Published November, 2011

Soil Fertility & Crop Nutrition

Management Strategies for Increasing Soybean Yield on Soils Susceptible to Iron Deficiency

A. M. Liesch, D. A. Ruiz Diaz,* K. L. Martin, B. L. Olson, D. B. Mengel, and K. L. Roozeboom

ABSTRACT

Soybean [*Glycine max* (L.) Merr.] production has increased by more than 55,000 ha in the last 25 yr in the western third of Kansas, a region with soils that can be prone to Fe chlorosis. The objective of this study was to evaluate the relative effectiveness of varietal selection, seed-applied Fe fertilizer, and foliar Fe application to reduce the incidence of Fe chlorosis under irrigated soybean production. Seven locations with a history of Fe deficiency in soybeans were selected. The study consisted of a factorial design with three foliar treatments (two chelated Fe fertilizer forms and no foliar), two seed-applied Fe fertilizer treatments (with and without chelated Fe fertilizer), and two different varieties (a nontolerant and tolerant commercial variety). Plant population, chlorophyll meter (CM) readings (V3 and V6 growth stage), plant height at maturity, and grain yield were measured. Foliar Fe application did not affect any plant parameter except for CM reading and grain yield at one location. However, the use of seed-applied chelated Fe fertilizer significantly increased CM readings at the V3 and V6 growth stages, plant height at maturity, and grain yield across all locations. Given soil conditions conducive to the development of severe iron chlorosis, seed-applied chelated Fe fertilizer increased yields by approximately 55% for both varieties. Chlorosis quantified as CM readings at V3 to V6 growth stage may not be correlated to the yield potential of a variety in all environments. This suggests that producers should choose the best varieties primarily based on yield potential if supplemental seed-applied Fe fertilizer will be used.

Advances in breeding, irrigation technology, and weed control have extended the corn (*Zea mays* L.)–soybean rotation westward into regions traditionally dominated by winter wheat (*Triticum aestivum* L.) production in the U.S. Great Plains. In the western third region of Kansas (area included in the three westernmost agricultural statistics districts), between 1990 and 2010, soybean production increased from 46,000 ha to approximately 83,000 ha annually (NASS, 2010). Production of soybean in these soils, often alkaline or calcareous, is frequently affected by Fe chlorosis. Iron chlorosis is thought to impact 30% of the world's semiarid crop production areas (Yousfi et al., 2007). In the North Central United States (an area including North Dakota, South Dakota, Minnesota, and Iowa), Fe chlorosis is estimated to cause $120 million worth of yield loss annually (Hansen et al., 2004). Low Fe availability decreases the synthesis of chlorophyll (Taylor et al., 1982). Symptoms of plants experiencing Fe chlorosis can vary from interveinal yellowing in the uppermost leaves of the plant, to necrosis and plant death in severe cases (Lingenfelser et al., 2005).

Iron chlorosis is a complex nutrient deficiency. Iron is the fourth most abundant element in the earth's crust; however, plant availability in high pH soils can be limiting (Rogers et al., 2009). One of the mechanisms used by plants to avoid Fe chlorosis is the exudance of H^+ or organic acid ions through the root membrane (Römheld, 1987). This process is controlled by cation-anion regulations and acidification of the rhizosphere, making Fe more available for plant uptake (Dakora and Phillips, 2002).

High spatial variability of Fe chlorosis often occurs within a field. Different weather patterns can also make Fe chlorosis more or less prevalent each year (Godsey et al., 2003). Iron chlorosis also differs under different soil conditions. In general, high soil pH impacts $CaCO_3$ and HCO_3^- availability, especially in wetter springs. Bicarbonates can reduce plant ion absorption (including Fe ions) in absorbing cells (Wadleigh and Brown, 1952).

Several management strategies have been suggested for management of Fe chlorosis in soybean systems. These management strategies involve varietal selection (Goos and Johnson, 2000; Helms et al., 2010), soil-applied in furrow Fe fertilizer (Godsey et al., 2003; Hergert et al., 1996), seed-applied Fe fertilizer (Karkosh et al., 1988; Goos and Johnson, 2001; Wiersma, 2005), and foliar application of Fe fertilizer (Godsey et al., 2003; Modaihsh, 1997). The results from application of fertilizers for Fe chlorosis are mixed because they can be highly affected by soils and environments; therefore, showing little or no improvement in yield or increased plant greenness (Cihacek, 1984). Most studies suggest that using a tolerant variety can generate the most consistent positive results and costs the least. Goos and Johnson (2000) and Wiersma (2007) found that growing Fe chlorosis-tolerant varieties resulted in greater yields and CM readings compared to a nontolerant variety.

Foliar application of chelated Fe fertilizer sources has been inconsistent: It has been successful in reducing signs of chlorosis in soybean at some locations (Goos and Johnson, 2000), increasing yield in some cases (Penas et al., 1990), and has had no effect

Dep. of Agronomy, Kansas State Univ., Manhattan, KS 66506. Contribution no. 11-279-J from the Kansas Agricultural Experiment Station. Received 17 June 2011. *Corresponding author (ruizdiaz@ksu.edu).

Published in Agron. J. 103:1870–1877 (2011)
Posted online 6 Oct 2011
doi:10.2134/agronj2011.0191

Abbreviations: CM, SPAD chlorophyll meter reading; EDDHA, ethylene diamine-N,N'-bis (hydroxy phenyl) acetic acid; HEDTA, hydroxyethylethylenediaminetriacetic acid; OM, soil organic matter; STK, soil test potassium; STP, soil test phosphorus.

at other locations in soybean (Lingenfelser et al., 2005) and corn (Godsey et al., 2003). Chelated forms of Fe fertilizer often are considered best because they are soluble and readily available to plants, and can be translocated to the leaves better than inorganic forms (Wittwer et al., 1965). However, the economic benefit are questionable in field-scale production of row crops, especially when applied as foliar applications that often need to be repeated.

Applying a Fe source to the soil in furrow has proven successful in corn using Fe sulfate fertilizer (Hergert et al., 1996; Godsey et al., 2003); however, sulfate forms of fertilizer become insoluble quickly at high soil pH levels. In Kansas, Fe sulfate fertilizer did not reduce the prevalence of chlorosis in soybean (Lingenfelser et al., 2005). However, the application of EDDHA [ethylene diamine-N,N′-bis (hydroxy phenyl) acetic acid] Fe fertilizer to the soil has reduced chlorosis in calcareous soils in other regions of the United States (Heitholt et al., 2003). The use of chelated Fe (EDDHA) applied to the seed has been successful in the North Central United States in some cases (Karkosh et al., 1988; Wiersma, 2005), and unsuccessful in other cases (Goos and Johnson, 2001). Even though Lingenfelser et al. (2005) and Godsey et al. (2003) studied the impact of adding Fe to the soil in Kansas in soybean and corn, respectively, they did not use chelated Fe sources. Furthermore, research on soybean Fe chlorosis in the United States has been focused on rainfed production systems, with limited research for irrigated conditions in the Great Plains region. The objective of this study was to evaluate the effect of chelated Fe fertilizer application using foliar and seed-applied methods in combination with variety selection for Fe chlorosis management of soybean grown under irrigated conditions.

MATERIALS AND METHODS

During 2009 and 2010, studies were conducted at producers' fields and research experiment fields with a history of Fe chlorosis in a total of seven locations in western Kansas. Small plots were established in areas of the field with a history of severe iron chlorosis. Descriptions for each location can be found in Table 1. Soybean was planted at 0.76 m row spacing with a seeding density of approximately 370,000 to 420,000 plants ha^{-1}. All locations were irrigated with center pivot sprinkler irrigation systems. Irrigation was applied as needed during the growing season.

Four varieties of maturity group II or III soybean were selected with varying tolerance to Fe chlorosis based on ratings provided by the commercial seed provider. Two varieties were selected to represent very good tolerance (Asgrow 2906 in 2009 and Asgrow 3039 in 2010) and low tolerance (Asgrow 3205 in 2009 and Asgrow 3005 in 2010). Treatments included two varieties differing in reaction to Fe chlorosis (nontolerant and tolerant); three foliar Fe treatments (Fe-EDDHA 6%, Fe-HEDTA 4.5% [4.5% Fe hydroxyethylethylenediaminetriacetic acid], and no foliar application); and two seed-applied Fe fertilizer treatments (with and without Fe-EDDHA). The variety, seed-applied fertilizer, and foliar fertilizer were arranged in a complete factorial treatment structure in a randomized complete block design and replicated four times. Plots were 4 rows wide by 7.6 m long.

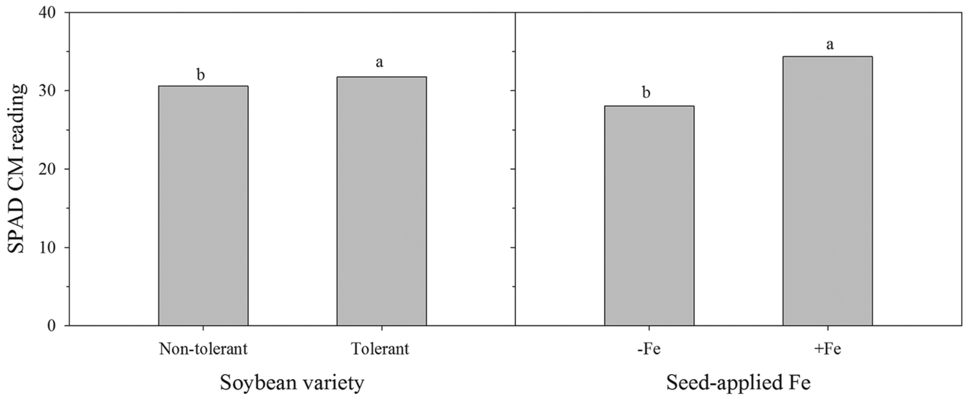
A dry chelated Fe fertilizer (EDDHA) product was mixed with water into slurry and applied to the seeds at a rate totaling approximately 0.2 kg Fe ha^{-1}. Seeds were dropped into a cement mixer, and the fertilizer slurry applied until seeds were completely covered; seeds were air-dried before planting. Two different chelated Fe fertilizers (EDDHA and HEDTA) were foliar-applied at 0.11 kg Fe ha^{-1} at the V3 growth stage (Pedersen, 2004) and a second application repeated approximately 2 to 4 wk later if chlorosis persisted (only in location 2). Foliar Fe fertilizer was applied using a hand-held CO_2–powered sprayer adjusted to a pressure of 0.11 MPa and diluted into 180 L ha^{-1} of water. Ammonium sulfate was used as an adjuvant with the foliar-applied fertilizer at 20 g L^{-1} water spray solution. The application rates and fertilizer sources were selected based on previous research and recommendations from other areas in the North Central and Great Plains Region.

Soil samples were collected at the 0- to 15-cm depth before planting from each block. Samples were analyzed for pH using a 1:1 soil/water ratio (Watson and Brown, 1998). Soil organic matter (OM) was measured using the Walkley–Black method (Combs and Nathan, 1998). Iron DTPA (diethylene-triamine-penta-acetate) extraction used the method of Whitney (1998) on an ICP Spectrometer. Soil test phosphorus (STP) was determined with the Mehlich-3 P method (Frank et al., 1998). Extractable K was determined by ammonium acetate extraction and analyzed on an ICP Spectrometer (Warncke and Brown,

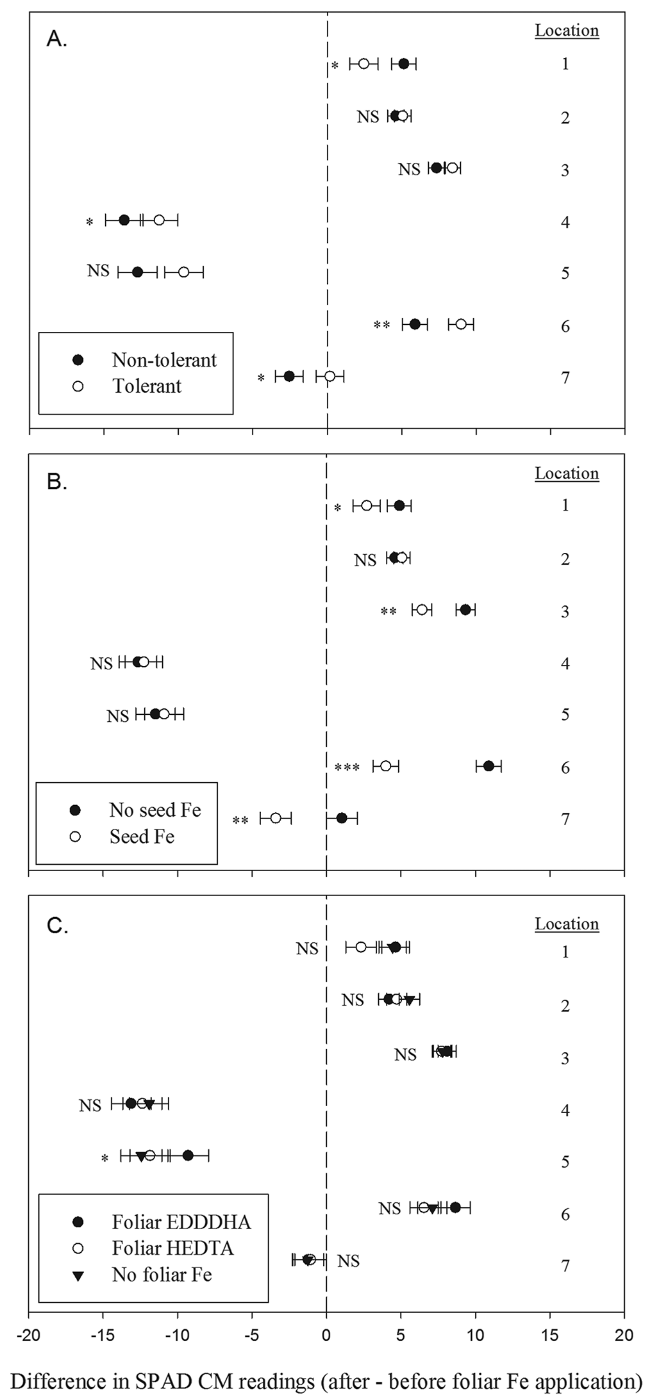
Table 1. Soil classification and initial soil test information for each location. Samples were collected at the 0- to 15-cm depth before planting. Samples for NO_3–N analysis were collected at the 0- to 60- cm depth.

		Predominant soil		Soil chemical analysis†						
Location	County	Series	Subgroup	pH	CCE‡	OM§	Fe¶	NO_3–N	STP#	STK#
					g kg^{-1}		mg kg^{-1}			
				2009						
1	Finney	Ulysses	Aridic Haplustolls	8.1	93	22	2.5	17.4	27	822
2	Lane	Richfield	Aridic Argiustolls	8.3	61	19	2.8	6.9	19	1050
3	Lane	Richfield	Aridic Argiustolls	8.2	45	18	3.3	8.8	20	1018
				2010						
4	Thomas	Ulysses	Aridic Haplustolls	8.3	97	21	1.7	7.0	53	923
5	Thomas	Ulysses	Aridic Haplustolls	8.5	138	17	2.3	5.1	60	958
6	Finney	Ulysses	Aridic Haplustolls	8.2	114	20	2.3	14.5	24	657
7	Lane	Richfield	Aridic Argiustolls	8.1	140	27	2.5	11.5	117	898

† Mean values of the initial samples collected from each block at the 0–15 cm (0–60 cm for NO_3–N).
‡ CCE, calcium carbonate equivalent.
§ OM, organic matter.
¶ Fe, soil-extractable Fe determined by DTPA extraction.
# STP, soil test phosphorus determined by Mehlich-3; STK, soil test potassium determined by ammonium acetate extraction.



◂ Fig. 1. Marginal means of the effect of soybean variety and seed-applied Fe fertilizer on chlorophyll meter (CM) readings at the V3 growth stage before foliar Fe application, averaged across all locations. Interaction effect was not statistically significant at the 0.05 level (*P* = 0.142). Means with different letters are statistically different at 0.05 probability level.



◂ Fig. 2. Changes in chlorophyll meter (CM) readings after foliar Fe fertilizer application for the main treatment effects of soybean variety, seed-applied Fe fertilizer, and foliar-applied Fe fertilizer. Mean values are the difference between CM readings after foliar Fe application minus readings before foliar Fe fertilizer application. Statistical differences between treatments for each location are indicated by ns,*, **, and *; not significant at 0.05 level, significant at the 0.05, 0.01, and 0.001 level. Error bars represent the standard error of the mean.**

1998). Calcium carbonate equivalent (CCE) was measured by adding dilute HCl to the soil and measuring CO_2 gas displacement. This displacement percentage is compared to the total displacement of pure $CaCO_2$, a method adapted from that of Huang et al. (2007). Soil samples for nitrate N were collected at the 0- to 60- cm depth and measured with a 1 M KCl extraction (Gelderman and Beegle, 1998) and using a Rapid Flow Analyzer (Alpkem, College Station, TX).

Chlorophyll meter readings were recorded at approximately V3 growth stage at all locations with a SPAD 502 CM (Minolta, Ramsey, NJ) immediately before foliar Fe fertilizer application. Readings were collected from 20 randomly selected plants within each plot using the uppermost fully developed leaflets and averaged into one value per plot. A second set of CM readings were collected to monitor the effectiveness of foliar-applied Fe fertilizer within 2 to 3 wk after foliar fertilizer application and first CM readings (approximately V6 growth stage). Plant population was recorded after emergence at the V3 growth stage. Plant height was recorded at maturity (R7 growth stage). Grain yield was determined by harvesting the two center rows using a plot combine or cutting plants from the two center rows and threshing with a stationary thresher. Grain moisture was measured by weighing approximately 500 g of field-moist grain and weighing the grain again after drying it at 65°C for 6 d. Moisture content was recorded and used to adjust grain yield to a moisture content of 130 g kg^{-1}.

Analysis of variance was completed using the method of restricted maximum likelihood, using the PROC GLIMMIX procedure in SAS 9.2 (SAS Institute, 2010). Separate analyses were completed for each location, considering block as a random factor in the model and using plant population as a covariate in the analysis. Data also were analyzed across locations to determine the effectiveness of treatments across locations, using location and block as random factors. The KENWARDROGER option was used for computing the denominator degrees of freedom for tests of fixed effects (Kenward and Roger, 1997). Values were deemed significant at the 0.05 probability level.

Table 2. Significance of *F* values for the fixed effects of soybean variety, seed applied Fe fertilizer, and foliar applied Fe fertilizer on chlorophyll meter (CM) reading within 2 wk after foliar Fe application, plant height at maturity, and grain for each location.

Location	Fixed effects						
	Variety (V)	Seed-Fe (S)	V × S	Foliar-Fe (F)	V × F	S × F	V × S × F
	$p > F$						
	CM reading						
1	0.856	0.778	0.021	0.808	0.582	0.917	0.451
2	0.684	0.511	0.911	0.763	0.392	0.808	0.568
3	0.680	0.249	0.555	0.967	0.201	0.276	0.712
4	<0.001	<0.001	0.233	0.752	0.772	0.967	0.793
5	<0.001	<0.001	0.959	0.393	0.959	0.944	0.851
6	<0.001	0.147	0.628	0.588	0.169	0.201	0.468
7	0.005	<0.0001	0.192	0.763	0.652	0.655	0.787
	Plant height						
1	0.002	0.357	<0.001	0.602	0.865	0.739	0.693
2	<0.001	<0.001	0.082	0.505	0.834	0.546	0.661
3	<0.001	<0.001	0.273	0.352	0.299	0.887	0.873
4	0.365	<0.001	0.688	0.222	0.893	0.313	0.365
5	0.017	<0.001	0.868	0.958	0.855	0.699	0.381
6	0.110	<0.001	0.749	0.658	0.392	0.462	0.350
7	<0.001	<0.001	0.079	0.556	0.484	0.118	0.684
	Grain yield						
1	0.756	0.691	0.295	0.799	0.575	0.665	0.436
2	0.001	<0.001	0.002	0.610	0.483	0.924	0.874
3	0.381	0.012	0.084	0.349	0.082	0.602	0.130
4	0.469	0.010	0.421	0.277	0.541	0.628	0.602
5	0.019	<0.001	0.002	0.165	0.448	0.252	0.615
6	0.031	0.024	0.257	0.006	0.682	0.443	0.170
7	<0.001	0.001	0.073	0.271	0.420	0.759	0.198

RESULTS AND DISCUSSION

Soil collected from the 0- to 15-cm depth at the study locations showed overall high pH and CCE levels that can be considered common of some western Kansas soils (Table 1). Soil test K and STP ranged from levels considered optimum to high (Leikam et al., 2003).

Chlorosis as measured by the CM readings at the V3 growth stage indicated greater greenness for the tolerant variety across all locations (Fig. 1). The seed-applied Fe fertilizer treatment also increased the overall plant greenness early in the season across both varieties. Iron chlorosis is often more severe early in the season and in many cases, plants may have the ability to partially recover when environmental conditions improve later in the season (Barker and Pilbeam, 2007). A second set of CM readings were collected after foliar Fe fertilizer application, and the values collected before and after foliar Fe application were compared (Fig. 2). All locations, except locations 4 and 5 showed an increase in CM readings by the V6 growth stage. After the first CM reading at V3 growth stage, locations 4 and 5 were treated with herbicides that caused some leaf damage and may have contributed to lower CM readings later in the season. Changes in CM readings at location 7 were not statistically different from zero; all other changes in CM values were statistically different from zero (data not shown). Foliar Fe fertilizer application did not change CM readings, except for location 5 where foliar-applied Fe EDDHA increased CM readings (Fig. 2). The second set of CM readings (Fig. 2) was collected approximately 2 wk later that the first CM reading (Fig. 1) and it is likely that plants naturally increased plant greenness later in the season. These results are similar to those of Goos and Johnson (2000) documenting that visual iron chlorosis ratings were not affected by foliar Fe application in three of four locations in North Dakota. Changes in CM readings were generally larger for the tolerant variety, suggesting that plants with higher level of tolerance to iron chlorosis can increase plant greenness more rapidly when compared to nontolerant varieties (Fig. 2).

On the other hand, the seed-applied Fe fertilizer treatment showed an overall smaller difference in CM readings later in the season compared to no-seed Fe fertilizer (Fig. 2). Chlorophyll meter readings were initially higher for the seed-applied Fe fertilizer treatment at the V3 growth stage (Fig. 1); and therefore additional increases in plant greenness are likely to be relatively smaller with seed-applied Fe fertilizer.

No interactions occurred between foliar applications and other main effects for CM readings, plant height at maturity, and grain yield (Table 2), and therefore means for these interactions were not shown. However, the variety by seed-applied Fe interaction was significant at location 1 for CM readings and plant height, and locations 2 and 5 for grain yield (Tables 2 and 3). Average CM readings, and plant height at location 1 did not follow the trend observed at other locations, and seed-applied Fe fertilizer seemed to generate opposite results for the different varieties (Table 3). At locations 2 and 5, the overall grain yield response to seed-applied fertilizer was greater for the tolerant variety when compared to the nontolerant variety (Table 3). However, different varietal response to seed-applied Fe fertilizer was not consistent across all locations in our study.

Variety selection affected chlorophyll meter readings significantly with an overall higher value for the tolerant variety for four

Table 3. Chlorophyll meter (CM) readings within 2 wk of foliar Fe application, plant height at maturity, and grain yield as affected by the interaction of variety and seed-applied Fe fertilizer.

Location	Nontolerant variety		Tolerant variety	
	–Fe†	+Fe†	–Fe	+Fe
	CM reading			
1	38.57b‡	40.70a	40.64a	38.92ab
2	35.25	35.60	35.45	35.94
3	38.38	38.91	38.33	39.37
4	12.11	20.33	14.70	25.26
5	8.68	20.35	13.52	25.09
6	34.48	35.12	41.04	42.30
7	24.65	33.18	29.52	34.59
	plant height, cm			
1	34.16bc	40.86a	35.24b	30.80c
2	64.26	75.91	50.94	66.58
3	60.51	74.54	53.18	63.78
4	21.39	40.90	25.97	42.59
5	7.69	33.78	17.31	42.01
6	41.50	48.32	44.16	50.12
7	25.25	50.70	10.80	23.45
	grain yield, kg ha^{-1}			
1	2009	2434	2236	2025
2	3028b	3990a	2234c	3949a
3	3365	3761	3241	4220
4	553	1041	518	1467
5	145c	760b	0c	1488a
6	956	1052	693	966
7	291	1249	32	315

† Seed applied Fe fertilizer:-Fe, without seed Fe fertilizer; +Fe, with seed Fe fertilizer.

‡ Numbers in the same row followed by different letters are statistically different at the 0.05 probability level.

Table 4. Chlorophyll meter (CM) reading within 2 wk of foliar Fe application, plant height at maturity, and grain yield as affected by soybean variety.

Location	Variety†		Significance‡
	NT	T	
	CM reading		
1	39.63	39.78	ns§
2	35.43	35.69	ns
3	38.65	38.85	ns
4	16.22	19.98	***
5	14.52	19.31	***
6	34.80	41.67	***
7	28.92	32.06	**
	plant height, cm		
1	37.51	33.02	**
2	70.08	58.76	***
3	67.52	58.48	***
4	31.15	34.28	ns
5	20.73	29.66	*
6	44.91	47.14	ns
7	37.97	17.13	***
	grain yield, kg ha^{-1}		
1	2222	2131	ns
2	3509	3092	***
3	3563	3730	ns
4	797	993	ns
5	452	744	*
6	1004	830	*
7	770	173	***

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Soybean variety: NT, nontolerant variety; T, tolerant variety.

‡ Statistical significance of the main effect of soybean variety.

§ ns, not significant at the 0.05 probability level.

of the seven locations (Table 4). On the other hand, plant height was generally greater for the non-tolerant variety at most locations (Table 4). This may be attributed to genotypic differences and greater growth potential of the nontolerant variety. Soybean plant height was highly variable by location and is likely due to the confounding effects of environment and varieties. Likewise, grain yield response due to the effect of variety selection was inconsistent and varied for each location (Table 4).

The marginal means for the main effect of the seed-applied Fe fertilizer treatment (+Fe) shows significantly greater CM values than the control (-Fe) for locations 4, 5, and 7 (Table 5). Other locations also show slightly greater CM readings for the +Fe treatment (Table 5). Plant height at maturity and grain yield were significantly greater with the +Fe treatment at all locations except location 1 (Table 5). On the other hand, the marginal means of the foliar-applied Fe fertilizer treatments shows no difference from the control for CM readings, plant height, and grain yield (Table 6). The only significant response to foliar application was grain yield at location 6 with a significantly higher yield for the Fe HEDTA foliar treatment (Table 6). Foliar nutrient applications are usually considered most successful when applied under high relative humidity and moderate temperatures (Fernandez and Eichert, 2009). At all locations where this study was conducted in western Kansas, the air during the growing season was generally hot and dry, likely not optimum conditions for absorption of foliar-applied Fe fertilizer. Although weather data was not available at the field level for location 6; it is possible that conditions at that location were more favorable for leaf absorption of Fe fertilizer at the moment of foliar treatment application. In another study in the same region in western Kansas with corn, Godsey et al. (2003) found that foliar-applied chelated Fe (HEDTA) was not effective in increasing grain yield, indicating that this region is likely not conducive to responses from foliar Fe applications, and using alternative Fe fertilizer application methods should be considered.

Chlorophyll meter readings, plant height, and grain yield averaged across all locations showed nonsignificant responses to foliar Fe application. Even though it is suggested that plants can outgrow chlorosis in the youngest leaves, the consequences seem to remain in terms of reduced yield (Schenkeveld et al., 2008; Naeve and Rehm, 2006). On the other hand, variety selection and seed-applied Fe fertilizer treatments significantly affected soybean response (Table 7). Marginal means of the effect of soybean variety across all locations show significantly greater CM readings for the tolerant variety (Fig. 3). However, overall plant height across locations was significantly greater for the nontolerant variety. Grain yield response as affected by variety selection was highly variable by location (Tables 2 and 4), and analysis across locations indicated no statistically significant difference between varieties (Table 7 and Fig. 3). However, a slightly higher average grain yield was observed for the nontolerant variety. This may have the potential to be economically important to the producer, particularly if this variety is best suited for the region in

Table 5. Chlorophyll meter (CM) readings within 2 wk of foliar Fe application, plant height at maturity, and grain yield as affected by seed-applied Fe fertilizer.

Location	Seed-applied Fe†		Significance‡
	–Fe	+Fe	
	CM reading		
1	39.61	39.81	ns§
2	35.35	35.77	ns
3	38.35	39.14	ns
4	13.41	22.80	***
5	11.10	22.72	***
6	37.76	38.71	ns
7	27.09	33.88	***
	plant height, cm		
1	34.70	35.83	ns
2	57.60	71.24	***
3	56.84	69.16	***
4	23.68	41.74	***
5	12.50	37.90	***
6	42.83	49.22	***
7	18.03	37.08	***
	grain yield, kg ha^{-1}		
1	2123	2230	ns
2	2631	3970	***
3	3303	3991	*
4	536	1254	**
5	72	1124	***
6	825	1009	*
7	161	782	***

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Seed applied Fe fertilizer:-Fe, without seed Fe fertilizer; +Fe, with seed Fe fertilizer.

‡ Statistical significance of the main effect of seed applied Fe fertilizer.

§ ns, not significant at the 0.05 probability level.

Table 6. Chlorophyll meter (CM) readings within 2 wk after foliar Fe application, plant height at maturity, and grain yield as affected by the foliar Fe application main effect.

Location	Foliar Fe application		
	No foliar Fe	Fe-EDDHA	Fe-HEDTA
	CM reading		
1	40.01	39.53	39.59
2	35.81	35.62	35.25
3	38.82	38.73	38.69
4	18.39	17.66	18.26
5	16.42	17.66	16.66
6	37.93	38.72	38.06
7	30.12	30.38	30.96
	plant height, cm		
1	35.84	35.42	34.54
2	65.26	63.56	64.44
3	61.42	63.95	63.64
4	35.58	28.90	33.65
5	24.63	25.69	25.27
6	45.84	45.38	46.85
7	29.17	27.74	25.75
	grain yield, kg ha^{-1}		
1	2222	2241	2065
2	3358	3221	3323
3	3535	3589	3817
4	1134	640	911
5	527	756	511
6	909b†	758b	1084a
7	459	609	346

† Numbers in the same row followed by different letters are statistically different at the 0.05 probability level.

Table 7. Significance of *F* values for the fixed effects of soybean variety, seed applied Fe fertilizer, and foliar applied Fe fertilizer on chlorophyll meter (CM) reading within 2 wk after foliar Fe application, plant height at maturity and grain yield across all locations. Locations and blocks within locations were considered random effects.

Fixed effects	CM reading	Plant height	Grain yield
		$p > F$	
Variety (V)	<0.001	<0.001	0.055
Seed-Fe (S)	<0.001	<0.001	<0.001
V × S	0.824	0.308	0.770
Foliar-Fe (F)	0.896	0.837	0.605
V × F	0.807	0.631	0.100
S × F	0.935	0.883	0.975
V × S × F	0.637	0.495	0.319

the absence of Fe chlorosis, or if the incidence of iron chlorosis is limited to certain parts of the field. Areas with high incidence of Fe chlorosis within a field can potentially be treated with seed-applied Fe fertilizer or planted with a tolerant variety. Varietal selection has been proven to be successful management strategy in relieving the pressures of Fe chlorosis (Goos and Johnson, 2000; Wiersma, 2007). However, our results also suggest that when seed-applied Fe fertilizer is used, the nontolerant variety can yield similar, or in some cases more, than the tolerant variety (Table 3). This is contrary to the results of Karkosh et al. (1988), who found that the overall response of soybean to Fe fertilizer application is significantly greater in tolerant varieties resulting in significantly greater yields; while in nontolerant varieties, no increase in grain yield occurred in response to the seed-applied Fe fertilizer.

Our results showed equal grain yields in the tolerant and nontolerant varieties, even though early season CM readings reflected a lower CM value in the nontolerant variety (Fig. 3). One possible explanation of these grain yield trends is in the screening methods traditionally used for determining chlorosis tolerance in soybean varieties. Greenness is usually measured visually at the V3 to V6 growth stage and green plants are considered to have a greater tolerance to Fe chlorosis. These evaluations have no yield component under chlorotic and nonchlorotic conditions (Naeve and Rehm, 2006). Therefore, it is possible that yields are similar, even though one variety was yellower than the other early in the season. This suggests that, although areas prone to chlorosis are planted to tolerant varieties, such an approach may not always be advantageous to the entire field. In a study done with tolerant soybean varieties spread over the Great Plains, Helms et al. (2010) reported that planting a chlorosis-tolerant variety leads to increased cosmetic effects (greenness), but this does not necessarily maximize the yield in the entire field. In fact, selection for chlorosis tolerance often selected lower-yielding or mediocre varieties in the absence of Fe chlorosis. Similar results were found by Froechlich and Fehr (1981). With the advancements in precision technology, farmers may be able to plant different varieties within a field to maximize yield potential.

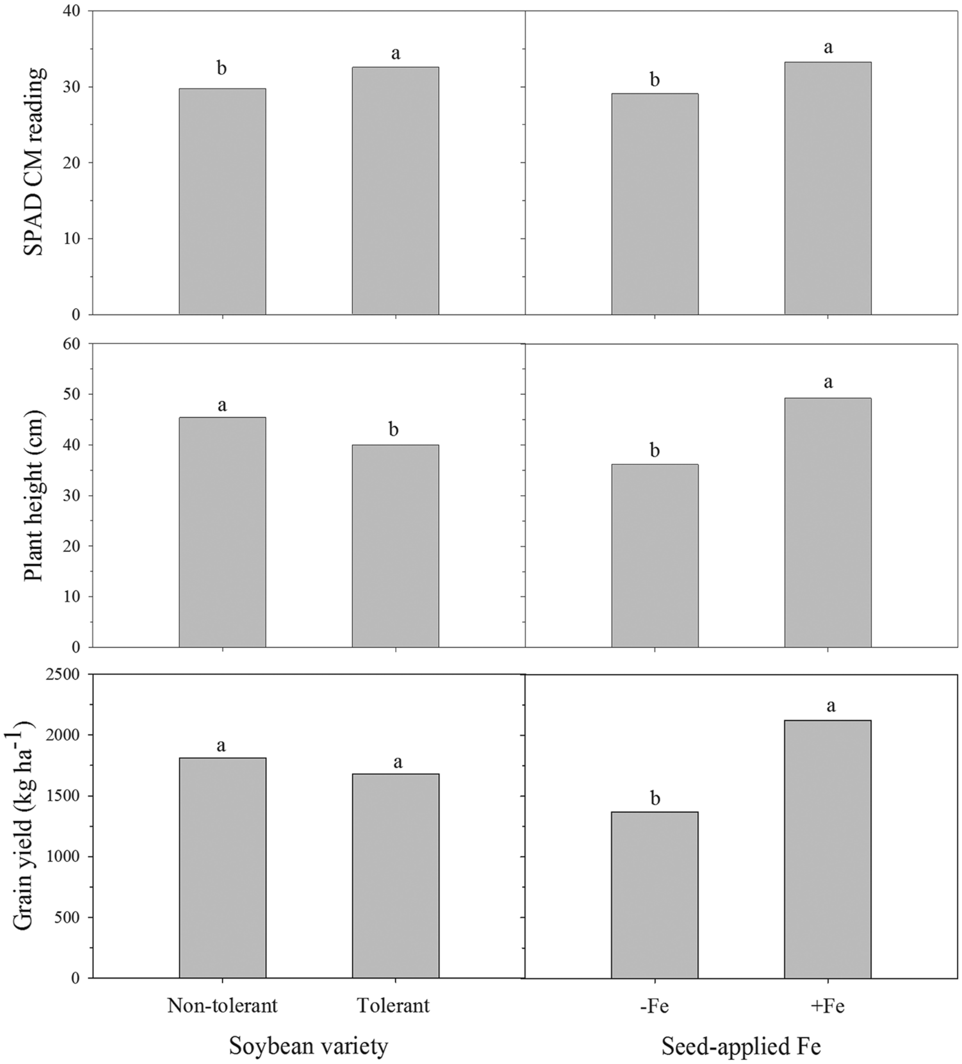


Fig. 3. Main effect of soybean variety and seed-applied Fe fertilizer on chlorophyll meter (CM) readings at the V6 growth stage within 2 wk after foliar Fe application, plant height at maturity, and soybean grain yield averaged across all locations. Interaction effects and foliar treatment main effect were not statistically significant (Table 7). Means with different letters are statistically different at 0.05 probability level.

Seed-applied Fe fertilizer significantly increased overall CM readings, plant height, and grain yield across all locations (Table 7 and Fig. 3). These results are in agreement with those of Karkosh et al. (1988) and Wiersma (2005), who found that applying Fe fertilizer to the seed significantly reduced visual chlorosis scores. The nontolerant variety was generally taller; however, in the absence of seed-applied Fe fertilizer, both varieties were severely stunted. Therefore seed-applied Fe fertilizer did influence the final plant height across varieties (Table 7 and Fig. 3).

Increases in soybean grain yield with seed-applied Fe fertilizer were comparable to results from other studies. Wiersma (2007) found that applying chelated Fe fertilizer (EDDHA) at planting resulted in the reduction of early season chlorosis, and resulted in approximately 15% increase in grain yield. This is much lower than the 55% increase we observed in our study (Fig. 3). Not all additions of Fe to the soil near the seed were significant. Heitholt et al. (2003) found that Fe fertilizer treatments applied to soil (DTPA, EDDHA, and Iron sulfate) increased yield by 13%, but this was not statistically significant in a Vertisol prairie experiment from Texas. However, these Fe sources were applied to the soil, and thus were likely not available to the plant as quickly as fertilizer applied in direct seed contact.

CONCLUSIONS

Soybean response to seed-applied chelated Fe fertilizer (EDDHA) was significant, with increases in grain yield, plant height, and CM readings at the V3 and V6 growth stage in both the tolerant and nontolerant varieties. Chlorophyll meter readings at the V6 growth stage (after foliar Fe application) were significantly increased; however, this increase was not associated with foliar Fe application. The tolerant variety showed consistently higher CM readings, particularly at the V6 growth stage. Grain yield response to seed-applied Fe fertilizer was the same for both varieties, which suggests that early season greenness is not always correlated to potential grain yield response. This indicates that chlorosis evaluation at the V3 toV6 growth stage may not be correlated to the yield potential of a variety in a certain environment. Foliar application of both Fe fertilizer sources (EDDHA and HEDTA) showed no significant effect on CM value, plant height, and grain yield overall in western Kansas.

Given soil conditions that were conducive to the development of severe iron chlorosis, seed-applied chelated Fe fertilizer (HEDTA) increased grain yield by approximately 55% for both tolerant and nontolerant varieties. This suggests that producers should consider choosing varieties primarily based on yield potential if supplemental seed-applied Fe fertilizer will be applied in soils prone to Fe chlorosis.

ACKNOWLEDGMENTS

This project was supported in part by the Kansas Soybean Commission. We would like to thank Maurice Miller for his contribution with field trials. Appreciation is extended to Nathan Mueller, Weston Grove, and Carlos Narvaez for assistance with field and laboratory work.

REFERENCES

Barker, A.V., and D.J. Pilbeam. 2007. Handbook of plant nutrition. CRC Press, Boca Raton, FL.

Cihacek, L.J. 1984. Economic soil treatment of Fe chlorosis in grain sorghum grown on gypsum affected soil. J. Plant Nutr. 7:329–340. doi:10.1080/01904168409363200

Combs, S.M., and M.V. Nathan. 1998. Soil organic matter. p. 53–58. *In* J.R. Brown (ed.) Recommended chemical soil test procedures for the North Central Region. North Central Reg. Res. Publ. 221 (Rev.). SB 1001. Missouri Agric. Exp. Stn., Columbia.

Dakora, F.D., and D.A. Phillips. 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245:35–47. doi:10.1023/A:1020809400075

Fernandez, V., and T. Eichert. 2009. Uptake of hydrophilic solutes through plant leaves: Current state of knowledge and perspectives of foliar fertilization. Crit. Rev. Plant Sci. 28:36–68. doi:10.1080/07352680902743069

Frank, K., D. Beegle, and J. Denning. 1998. Phosphorus. p. 21–30. *In* J.R. Brown (ed.) Recommended chemical soil test procedures for the North Central Region. North Central Reg. Res. Publ. 221 (Rev.). SB 1001. Missouri Agric. Exp. Stn., Columbia.

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

Gelderman, R.H., and D. Beegle. 1998. Nitrate-nitrogen. p. 17–21. *In* J.R. Brown (ed.) Recommended chemical soil test procedures for the North Central Region. North Central Reg. Res. Publ. 221 (Rev.). SB 1001. Missouri Agric. Exp. Stn., Columbia.

Godsey, C.B., J.P. Schmidt, A.J. Schlegel, R.K. Taylor, C.R. Thompson, and R.J. Gehl. 2003. Correcting Fe deficiency in corn with seed row–applied Fe sulfate. Agron. J. 95:160–166. doi:10.2134/agronj2003.0160

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

Goos, R.J., and B.E. Johnson. 2001. Seed treatment, seeding rate, and cultivar effects on Fe deficiency chlorosis of soybean. J. Plant Nutr. 24:1255–1268. doi:10.1081/PLN-100106980

Hansen, N.C., V.D. Jolley, S.L. Naeve, and R.J. Goos. 2004. Fe deficiency of soybeans in the North Central U.S. and associated soil properties. Soil Sci. Plant Nutr. 50:983–987.

Heitholt, J.J., J.J. Sloan, C.T. MacKown, and R.I. Cabrera. 2003. Soybean growth on calcareous soil as affected by three Fe sources. J. Plant Nutr. 26:935–948. doi:10.1081/PLN-120018575

Helms, T.C., R.A. Scott, W.T. Schapaugh, R.J. Goos, D.W. Franzen, and A.J. Schlegel. 2010. Soybean Fe-deficiency chlorosis tolerance and yield decrease on calcareous soils. Agron. J. 102:492–498. doi:10.2134/agronj2009.0317

Hergert, G.W., P.T. Nordquist, J.L. Peterson, and B.A. Skates. 1996. Fertilizer and crop management practices for improving maize yields on high pH soils. J. Plant Nutr. 19:1223–1233. doi:10.1080/01904169609365193

Huang, J., P.R. Fisher, and W.R. Argo. 2007. A gasometric procedure to measure residual lime in container substrates. HortScience 42:1685–1689.

Karkosh, A.E., S.K. Walker, and J.J. Simons. 1988. Seed treatments for control of Fe deficiency chlorosis of soybean. Crop Sci. 28:369–370. doi:10.2135/cropsci1988.0011183X002800020039x

Kenward, M.G., and J.H. Roger. 1997. Small sample inference for fixed effects from Restricted Maximum Likelihood. Biometrics 53:983–997. doi:10.2307/2533558

Leikam, D., R.E. Lamond, and D.B. Mengel. 2003. Soil test interpretations and fertilizer recommendations, MF-2586. Kansas State Univ. Agric. Exp. Stn. and Coop. Ext., Manhattan, KS.

Lingenfelser, J.E., W.T. Schapaugh, Jr., J.P. Schmidt, and J.J. Higgins. 2005. Comparison of genotype and cultural practices to control Fe deficiency chlorosis in soybean. Commun. Soil Sci. Plant Anal. 36:1047–1062. doi:10.1081/CSS-200050493

Modaihsh, A.S. 1997. Foliar application of chelated and non-chelated metals for supplying micronutrients to wheat grown on calcareous soil. Exp. Agric. 33:237–245. doi:10.1017/S001447979700001X

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

National Agricultural Statistics Service. 2010. Kansas farm facts. Available at http://www.nass.usda.gov/Statistics_by_State/Kansas (cited 15 Dec 2010; verified 21 Sept. 2011). USDA, Washington, DC.

Pedersen, P. 2004. Soybean growth and development. PM1945. Iowa State Univ. Ext., Ames.

Penas, E.J., R.A. Wiese, R.W. Elmore, G.W. Hergert, and R.S. Moomaw. 1990. Soybean chlorosis studies on high pH bottomland soils. Univ. of Nebraska Inst. Agric. Nat. Res. Bull. 312. Univ. of Nebraska, Lincoln.

Rogers, E.E., X. Wu, G. Stacey, and H.T. Nguyen. 2009. Two mate proteins play a role in Fe efficiency in soybean. J. Plant Physiol. 166:1453–1459. doi:10.1016/j.jplph.2009.02.009

Römheld, V. 1987. Different strategies for Fe acquisition in higher plants. Physiol. Plant. 70:231–234. doi:10.1111/j.1399-3054.1987.tb06137.x

SAS Institute. 2010. The SAS system for Windows. Release 9.2. SAS Inst., 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 Fe chlorosis in soybean as a function of the o,o-FeEDDHA content. Plant Soil 303:161–176. doi:10.1007/s11104-007-9496-x

Taylor, S.E., N. Terry, and R.P. Huston. 1982. Limiting factors in phytosynthesis-III Effect of Fe nutrition on the activities of three regulatory enzymes of photosynthetic carbon metabolism. Plant Physiol. 70:1541–1543. doi:10.1104/pp.70.5.1541

Wadleigh, C.H., and J.W. Brown. 1952. The chemical status of bean plants afflicted with bicarbonate-induced chlorosis. Bot. Gaz. 113:373–392. doi:10.1086/335729

Warncke, D., and J.R. Brown. 1998. Potassium and other basic cations. p. 31–34. *In* J.R. Brown (ed.) Recommended chemical soil test procedures for the North Central Region. North Central Reg. Res. Publ. 221 (Rev.). SB 1001. Missouri Agric. Exp. Stn., Columbia.

Watson, M.E., and J.R. Brown. 1998. pH and lime requirement. p. 13–16. *In* J.R. Brown (ed.) Recommended chemical soil test procedures for the North Central Region. North Central Reg. Res. Publ. 221 (Rev.). SB 1001. Missouri Agric. Exp. Stn., Columbia.

Whitney, D.A. 1998. Micronutrients: Zinc, iron, manganese, and copper. p. 41–44. *In* J.R. Brown (ed.) Recommended chemical soil test procedures for the North Central Region. North Central Reg. Res. Publ. 221 (Rev.). SB 1001. Missouri Agric. Exp. Stn., Columbia.

Wiersma, J.V. 2005. High rates of Fe-EDDHA and seed Fe concentration suggest partial solutions to Fe 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

Wittwer, S.H., W.H. Jyung, Y. Yamada, and M.J. Bukovac. R. De S. Kannan, H.P. Rasmussen, and S.N. Haile-Mariam. 1965. Pathways and mechanisms for foliar absorption of mineral nutrients as revealed by radioisotopes. p. 387–403. *In* Isotopes and radiation in soil plant nutrition studies. Proc. Symp. IAEA (Vienna), Ankara, Turkey. 28–2 July. Int. Atomic Energy Agency, Vienna, Austria.

Yousfi, S., M. Wissal, H. Mahmoudi, C. Abdelly, and M. Gharsalli. 2007. Effect of salt on physiological responses of barley to Fe deðciency. Plant Physiol. Biochem. 45:309–331. doi:10.1016/j.plaphy.2007.03.013