiting at BBREC compared to GIV.

The soil NO_3^- concentration averaged at 10.2 mg kg^{-1} for BBREC and 17.1 mg kg^{-1} for GIV. Aktas and van Egmond (1979) did not observe a long-term effect of NO_3^- on chlorosis at NO_3^- levels of 83 mg kg^{-1}, but at 170 mg kg^{-1} plants remained chlorotic until harvest. While uptake of NO_3^- may reduce the plant's Fe-deficiency acquisition strategies, the NO_3^- levels in the current study were likely not high enough to contribute to IDC.

Visual Chlorosis Scoring

Visual chlorosis scores ranged from 3.8 to 6.6 at BBREC and 2.8 to 4.6 at GIV. The degree of chlorosis was consistently greater at BBREC compared to GIV and can be partially explained by soil test results that indicated lower plant-available Fe and greater CCE at BBREC. Similarly, Wiersma (2007) observed more severe chlorosis with decreased levels of DTPA-Fe and increased CCE in soils. A location × treatment interaction was observed for VCS scores at the V3 and V5 growth stage (Table 2). Visual chlorosis scores were improved for Fe-EDDHA in-furrow treatments ($FeEDDHA-AP_1$,$-AP_2$,$-AP_3$) at BBREC during the V3 growth stage (all $P < 0.038$) (Table 3). Karkosh et al. (1988) and Wiersma (2007) also observed improvements in chlorosis during the V3 growth stage when Fe-EDDHA was applied as a seed treatment. Treatments that contained only a foliar spray application in the current study did not show a reduction in chlorosis at the V3 stage; this was expected since foliar sprays were applied after sampling at the V3 stage.

At both locations, improvements in chlorosis were more commonly observed at the V5 growth stage. All treatments receiving Fe-EDDHA in-furrow (FeEDDHA-AP1,-AP2,-AP3,-SP) had a lower degree of chlorosis than the untreated check at the V5 stage (Table 3); however, results were only significant at BBREC (all $P < 0.028$). Foliar application of $FeSO_4$–FS was also effective at reducing degree of chlorosis at GIV ($P < 0.013$) during the V5 growth stage (Table 3). Improvements in degree of chlorosis with application of $FeSO_4$–FS suggest that foliar treatments of $FeSO_4$–FS may be plant available. Hecht-Buchholz and Ortmann (1986) also observed improvements in chlorosis 6 d after applying a foliar spray of $FeSO_4$ at the V5 growth stage of soybean. Improvements in degree of chlorosis were not observed in the other foliar treatments (i.e., FeCitrate-SP and FeEDDHA-FS) or in the FeCitrate-AP application. In contrast, Aly and Soliman (1998) reported increased chlorophyll content with the application of citric acid coupled with soil-applied $FeSO_4$ in a greenhouse experiment.

Treatments of Fe-EDDHA applied in-furrow at planting were the most effective for reducing degree of chlorosis. Responses to Fe application were observed at the location where chlorosis was most prevalent at BBREC (Table 3). Even when Fe-EDDHA treatments were effective, VCS ratings were often <1 VCS unit lower for treated compared to untreated soybean and were not enough to reduce chlorosis to that of a non-chlorotic plant. Wiersma (2005) reported that rates greater than 4.5 kg product (6% Fe as Fe-EDDHA) ha^{-1} were required to decrease chlorosis ratings to that of a non-chlorotic plant. However, high product rates may reduce net returns and may not be economically feasible for producers. Iron deficiency chlorosis is a difficult problem to ameliorate, and slight improvements in chlorosis may have the potential to affect yield and net return. Inskeep and Bloom (1987) observed a 35 to 40% yield reduction when visual ratings were above 2.5 (1–5 scale) at the V4 growth stage, which was a similar degree of chlorosis observed in the current study.

Table 3. Soybean visual chlorosis scores (VCS) at V3 and V5 growth stages (Fehr and Caviness, 1977) at the Blackbelt Research and Extension Center (BBREC) and the Givhan Farm (GIV) in Alabama from 2010 to 2012. Larger numbers indicate greater incidence of chlorosis.

	VCS			
	V3		V5	
Treatment	BBREC	GIV	BBREC	GIV
Untreated	5.1	3.6	6.6	4.6
FeCitrate-SP	5.3	3.2	6.2	4.3
FeCitrate-AP	5.3	3.3	6.6	3.9
$FeSO_4$–FS	5.3	3.5	6.1	3.7*
$FeEDDHA-AP_1$	4.3*	3.0	5.7*	4.0
$FeEDDHA-AP_2$	4.1*	3.3	5.5*	4.1
$FeEDDHA-AP_3$	3.8*	3.4	5.3*	4.1
FeEDDHA-FS	5.1	3.5	6.2	4.1
FeEDDHA-SP	4.4	2.8	5.7*	4.1
Standard Error	0.23		0.22	

* Value is significantly different from untreated soybean ($P < 0.05$) using Dunnett's test.

Normalized Difference Vegetation Index and Relative Chlorophyll Content

No significant differences occurred between treatments or locations for NDVI or relative chlorophyll content (SPAD analysis), although readings were consistently greater for the GIV location (data not shown). Although NDVI declined with VCS ratings ($r = -0.63$, $P < 0.001$ at V3 growth stage; $r = -0.84$, $P < 0.001$ at V5 growth stage), NDVI was not able to detect differences from untreated soybean determined by visual scoring and was therefore not sensitive enough to determine degree of chlorosis. Lack of differences was most likely due to minimal ground cover provided by the soybean crop. It is not uncommon for soil background to interfere in remote sensing measurements when minimal canopy cover is apparent (Hong et al., 2007). Because Greenseeker measures light reflectance above the plant canopy, the low ground cover may have affected NDVI readings. Relative chlorophyll (SPAD) readings also did not detect differences observed by visual ratings. Visual scores were better able to assess chlorosis and stunting simultaneously, while SPAD readings were only able to assess chlorosis. Wiersma (2005) observed that relative chlorophyll content increased with increasing rate of seed-applied Fe-EDDHA, and that this response was greater in IDC-susceptible cultivars than in IDC-tolerant. The soybean variety used in this study was moderately IDC-susceptible, and changes may have not been significant enough to be read by SPAD. This data suggests visual ratings were more effective for assessing chlorosis compared to NDVI and SPAD measurements.

Yield and Seed Mass

Soybean grain yield at GIV was twice as large (1680 vs. 810 kg ha^{-1}, $P = 0.001$) as the yield at the BBREC across this 3-yr study. A location × treatment interaction was not significant in this study, but treatments did affect grain yield (Table 2). These results indicated that responses to treatments were similar whether applied to highly or moderately chlorotic soybean. Significantly greater grain yields were observed across locations for FeEDDHA-AP$_3$ and FeEDDHA-SP ($P = 0.031$, 0.014) compared to the untreated soybean (Table 4). Yield was somewhat related to VCS ratings at the V3 stage ($r = -0.55$, $P < 0.001$) and at the V5 stage ($r = -0.66$, $P < 0.001$). Both of these treatments (i.e., Fe-EDDHA-AP$_3$ and FeEDDHA-SP) corresponded to treatments with improved VCS ratings. Thus, VCS ratings have potential to predict yield improvements in chlorotic soybean even with small improvements in chlorosis.

Table 4. Soybean grain yield and seed mass in response to Fe treatment at the Blackbelt Research and Extension Center (BBREC) and Givhan Farm (GIV) in western Alabama from 2010 to 2012.

Treatment	Yield†	Seed mass	
		BBREC	GIV
	kg ha^{-1}	g 100 seeds^{-1}	
Untreated	1120	15.2	13.4
FeCitrate-SP	1220	15.1	13.1
FeCitrate-AP	1210	15.1	12.7
$FeSO_4$–FS	1180	15.0	12.7
FeEDDHA-AP$_1$	1240	15.6	13.3
FeEDDHA-AP$_2$	1290	15.1	13.0
FeEDDHA-AP$_3$	1320*	15.4	13.6
FeEDDHA-FS	1270	15.6	12.8
FeEDDHA-SP	1340*	14.9	13.2
Standard Error	70	0.22	

* Value is significantly different from untreated soybean with ($P < 0.05$) using Dunnett's test.

† Means for grain yield are averages for 3 yr (2010, 2011, and 2012) and two locations (BBREC and GIV).

Yield increased up to 20% with treatments of FeEDDHA compared to the untreated check. However, increases in grain yield did not follow a trend of linear response to seed-applied Fe-EDDHA as observed by Wiersma (2005). Results from this study suggest 4.5 kg product ha^{-1} Fe-EDDHA applied in-furrow (i.e., FeEDDHA-AP$_3$) or as a split application (i.e., FeEDDHA-SP) was effective at improving yield. Other studies have shown yield improvements for soybean using Fe-EDDHA as a soil application (Schenkeveld et al., 2008) and as a foliar spray (Goos and Johnson, 2000).

Although yield improvements with Fe-EDDHA were slight, they may be enough to justify the expense of the product and thus benefit soybean growers in the Blackbelt. The average unit prices of soybean were US\$0.41, \$0.44, and \$0.54 kg^{-1} for 2010, 2011, and 2012, respectively (USDA-NASS, 2013). Based on the price (approximately \$15 kg^{-1}) and rate (4.5 kg product ha^{-1}) of FeEDDHA, returns due to yield improvements over the cost of chlorosis treatment observed in this study would have resulted in an additional \$15 to 41 ha^{-1} for the FeEDDHA-AP$_3$ treatment and \$23 to 52 ha^{-1} for the FeEDDHA-SP treatment, depending on the price per hectare used. The extra cost of application for the second application of the product for FeEDDHA-SP was not included in these values. However, data indicates that application of Fe-EDDHA may be beneficial to producers in the southeastern United States.

Seed mass was greater at BBREC (15.2 g 100 seeds^{-1}), where IDC was most severe, than at GIV (13.1 g 100 seeds^{-1}; $P = 0.046$; Table 4). It is likely that poor plant growth during early growth stages resulted in fewer seeds being produced per plant. Poor mineral nutrition, particularly micronutrient nutrition, is known to affect plant reproduction (Marschner, 1995). Plants were observed to recover slightly following the late summer rainfall, which may have resulted in photosynthates being allocated to fewer seed, thus producing more dense seed.

Leaf and Seed Iron Concentrations

The concentration of Fe in the leaf ranged from 69.5 to 112.3 mg kg^{-1} at the V3 growth stage and 105.0 to 173.5 mg kg^{-1} at the V5 growth stage. These values are slightly lower than those detected for plant material collected by Aly and Soliman (1998), which ranged between 203 and 864 mg kg^{-1} after 45 d of growth. In the current study, a linear response of leaf Fe concentration to increasing rates of in-furrow Fe-EDDHA was observed for the V5 growth stage at BBREC (Fig. 1). Results suggest that Fe was supplied to the leaves when Fe-EDDHA was applied in-furrow at planting. However, treatments with greater leaf Fe concentration did not always correspond to treatments with improved VCS ratings or grain yields. The concentration of Fe in the leaf was greater for $FeSO_4$–FS, FeEDDHA-AP$_3$, and FeEDDHA-FS than for untreated plants at the V5 growth stage at BBREC (Table 5). Of these, only FeEDDHA-AP$_3$ improved grain yield. It is not uncommon to observe little relation between leaf Fe concentration and improved chlorosis. Inskeep and Bloom (1987) observed that plant tissue Fe

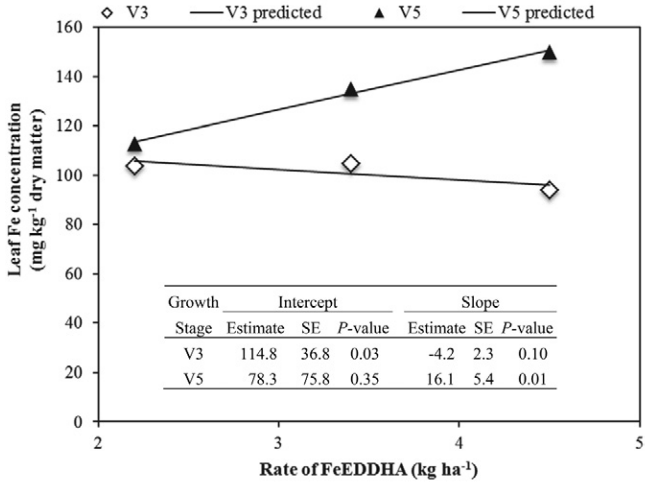

Fig. 1. Response of leaf Fe concentration to increasing rates of FeEDDHA applied at planting in-furrow at V3 and V5 growth stages (Fehr and Caviness, 1977) at Blackbelt Research and Extension Center (BBREC) from 2010 to 2012.

was often greater in chlorotic than non-chlorotic plants. At GIV, there was no difference in leaf Fe concentration between untreated and treated soybean at either V3 or V5.

Values for seed Fe concentration ranged from 38.3 to 43.1 mg kg^{-1} and there was no difference among locations (Table 2). These values are similar to those obtained by Wiersma (2007), who observed seed Fe concentration of approximately 47 mg kg^{-1} seed in soils that exhibited a moderate degree of chlorosis; however, Wiersma (2007) observed small, but linear, increases in seed Fe concentrations with increasing rate of FeEDDHA seed treatment. This was not observed in this study with increasing rates of FeEDDHA in-furrow treatments.

CONCLUSIONS

Applications of chelated Fe-EDDHA at planting were the most effective treatments for improving IDC, but no treatment completely eliminated chlorosis. Reduction in IDC due to Fe-EDDHA was most commonly observed when IDC was most severe. Iron acquisition in leaves at the V5 stage responded linearly to increasing rates of Fe-EDDHA at planting suggesting that chelated Fe may be plant available. The highest rate of Fe-EDDHA reduced chlorosis and increased yield in the alkaline soils of the Blackbelt region of Alabama, demonstrating that this chelate has the potential to be effective in a southeastern climate. Objective techniques such as SPAD and Greenseeker were not effective at assessing changes in chlorosis compared to a visual rating system. These sensor-based techniques may not accurately capture both the chlorosis and stunting symptoms that are more easily rated by visual inspection.

Based on current soybean and chelate product prices, net return from soybean produced in this study increased approximately \$30 ha^{-1} using the highest product rate applied either in-furrow or as a split application. This study contributes to the research performed in the north-central United States that has demonstrated the effectiveness of Fe-EDDHA to improve IDC in soybean at similar rates. Use of other strategies to reduce IDC, such as selection of IDC-resistant cultivars, may further enhance the effectiveness of Fe chelates.

Table 5. Concentration of Fe in soybean leaves at V3 and V5 growth stages (Fehr and Caviness, 1977) and seed Fe at harvest at Blackbelt Research and Extension Center (BBREC) and Givhan Farm (GIV) in western Alabama from 2010 to 2012.

	Leaf Fe				Seed Fe	
	V3		V5		Harvest	
Treatment	BBREC	GIV	BBREC	GIV	BBREC	GIV
	mg kg^{-1} dry weight					
Untreated	112.3	73.8	105.5	149.7	41.0	38.3
FeCitrate-SP	95.4	74.3	105.0	165.0	41.1	41.8
FeCitrate-AP	97.7	75.0	118.6	135.8	40.1	41.1
$FeSO_4$–FS	97.1	76.9	173.5*	163.0	40.9	41.6
FeEDDHA-AP_1	103.8	77.1	112.8	154.1	38.7	41.1
FeEDDHA-AP_2	104.6	73.0	134.8	146.3	40.2	43.1
FeEDDHA-AP_3	94.0	69.5	149.8*	135.5	39.2	40.1
FeEDDHA-FS	97.5	78.8	144.3*	159.9	39.8	40.7
FeEDDHA-SP	86.9	71.7	117.1	147.8	40.2	40.6
Standard Error	4.96		12.62		2.03	

* Value is significantly different from untreated soybean with ($P < 0.05$) using Dunnett's test.

REFERENCES

Aktas, M., and F. van Egmond. 1979. Effect of nitrate nutrition on iron utilization by an Fe-efficient and an Fe-inefficient soybean cultivar. Plant Soil 51:257–274. doi:10.1007/BF02232888

Allison, L.E., and C.D. Moodie. 1965. Carbonate. In: C.A. Black, editor, Methods of soil analysis. Part 2. ASA, Madison, WI. p. 1388–1389.

Aly, S.S.M., and S.M. Soliman. 1998. Impact of some organic acids on correcting iron chlorosis in two soybean genotypes grown in calcareous soil. Nutr. Cycling Agroecosyst. 51:185–191. doi:10.1023/A:1009740601479

Barker, D.W., and J.E. Sawyer. 2010. Using active canopy sensors to quantify corn nitrogen stress and nitrogen application rate. Agron. J. 102:964–971. doi:10.2134/agronj2010.0004

Batchelor, W. 2013. Nutrient recommendations for Alabama crops. Ser. no. 324B. Dep. of Agronomy and Soils, Auburn Univ., Auburn, AL.

Campbell, J.J.N., and W.R. Seymour. 2011. A review of native vegetation types in the Black Belt of Mississippi and Alabama, with suggested relationships to the catenas of soil series. J. Miss. Acad. Sci. 56:166–184.

Everest, J., C. Ray, K. Flanders, S.F. Enloe, A. Hagan, B. Ortiz et al. 2014. Alabama pest management handbook, 2014. Alabama Coop. Ext. System, Auburn.

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. Spec. Rep. 80. Iowa Agric. and Home Econ. Exp. Stn. Iowa State Univ., Ames.

Franzen, D.W., J.H. O'Barr, and R.K. Zollinger. 2004. Influence of certain postemergence herbicides on soybean stressed from iron deficiency chlorosis. Agron. J. 96:1357–1363. doi:10.2134/agronj2004.1357

Froechlich, D.M., and W.R. Fehr. 1981. Agronomic performance of soybean with differing levels of iron deficiency chlorosis on calcareous soil. Crop Sci. 21:438–441. doi:10.2135/cropsci1981.0011183X002100030021x

Glass, K.M., D.P. Delaney, and E. van Santen. 2012. Performance of soybean varieties in Alabama, 2012. Ser. no. 327. Dep. of Agronomy and Soils, Auburn Univ., Auburn, AL.

Goos, R.J., and B.E. Johnson. 2000. A comparison of three methods for reducing iron-deficiency chlorosis in soybean. Agron. J. 92:1135–1139. doi:10.2134/agronj2000.9261135x

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. doi:10.2134/agronj2003.1595

Hecht-Buchholz, C., and U. Ortmann. 1986. Effect of foliar iron application on regreening and chloroplast development in iron-chlorotic soybean. J. Plant Nutr. 9:647–659. doi:10.1080/01904168609363471

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. doi:10.2134/agronj2009.0317

Hong, S., J.S. Schepers, D.D. Francis, and M.R. Schlemmer. 2007. Comparison of ground-based remote sensors for evaluation of corn biomass affected by nitrogen stress. Commun. Soil. Sci. Plant Anal. 38:2209–2226. doi:10.1080/00103620701549157

Hue, N.V., and C.E. Evans. 1979. Procedures used by the Auburn University soil testing laboratory. Ser. no. 16. Dep. of Agronomy and Soils, Auburn Univ., Auburn, AL. p. 8.

Inskeep, W.P., and P.R. Bloom. 1987. Soil chemical factors associated with soybean chlorosis in Calciaquolls of western Minnesota. Agron. J. 79:779–786. doi:10.2134/agronj1987.00021962007900050005x

Karkosh, A.E., A.K. Walker, and J.J. Simmons. 1988. Seed treatment for control of iron-deficiency chlorosis of soybean. Crop Sci. 28:369–370. doi:10.2135/cropsci1988.0011183X002800020039x

Kitchen, N.R., K.A. Sudduth, S.T. Drummond, P.C. Scharf, H.L. Palm, D.F. Roberts, and E.D. Vories. 2010. Ground-based canopy reflectance sensing for variable-rate nitrogen corn fertilization. Agron. J. 102:71–84. doi:10.2134/agronj2009.0114

Loeppert, R.H., and W.P. Inskeep. 1996. Iron. In: D.L. Sparks, editor, Methods of soil analysis. Part 3. SSSA and ASA, Madison, WI. p. 654–657.

Lucena, J.J., M. Manzanaras, and A. Gàrate. 1992. A test to evaluate the efficacy of commercial Fe-chelates. J. Plant Nutr. 15:1553–1566. doi:10.1080/01904169209364421

Markwell, J., J.C. Osterman, and J.L. Mitchell. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosynth. Res. 46:467–472. doi:10.1007/BF00032301

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, London.

Marschner, H., and V. Römheld. 1994. Strategies of plants for acquisition of iron. Plant Soil 165:261–274. doi:10.1007/BF00008069

Marschner, H., V. Römheld, and M. Kissel. 1986. Different strategies in higher plants in mobilization and uptake of iron. J. Plant Nutr. 9:3–7.

Naeve, S.L., and G.W. Rehm. 2006. Genotype × environment interactions within iron deficiency chlorosis-tolerant soybean genotypes. Agron. J. 98:808–814. doi:10.2134/agronj2005.0281

Niebur, W.S., and W.R. Fehr. 1981. Agronomic evaluation of soybean genotypes resistant to iron deficiency chlorosis. Crop Sci. 21:551–554. doi:10.2135/cropsci1981.0011183X002100040019x

Norvell, W.A. 1972. Equilibria of metal chelates in soil solution. In: J.J. Mortvedt, P.M. Giordano, and W.L. Lindsay, editors, Micronutrients in agriculture. SSSA, Madison, WI. p. 115–138.

O'Rourke, J.A., D.O. Gonzalez, L.O. Vodkin, M.A. Graham, S.R. Cianzio, M.A. Grusak, and R.C. Shoemaker. 2007. Microarray analysis of iron deficiency chlorosis in near-isogenic soybeans lines. BMC Genomics 8:476–488. doi:10.1186/1471-2164-8-476

Reed, D.W., C.G. Lyons, Jr., and G.R. McEachern. 1988. Field evaluation of inorganic and chelated iron fertilizers as foliar sprays and soil application. J. Plant Nutr. 11:1369–1378. doi:10.1080/01904168809363894

Römheld, V., and H. Marschner. 1986. Evidence for a specific uptake system for iron phytosiderophore in roots of grasses. Plant Physiol. 80:175–180. doi:10.1104/pp.80.1.175

SAS Institute. 2011. The SAS system for windows. Version 9.3. SAS Inst. Inc., Cary, NC.

Schenkeveld, W.D.C., R. Dijcker, A.M. Reichwein, E.J.M. Temminghoff, and W.H. van Riemsdijk. 2008. The effectiveness of soil-applied FeEDDHA treatments in preventing iron chlorosis in soybean as a function of the o,o FeEDDHA content. Plant Soil 303:161–176. doi:10.1007/s11104-007-9496-x

Scharf, P.C., D.K. Shannon, H.L. Palm, K.A. Sudduth, S.T. Drummond, N.R. Kitchen et al. 2011. Sensor-based nitrogen applications outperformed producer-chosen rates for corn in on-farm demonstrations. Agron. J. 103:1683–1691.

Solari, F., J. Shanahan, R. Ferguson, J. Schepers, and A. Gitelson. 2008. Active sensor reflectance measurements of corn nitrogen status and yield potential. Agron. J. 100:571–579. doi:10.2134/agronj2007.0244

Teal, R.K., B. Turbana, K. Girma, K.W. Freeman, D.B. Arnall, O. Walsh, and W.R. Raun. 2006. In-season prediction of corn grain yield potential using normalized difference vegetation index. Agron. J. 98:1488–1494. doi:10.2134/agronj2006.0103

Teem, D.H. 1986. Procedures used for soil and plant analysis by the Auburn University soil testing laboratory. Ser. no. 106. Dep. of Agronomy and Soils, Auburn Univ., Auburn, AL. p. 20.

USDA-NASS. 2013. USDA-NASS Quick Stats. USDA-NASS. http://quickstats.nass.usda.gov/ (accessed 27 Jan. 2014).

USEPA. 2007. Method 3051a- Microwave assisted acid digestion of sediments, sludges, soils and oils. USEPA. www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/3051a.pdf (accessed 27 Jan. 2014).

Varvel, G.E., J.S. Schepers, and D.D. Francis. 1997. Ability for in-season correction of nitrogen deficiency in corn using chlorophyll meters. Soil Sci. Soc. Am. J. 61:1233–1239. doi:10.2136/sssaj1997.03615995006100040032x

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. doi:10.2134/agronj2004.0309

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. doi:10.2134/agronj2006.0271