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Comparison of Field Management Strategies for Preventing Iron Deficiency Chlorosis in Soybean

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ABSTRACT

Iron deficiency chlorosis (IDC) is a serious management issue for soybean [*Glycine max* (L.) Merr.] grown on calcareous soils. Strip trials were established on calcareous Mollisols to study the effects of Fe-ethylene diamine-*N*,*N*′-bis (hydroxy phenyl) acetic acid (EDDHA) in-furrow (IF-Fe) and of an oat (*Avena sativa* L.) companion crop on two soybean varieties either tolerant or susceptible to IDC. The severity of IDC varied from low to severe within sites. The susceptible variety produced the highest yield in the absence of IDC. In-furrow Fe increased the yield of a variety susceptible to IDC under moderate to severe IDC. The oat companion crop increased yield consistently for the susceptible variety under severe IDC and sometimes reduced yield when oat grew beyond 25 cm in height. The tolerant variety without IDC management produced yields similar to those of the susceptible variety with IF-Fe or an oat companion crop. Oat reduced trifoliate nitrate N and Fe concentration regardless of IDC severity. Trifoliate Fe concentration lowered with IF-Fe, but only when oat was not planted. Grain protein and oil concentration were affected by variety, but were not affected by IDC management. Soil test factors such as soil organic matter (SOM), pH, electrical conductivity (EC), or diethylene triamine phentaacetic acid Fe (Fe-DTPA) were poor predictors of the severity of IDC. Variety selection is the most important strategy for lessening the severity of IDC. In-furrow application of Fe-EDDHA provides a solution for mitigating moderate to severe IDC and provides less risk than an oat companion crop.

Iron deficiency chlorosis is a serious management problem for soybean grown on soils formed in calcareous parent materials in the western Corn Belt and Great Plains. A review by Hansen et al. (2004) indicated that the impact of IDC cost soybean growers 120 million U.S. dollars in lost profit in North and South Dakota and areas of western Minnesota. In 2011, 2.95×10^6 ha of soybean were planted in Minnesota, which ranks third among states in the U.S. Nine of the top ten soybean-producing counties within Minnesota contain soils where IDC may occur (USDA-NASS, 2014). With the great potential for losses, soybean growers have been looking for management strategies to mitigate IDC and increase soybean grain yield.

Iron deficiency chlorosis is not related to an absence of Fe in the soil (Hansen et al., 2003). Factors such as DTPA-extractable Fe, soluble salt concentration, carbonates in the soil, soil pH, and soil NO_3–N can influence the incidence of IDC (Morris et al., 1990; Hansen et al., 2003; Rogovska et al., 2007; Liesch et al., 2012; Bloom et al., 2011), but in the end, IDC results from low Fe solubility preventing transport of Fe^{3+} to the root surface where it is reduced to Fe^{2+} to be taken up and used by the plant. In field studies, Inskeep and Bloom (1987) identified that IDC severity increased with greater concentrations of bicarbonate (HCO_3^-) in soil. Buffering caused by HCO_3^- in high pH soils can limit the reduction of Fe^{3+} to Fe^{2+} by Strategy 1 plants (like soybean) by inhibiting the ability of the plant to lower soil pH near the root through exudation of protons or acids (Lucena, 2000). In addition, CO_2 produced through respiration that is trapped in soil water can dissolve, forming HCO_3^- (Bloom et al., 2011). Inskeep and Bloom (1986) found if soils prone to IDC allowed significant gas exchange to the atmosphere, severe chlorosis in soybean did not occur. Routine soil tests do not directly measure HCO_3^-, making it difficult to predict where IDC will occur.

Management strategies for mitigating IDC have been researched for many years. Planting a variety tolerant to IDC has long been identified as the best strategy for mitigation (Goos and Johnson, 2000; Naeve and Rehm, 2006; Helms et al., 2010). Chelated sources of Fe have previously been researched to correct IDC, but can vary in effectiveness. Lucena (2003) indicated that Fe-EDDHA and Fe-DTPA applied at similar rates of Fe were shown to increase the grain yield of soybean on calcareous soils. Other Fe chelates such as Fe-EDTA (ethylene diamine tetraacetic acid) and

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Abbreviations: CCE, calcium carbonate equivalency; DTPA, diethylene triamine phentaacetic acid; EC, electrical conductivity; EDDHA, ethylene diamine-*N*,*N*′-bis (hydroxy phenyl) acetic acid; HCO_3^-, bicarbonate; IDC, iron deficiency chlorosis; IF, in-furrow; IF-Fe, in-furrow iron; SOM, soil organic matter.

Table 1. Site location, predominant and secondary soil series, and planting date information for the experimental areas from six locations in western–central and southwestern Minnesota.

			Predominant soil		Secondary soil		
Site	Year	County	Series	Classification	Series	Classification	Planting date
1	2010	Renville	Amiret	fine loamy mixed, superactive, calcic Hapludolls	Canisteo	fine loamy mixed, superactive, calcareous, mesic typic Endoaquolls	6 May
2	2010	Renville	Okoboji	fine smectic mesic, cumulic, vertic Endoaquolls	Leen	fine silty mixed superactive, mesic Calciaquolls	6 May
3	2011	Kandiyohi	Canisteo	fine loamy mixed, superactive, calcareous, mesic, typic, Endoaquolls	Seaforth	fine loamy mixed, superactive, mesic aquic Calciudolls	26 May
4	2011	Renville	Amiret	fine loamy mixed, superactive, calcic Hapludolls	Okoboji	fine smectic mesic, cumulic, vertic Endoaquolls	8 June
5	2012	Lyon	Jeffers	mixed, superactive, calcareous, mesic typic Endoaquolls	Calco	mixed, superactive, calcareous, mesic cumulic Endoaquolls	16 May
6	2012	Laq Qui Parle	Dovray	frigid cumulic vertic Epiaquolls	Colvin	mxed, superactive frigid typic Calciaquolls	15 May

Fe-hydroxy ethylene diamine triacetic acid were shown to not increase soybean grain yield. Lindsay and Schwab (1982) found that Fe-EDDHA kept all of the Fe in a chelated form over a range of pH values, whereas Fe-ethylene diamine tetraacetic acid and Fe-DTPA could only maintain a fraction of Fe in solution at pH of 7.5. It is important to note that products that are sold as Fe-EDDHA do not comprise a single substance but a mixture of isomers and oligomers that vary in their ability to keep Fe in solution and deliver Fe to plants. The *o,o*-Fe-EDDHA isomers have the most stable pH and are the least sensitive to processes like adsorption. Iron-EDDHA products can strongly vary in composition of their different components. The *o,o*-Fe-EDDHA fraction is an important parameter, because on highly calcareous soils, only these components seem to perform well as Fe fertilizer (Schenkeveld et al., 2008, 2010).

Research with foliar application of Fe-EDDHA has shown mixed results following multiple applications. Randall (1977) indicated soybean grain yield could be increased with 18 to 28 g ha^{-1} of foliar-applied Fe (as a 6% Fe-EDDHA chelate) when the second trifoliate emerged. Liesch et al. (2011) found that foliar-applied Fe-EDDHA increased soybean grain yield at one of seven locations. Goos and Johnson (2000) showed that Fe-EDDHA increased soybean seed yield. The increase in seed yield occurred for a variety less tolerant to IDC at three out of four sites and for two varieties that are more tolerant to IDC at one site. Seed-placed Fe-EDDHA either as a seed coating (Karkosh et. al., 1988; Wiersma, 2007; Liesch et al., 2011) or as a combination of a seed coating and a band application directly with the soybean seed (Wiersma, 2005), have shown promise for increasing soybean grain yield. However, Wiersma (2005) stated that the use of Fe-EDDHA was not a cost-effective management strategy when considering the manufacture cost of the product.

Innovations in the manufacture process of Fe-EDDHA have reduced the cost to soybean growers. The major question from growers has been the method of application, either seed placement or foliar. Since many farmers in central and western Minnesota have planters equipped to apply liquid fertilizers on the seed for other crops, application of Fe-EDDHA in a liquid suspension is a viable option. Application of fertilizer with the planter directly on the soybean seed is discouraged due to the potential risk for stand loss due to high concentrations of salts in contact with the seed (Rehm and Lamb, 2010). The application of Fe-EDDHA with the seed has not been shown to reduce a soybean population (Wiersma, 2007).

Excess application of N has been shown to increase the severity of IDC on susceptible soils (Aktas and van Egmond, 1979). Wiersma (2010) found that the severity of IDC increased for susceptible varieties with the addition of NO_3–N in northwestern Minnesota, although there was no effect on varieties that are moderately tolerant and tolerant to IDC. Bloom et al. (2011) studied field areas compacted by wheel traffic where IDC severity was reduced. Soil NO_3–N was lower in the compacted areas compared to adjacent field areas where IDC was severe. Bloom et al. (2011) concluded that NO_3–N was a causative factor for IDC in calcareous Mollisols in western Minnesota. Use of a companion crop to take up excess NO_3–N and to reduce excess soil moisture has been previously studied (Naeve, 2006; Bloom et al., 2011). Oat has been used due to its relatively low cost and susceptibility to glyphosate. Oat is a Strategy 2 plant with regard to Fe acquisition. Strategy 2 plants release phytosiderophores, which are chelating agents that aid in the mobilization and acquisition of Fe. Zuo et al. (2000) theorized that phytosiderophores released from Strategy 2 plants may aid in the acquisition of Fe of intercropped Strategy 1 plants. Naeve (2006) found that an oat companion crop improved visual IDC scores, but concluded that the oat companion crop was not likely to be profitable, as soybean grain yield was not consistently increased. Bloom et al. (2011) found that an oat companion crop increased yields at one out of four locations and also decreased at one location due to overcompetition for resources, such as soil moisture.

The severity of IDC varies annually, making locating a large enough area with a consistent level of chlorosis difficult for small-plot research. If a field area does not vary in the severity of IDC, it is difficult to address whether management strategies should be changed based on the severity of IDC. Strip trials have an advantage over traditional small plots since they encompass more field area, which allows for testing of treatments under varying soil chemical properties. Soybean producers are questioning whether higher yielding varieties that are more susceptible to IDC can be planted in areas prone to severe IDC if Fe-EDDHA or an oat companion crop is used. Although other management options are available, planting a tolerant variety is still recommended (Kaiser et al., 2011). Replicated strip trials were established to study the impact of variety selection, application of Fe-EDDHA directly on the seed with the planter (in-furrow), and an oat companion crop among field areas with differing severity of IDC on trifoliate NO_3–N and Fe concentration, soybean grain yield, and seed protein and oil concentration. Using yield differences among varieties tolerant or susceptible to IDC, we aimed to determine if routine soil test factors can be used to predict IDC severity in calcareous Mollisols in western Minnesota.

MATERIALS AND METHODS

Six strip trials were conducted from 2010 to 2012 on soils formed in glacial till in the western part of central Minnesota (soil descriptions are given in Table 1). Field sites were selected that had a previous history of IDC but with some variability ranging from no to low, moderate, and severe levels. Treatments were arranged as a split-plot within a randomized complete block design and were replicated five times. Main plots consisted of: (i) a control (no IDC treatment), (ii) a solution containing 6% Fe [trade name Soygreen (West Central Inc., Willmar, MN)] applied on the seed at the time of planting (IF-Fe), (iii) an oat companion crop, and (iv) a combination of IF-Fe and an oat companion crop. In total, 80 to 83% of the Fe in the Fe-EDDHA fertilizer was present as *o-o*-Fe-EDDHA. The Fe-EDDHA product was applied at a rate of 3.36 kg ha^{-1} and was mixed with water. The Fe-EDDHA and water mixture was applied at 56 L ha^{-1} directly on the seed (IF application) with a planter (John Deere Max-Emerge 7200 (John Deere Co., Moline, IL) equipped with a liquid delivery system (Raven Industries, Sioux Falls, SD). Oat seed was broadcast at 134 kg ha^{-1} 1 d before soybean planting in 2010 and the day of soybean planting in 2011 and 2012. A spike tooth harrow was used to slightly bury the oat seed before soybean was planted. The oat crop was terminated with a single application of glyphosate when the oat crop was approximately 25 cm tall and soybean was at the V3 to V8 growth stage (Ritchie et al., 1994).

Subplots consisted of two soybean varieties, one rated as tolerant to IDC and the other less tolerant but higher yielding when IDC is not present. All varieties selected were of similar maturity groups, were glyphosate resistant, and had similar disease resistance packages. The tolerant varieties used were Gold Country brand '3517' in 2010 and '1448' in 2011 and 2012. The nontolerant variety used was Gold Country brand '2717' in 2010 and 2011 and '1542' in 2012. Row width was 76 cm and seeding rate was 370,500 seeds ha^{-1} at all locations. Planting dates for each location are given in Table 1.

The individual main and subplot combinations (whole plots) were established as 146.4-m long strips within each study location. Main plot strips were 6 m wide and were subdivided into 3-m wide subplot (variety) strips (each subplot strip consisted of four rows). Each strip was subdivided into eight segments, each 18.3 m long. These segments represented individual sampling points for in-season plant samples and yield measurements. An example of an individual replication with subsampling points is given in Fig. 1. The total area used in each study site was 1.78 ha. The previous crop was corn (*Zea mays* L.) at all locations except Site 2, which was previously planted to sugar beet (*Beta vulgaris* L.).

Multiple soil samples were collected within each replication to assess within-plot variability. Subdivisions represented an area the full width of the replication (24 m) and were

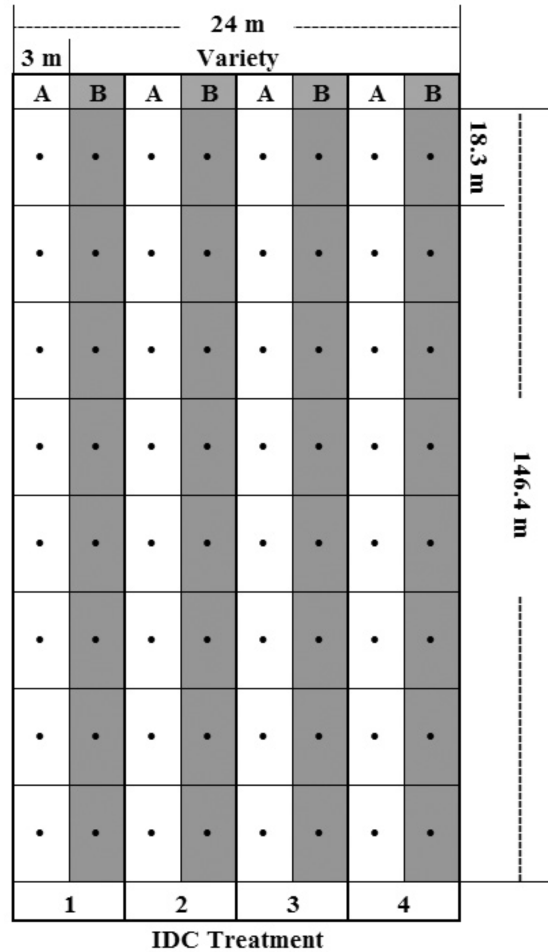

Fig. 1. Example of an individual replication within a study location showing main and subplot treatment arrangement. Closed in circles represent individual subsampling points along each strip. Main plot treatments were randomized separately for each replication. Subplots (variety) strips were randomly assigned within each main plot. Each study included five replications.

18.3 m long (0.044 ha area per soil sample). Each replication subdivision is termed an individual grid cell within the field and included a single repetition of all combinations of oat, IF-Fe, and variety treatments. A total of 40 grid cells were soil sampled per location (eight per replication). Soil samples were collected by taking 12 individual soil cores at a depth of 0 to 15 cm within 5 m of the center of each grid cell. Soils were dried at 40°C and ground to pass through a 2-mm sieve. Soils were analyzed for NO_3–N with 2M KCl (Gelderman and Beegle, 1998), Olsen P (Frank et al., 1998), ammonium acetate K (Warncke and Brown, 1998), soil pH [1:1 soil/water (Watson and Brown, 1998)], SOM [loss on ignition (Wang and Anderson, 1998)], soluble salts [by electrical conductivity (EC), 1:1 soil/water (Whitney, 1998b)], calcium carbonate equivalency (CCE) measured using the modified pressure calcimeter method (Sherrod et al., 2002), and DTPA-extractable Fe (Whitney 1998a).

Plant samples were collected from each location at the time oat was terminated. Oat plants were sampled by cutting all plants at the soil surface within a 1-m^2 area from the center of each oat main plot strip. Soybean plants were sampled by taking the uppermost fully developed trifoliate, with the petiole. Thirty individual plants were sampled at each sampling point at approximately the V3 growth stage. Samples were transported from each location in a cooler with ice then dried at 60°C for 7 d. Plant samples were ground to pass through a 1-mm sieve. Total N concentration in the oat samples was determined using a Variomax C and N analyzer (Elementar Americas Inc., Mt. Laurel, NJ). Oat N uptake was calculated using the product of the total biomass produced multiplied by total N concentration. Soybean trifoliate samples were analyzed for NO_3–N and Fe concentration. Nitrate N concentration was determined by shaking 100 mg of the sample in 20 mL of water for 30 min on a reciprocal shaker. Nitrate N in the plant tissue extract was determined using a Lachat flow-through analyzer (Hach Co., Loveland, CO). Total Fe in the plant tissue was determined with inductively coupled plasma mass spectroscopy following digestion with HNO_3 and H_2O_2 (Gavlak et al., 2003).

Visual chlorosis scores were taken at the R6 growth stage. Initial scores were taken at the V3 growth stage, but the visual differences were not readily apparent so these data were not used. All subplots within each cell were scored on a scale from 0 to 5. The scores used were: 0, no IDC present; 1, normal looking plants with yellow leaves consisting of <50% of the upper leaves; 2, normal looking plants with yellow leaves on >50% of the upper leaves; 3, plants were stunted and chlorotic with greater than one pod per node; 4, plants were stunted and chlorotic with less than one pod per node; 5, plants were severely affected by IDC and had no pods.

Trials were harvested using a research grade combine. A single yield measurement was taken from three rows every18.3 m along each subplot (variety) strip. A total of 320 individual observations were collected at each site (eight subdivisions per strip, two variety strips per treatment, four treatments per replication, and five replications per site). All yields are reported at 130 g kg^{-1} moisture content. A subsample of grain was collected at harvest and protein and oil concentration was determined on unground samples using a Perten 7250 diode array (Perten Instruments, Stockholm, Sweden).

Treatment significance was determined on relative means using PROC GLIMMIX in SAS version 9.3 (SAS Institute, Cary, NC) assuming fixed effects of IDC rating score, variety, oat, and IF-Fe application and random year, site, and block effects. Effects were considered significant at $P \leq 0.10$. Relative means within each location for all measured variables were calculated based on the mean of the tolerant variety with no IDC treatment (the control) in field areas with no IDC at each location (Table 2). This treatment was used because it represents the standard practice used in fields with IDC in western Minnesota. Significant interactions were investigated using the SLICE option of the LSMEANS statement; means separation was completed using the PDIFF option for the four-way interaction of rating score, variety, oat, and IF-Fe. Multiple regression was conducted in SAS using PROC REG with stepwise selection. Correlations among variables were assessed using PROC CORR. All mean values presented are least squares means.

RESULTS AND DISCUSSION

Soil series information is summarized in Table 1. Multiple soil series were mapped at each location. The major and minor soil series listed in the table encompassed 75% or more of the area within each plot and are soils typical of Mollisols where IDC occurs in western–central and southwestern Minnesota. Soil test variability across the plot areas is summarized in Table 3. All measured variables exhibited a significant amount of variability across the trial areas. The measured variable with the least variation was pH, which averaged near 7.4 for most locations and ranged down to as low as 5.9 and as high as 7.8. Soil test P ranged from low to very high according to current guidelines. No fertilizer P was applied directly to the soybean. However, fertilizer P was applied before the previous crop for soybean, which is common for rotations containing soybean in Minnesota. Since some areas still tested low in soil P (<8 mg kg^{-1} by the Olsen P test), there may have been some yield reductions within some fields due to a lack of P. Soil K tested in the very high classification (>160 mg kg^{-1} using the ammonium acetate test). Nitrate-N and soil test Fe were also measured but their relative sufficiency cannot be determined since neither test is calibrated to predict sufficiency for soybean grown in Minnesota (Kaiser and Lamb, 2012). Soil organic matter ranged from 21 to 107 g kg^{-1}.

Table 2. Mean of soybean trifoliate NO_3–N and Fe concentration and grain yield, protein, and oil concentration of the tolerant variety grown on soils with no or low iron deficiency chlorosis (IDC) severity and no IDC management.

	Trifoliate		Soybean grain		
Site	NO_3–N	Fe	Yield	Protein	Oil
	—— mg kg^{-1} ——		Mg ha^{-1}	—— g kg^{-1} ——	
1	221	196	3.4	382	210
2	222	701	3.1	389	208
3	119	298	2.9	371	199
4	54	177	2.5	382	193
5	81	213	2.2	401	208
6	229	170	2.2	392	214

Two factors that are generally considered to be important in identifying areas of severe IDC are CCE and soluble salts (measured by EC) (Hansen et al., 2003). There was a significant range in the EC measurements at Sites 1, 2, 5, and 6. Mean EC across Sites 1 and 2 was much closer to the minimum values, as there were fewer grid cells that tested toward the maximum values at these locations. Mean EC levels were much higher for Sites 5 and 6. This could have been a result of the dry soil conditions caused by the lower than normal precipitation between the fall of 2011 and early spring in 2012 (data not shown). There was substantial variation in CCE across and within the locations. The site with the highest mean CCE was Site 3 (225 g kg^{-1}) and the lowest was Site 5 (7 g kg^{-1}). If CCE alone was the best indicator of IDC severity, Site 3 would post the greatest risk followed by Sites 2, 1, 6, 4, and 5.

Average monthly temperature and total monthly precipitation data are summarized in Table 4. Precipitation in May was greater than the 30-year normal for all sites. During June and July, total precipitation was greater than normal for Sites 1 to 4 and was less for Site 6. Precipitation at Site 5 was similar to normal in June and much less than normal in July. These variations are reflective of precipitation differences in 2010 and 2011 vs 2012, which was much drier and warmer for most of the growing season. In August, precipitation was less than normal, except at Sites 3 and 6.

Table 3. Site minimum, mean, and maximum Olsen soil test P, ammonium acetate K, DTPA-extractable Fe, soil organic matter (SOM), calcium carbonate equivalency (CCE), electrical conductivity (EC) and 1:1 soil/water pH taken from grid cells sampled before treatment application. Samples are composed of 8 to 10 soil cores taken at a depth of 0 to 15 cm from a 10-m radius from the center of each grid cell.

Site	DStat†	NO_3–N	P	K	Fe	SOM	CCE	EC	pH
		mg kg^{-1}	mg kg^{-1}			g kg^{-1}		S m^{-1}	
1	Min.	6	4	165	7.8	21	9	0.03	7.0
	Mean	12	15	262	12.2	65	67	0.05	7.4
	Max.	24	47	596	20.8	107	117	0.18	7.8
2	Min.	5	11	161	8.4	45	30	0.05	7.0
	Mean	14	18	248	10.0	63	107	0.08	7.3
	Max.	28	30	361	12.2	90	207	0.20	7.7
3	Min.	6	8	167	9.2	37	88	0.03	6.7
	Mean	8	13	251	13.5	70	225	0.04	7.3
	Max.	18	20	351	20.9	86	357	0.05	7.6
4	Min.	3	5	214	12.5	37	0	0.02	5.9
	Mean	7	15	282	35.0	54	9	0.03	6.9
	Max.	12	38	480	96.4	80	38	0.05	7.5
5	Min.	5	3	191	7.7	30	0	0.03	7.2
	Mean	9	9	237	11.5	40	7	0.13	7.5
	Max.	20	27	306	18.7	49	38	0.33	7.8
6	Min.	4	4	166	9.0	35	25	0.03	6.3
	Mean	8	31	298	15.9	62	61	0.13	7.4
	Max.	12	60	460	55.2	74	90	0.19	7.8

† DStat, descriptive statistics; Min., minimum; Max., maximum.

Table 4. Monthly average temperature and total precipitation data at the four locations studied. Data are collected from the nearest reporting weather stations within 30 km of each location. Research was conducted in 2010 at Sites 1 and 2, in 2011 at Sites 3 and 4, and in 2012 at Sites 5 and 6.

	Precipitation data†								Temperature data‡							
	May		June		July		August		May		June		July		August	
Site	Tot.	DN	Tot.	DN	Tot.	DN	Tot.	DN	Avg.	DN	Avg.	DN	Avg.	DN	Avg.	DN
	mm								°C							
1	71	14	151	81	75	14	53	−06	14.1	−0.6	18.8	−1.1	20.9	−1.0	23.0	2.5
2	71	14	151	81	75	14	53	−06	14.1	−0.6	18.8	−1.1	20.9	−1.0	23.0	2.5
3	123	65	126	49	202	139	74	11	12.6	−1.9	18.4	−1.2	24.0	2.1	20.2	−0.2
4	132	76	91	21	178	117	31	−28	12.6	−2.2	18.6	−1.3	24.7	2.8	20.6	0.2
5	118	61	72	04	08	−52	43	−14	16.7	2.1	21.6	1.8	25.4	3.2	20.7	−0.2
6	216	159	50	−13	46	−15	112	56	17.2	1.2	22.3	1.5	26.3	3.3	21.8	−0.2

† Tot, total monthly precipitation; DN, departure from normal.
‡ Avg., average monthly temperature; DN, departure from normal.

Since direct soil moisture measurements were not taken, the precipitation data could provide some insight into the relative risk for severity of IDC. As previously stated, most growers use CCE and EC to determine where severe areas of IDC lie, but research by Inskeep and Bloom (1986, 1987) has indicated that these two measurements alone do not guarantee that IDC will develop. In their work, IDC developed only when a soil had a high water content that caused a build-up of HCO_3^- in the soil. Since all locations had at least one period of above-normal rainfall, determining when the critical timing of rainfall occurs that results in a high prevalence of IDC within a field is important. This will be discussed further, along with the relevance of soil test variables for predicting IDC severity.

Effects on Soybean Grain Yield

The visual IDC scores of the susceptible variety taken at the R6 growth stage were studied to separate the field areas into three categories (no or low IDC pressure, moderate IDC where plants are chlorotic and slight reductions in grain yield are expected, and severe IDC). Only data from Sites 1 and 2 could be used, since IDC symptoms were clearly visible when the scores were taken. Site 1 contained very little IDC pressure in large areas of the field. A comparison among IDC severities could not be made between yield and visual IDC score, so the Site 1 data were not used. Treatment differences were highly ambiguous at the remaining sites, making it impossible to use the visual scores themselves to divide the sites for analysis. Therefore, additional methods were needed to assess IDC variability across the research sites.

Two methods previously reported by Rogovska et al. (2007) and Naeve and Rehm (2006) were used to estimate the severity of IDC. Each method used multiple regression of the soil test variables with reductions in soybean grain yield due to IDC. Rogovska et al. (2007) proposed an alkalinity stress index based on soil pH + 0.14 (% CCE) of the soil. Since there was a high degree of variability in the yield potential of the susceptible variety and lower variation in soil pH, the alkalinity stress index value did not perform well for determining the severity of IDC and was not used in the

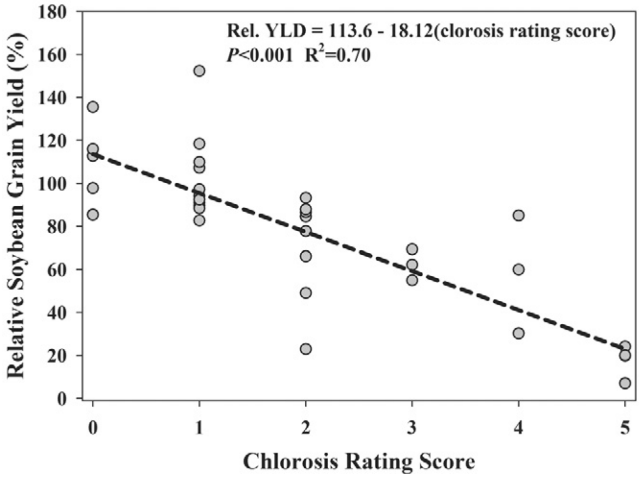

Fig. 2. Relationship between the relative yield of the susceptible variety as a percentage of the yield of the tolerant variety when no iron deficiency chlorosis (IDC) management was used vs visual IDC scores taken at the R6 growth stage within each grid cell from Site 2. The dashed line represents the linear regression with the best fit.

current study. Naeve and Rehm (2006) proposed an index using

$$0.77 - 2.25 \times \mathrm{EC} - 0.00572 \times \mathrm{CCE} + 0.0615 \times \text{Fe-DTPA}$$

where EC is in S m^{-1}, CCE is in g kg^{-1} and Fe-DTPA is in mg kg^{-1}. An index value of greater than 1 would indicate where severe IDC would be present and a value less than 0.05 would indicate no IDC. When calculated for the current study, the index proposed by Naeve and Rehm (2006) showed no clear relationship to yield or an ability to predict the severity of IDC.

The site with the greatest variability in IDC severity that could be visually scored (Site 2) was used to regress the relative yield of the susceptible variety (the grain yield of the susceptible divided by the grain yield of the tolerant under the control treatments) for each grid cell with the IDC rating score taken at the R6 growth stage. The relationship between the visual IDC score and relative yield was significant and linear (Fig. 2). To maximize the total number of grid cells per IDC rating, the six visual IDC scores were grouped into three classifications (IDC ratings) for analysis. A visual IDC score less than 1.5 was considered no or low IDC (identified hereafter as low), a score between 1.5 and 2.5 was considered moderate, and a score greater than 2.5 was severe. Using the linear regression, the predicted ranges for relative yield would be >90% for low IDC severity, 65 to 90% for moderate IDC, and <65% for severe IDC.

Each grid cell was given a severity rating using the relative yield of the susceptible variety (compared to the tolerant one) with no IDC management. Grain yield data were combined across four of the locations where IDC was severe (Sites 1, 2, 3, and 5). Sites 4 and 6 were analyzed separately, as both locations were negatively impacted by the oat treatment across each study and neither had field areas considered severe. Data were combined across all six locations for the remaining factors studied, as the impact of the oat treatment was similar for each location.

The significance of the main effect and their interactions for soybean grain yield is given in Table 5. For the four locations (Sites 1, 2, 3, and 5) where IDC was the most severe main effects of IDC rating, IF-Fe, and variety were significant ($P \leq 0.10$). The only main effect that was not significant at the accepted level was the oat companion crop. However, there were several significant two- and three-way interactions containing oat, indicating a potential effect that differed by one or more other factors. In addition, the four-way interaction was significant, indicating that the three-way interaction of variety, oat, and IF-Fe varied with IDC severity rating. Test effect slices showed that all three-way interactions were significant within each severity rating class, indicating differences in response to IDC management for differing levels of IDC severity.

Table 5. Summary of statistical significance of the main treatment effects (iron deficiency chlorosis (IDC) severity rating, oat, in-furrow Fe, and variety) and interaction effects for soybean grain yield collected from six locations.

	Grain yield by sites		Trifoliate		Grain	
	$P > F$		$P > F$		$P > F$	
Effect†	1, 2, 3, and 5	4 and 6	NO_3–N	Fe	Protein	Oil
R	***	0.33	0.47	0.06	0.21	0.87
O	0.20	**	***	***	0.23	0.57
R × O	**	0.06	0.32	*	0.13	0.17
Fe	***	0.13	0.26	*	0.87	0.81
R × Fe	***	0.34	0.56	0.45	0.81	0.48
Fe × O	0.77	0.25	*	***	0.57	0.62
R × Fe × O	0.12	0.13	0.06	*	0.74	0.29
V	**	0.35	***	***	***	***
R × V	***	*	0.23	0.06	***	0.14
V × O	***	0.75	***	***	0.32	0.69
R × V × O	***	*	0.22	0.07	0.18	0.28
V × Fe	***	0.06	0.24	0.45	0.47	0.20
R × V × Fe	*	0.44	0.10	0.52	0.22	0.67
V × O × Fe	*	0.73	0.11	0.90	0.83	0.42
R × V × O × Fe	*	0.63	0.36	0.47	0.29	0.11

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† R, IDC severity rating; O, oat; Fe, in-furrow iron; V, variety.

When IDC was not present, the susceptible variety out-yielded the tolerant one by an average of 9% across all IDC management treatments (Table 6). The only treatment that reduced yield in the susceptible variety was oat without IF-Fe, which resulted in a yield similar to the tolerant variety. There was no difference in yield among any of the IDC management treatments for the tolerant variety when no or moderate IDC was present. The yield of the susceptible variety in the treatment without oat and IF-Fe was reduced by 27% in the presence of moderate IDC. However, soybean grain yield increased by 15% with IF-Fe and resulted in yield levels similar to the tolerant variety without any IDC management. This indicates that there may be a greater potential grain yield response to IF-Fe for the susceptible variety but the yields achieved are no better than those of the tolerant varieties with or without IDC management treatment.

Soybean yield was greatly reduced for both varieties when IDC was severe. The greatest reduction in yield was for the susceptible variety without any IDC management, which was 34% less than the tolerant variety with severe IDC and was 62% less than the susceptible variety in field areas with low IDC. The susceptible variety benefited the most from both oat and IF-Fe, which increased yield by 23 to 25% when used alone and 39% when used together. The 39% increase produced a grain yield that was no greater than that of the tolerant variety with or without IDC management treatments. The combination of oat and IF-Fe was the only treatment for the tolerant variety that statistically differed from the control under severe IDC and resulted in yields similar to the control of the tolerant variety under low and moderate levels of IDC. The relative grain yield response to IF-Fe applied to the tolerant variety was no different from the response to the combination of oat plus IF-Fe and that to the control. The difference in yield for oat only vs oat plus IF-Fe indicates a greater potential for IF-Fe to increase yield than oat for the tolerant variety. The oat treatment may have enhanced the effect of IF-Fe for the tolerant variety but the effect was not as great as that found for the susceptible variety. Since the susceptible variety did not result in greater yield than the tolerant one under any IDC management treatments when grown under moderate to severe IDC, the use of a tolerant variety is the best management option for fields affected by IDC.

Soybean grain yield was reduced by the oat companion crop by an average of 16% at Sites 4 and 6 (Table 6). The reduction in soybean grain yield with the oat companion crop was a direct result of oat termination beyond the optimal time. The targeted termination of the oat crop was at 25 cm (Kaiser et al., 2011), which is based on previous unpublished research data. It was found that early termination did not benefit the soybean crop but if termination was too late, this caused over-competition and reduced soybean yield. Tractor-mounted sprayers were used due to the size of the studies. Heavy precipitation events near the optimum time of spraying delayed the time of termination until the oat crop was greater than 25 cm high at a few sites. Although heights were not measured, total oat biomass is indicative of plant size (height) at termination (Table 7). The greatest oat biomass produced was at Site 6, which coincided with the greatest reduction in yield (not shown). Although the total biomass produced was important, weather patterns shifted and precipitation decreased in June and July at Site 6 (Table 4), which magnified the negative effect of the oat companion crop.

There were two interactions of note that were significant for the data summarized from Sites 4 and 6, severity rating × variety × oat and variety × IF-Fe (Table 5). The severity rating × variety × oat interaction was caused by a reduction in yield caused by oat in field areas with low IDC severity (average reduction of 25%). Yields were similar with and without the oat treatment for both varieties when grown under moderate IDC. The significant two-way interaction between variety and IF-Fe indicated that IF-Fe slightly increased the yield of the susceptible variety. Similar to the other four sites, the yield level attained by

Table 6. Relative soybean grain yield and trifoliate NO_3–N and Fe concentration response (taken at approximately the V3 growth stage) relative to the tolerant variety grown on field areas with no or low iron deficiency chlorosis (IDC) severity without an IDC management treatment. Performance of the IDC management treatment [in-furrow Fe (IF-Fe) oat companion crop, and variety selection)] is summarized for field areas of differing IDC severity. Data were summarized across six locations unless otherwise noted.

			Relative soybean grain yield				Relative trifoliate NO_3–N†		Relative trifoliate-Fe†	
			Sites 1, 2, 3, and 5†		Sites 4 and 6†					
IDC severity	Oat	Fe	S	T	S	T	S	T	S	T
			%							
No or low	no	no	110ab	100c	105ab	100abc	160c	100de	110cde	100efg
		yes	111a	100c	108a	101abc	156c	92de	105def	93g
	yes	no	101c	95cde	75f	80ef	42f	51f	68h	66h
		yes	110ab	101c	81ef	78f	47f	37f	67h	64h
Moderate	no	no	83fgh	97cd	76f	92cde	202b	114d	129b	106def
		yes	102bc	100c	93abcd	98abcd	192bc	112de	120bc	92g
	yes	no	89defg	95cde	80ef	86def	38f	41f	70h	61h
		yes	100c	98c	84def	81ef	49f	49f	70h	67h
Severe	no	no	48j	82gh	–	–	282a	106de	151a	114bcd
		yes	71i	87efg	–	–	168bc	103de	111cde	96fg
	yes	no	73hi	76hi	–	–	42f	38f	58h	58h
		yes	87defg	93cdef	–	–	61ef	61ef	62h	65h

† Letters following numbers indicate significance of the treatment interaction between rating, oat, IF-Fe, and variety at $P \leq 0.10$ for the susceptible (S) and tolerant (T) varieties.

Table 7. Minimum, mean, and maximum oat biomass and total N uptake for the oat treatments at the six studied sites sampled when the oats were at least 25 cm in height.

Site	Oat biomass			Oat N uptake		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum
	kg ha^{-1}					
1	296	666	1232	9	21	51
2	0	718	1351	0	29	53
3	352	703	1114	11	21	34
4	106	334	762	4	14	33
5	163	484	1026	11	15	34
6	415	801	1213	4	20	33

the combination of IF-Fe and the susceptible variety was still no greater on average than the tolerant variety without IF-Fe.

A difference in grain yield among varieties was expected, as many previous studies have shown a clear relationship between grain yield potential and IDC severity (Goos and Johnson, 2000; Naeve and Rehm, 2006; Helms et al., 2010). In terms of risk, planting the tolerant variety without IDC treatment appears to be as good as planting the susceptible variety with either oat or IF-Fe, or the combination of the two. The exception would be field areas where IDC is not present, in which the susceptible variety without IDC treatment resulted in the greatest yield potential at Sites 1, 2, 3, and 5. These data support the practice of variable seeding of soybean by planting a higher yielding but susceptible variety where there is low risk for IDC and a tolerant variety in areas affected by moderate to severe IDC.

Increased soybean grain yield from the oat companion crop supports previous research by Naeve (2006) who indicated that small grains such as oat offered the greatest opportunity to mitigate the symptoms of IDC. However, Naeve (2006) did not document consistent yield responses from the use of oat interseeded with soybean. Although yield increases were documented across four sites for the susceptible variety, there was no statistical evidence that the use of oat increased the yield of the susceptible to a higher level than the yield average of the tolerant variety without oat. Thus using the tolerant variety alone provided the same yield and did not present the risks associated with decreased grain yield found at Sites 4 and 6. In addition to the reductions in yield at Sites 4 and 6, there was some evidence of a small reduction in yield when no IDC was present at Sites 1, 2, 4, and 5 for both varieties for the oat treatment without IF-Fe (data not shown). If oat is used, it appears that its application should be targeted to areas of severe IDC to limit the possibility of negative effects with this practice.

Past research has focused on treatment response for field areas with moderate to severe IDC. No previously published work could fully address the impact of severity of IDC on treatment performance within the same location. When comparing results from separate locations, Wiersma (2005) indicated that grain yield was increased by seed-placed Fe-EDDHA for varieties with differing tolerance to IDC in environments with moderate and severe IDC. Results from the current study agree with past research as to the potential benefits of Fe-EDDHA applied to the seed. The greater response from the less tolerant variety agrees with the results for seed-placed Fe-EDDHA (Wiersma, 2005) and foliar Fe-EDDHA (Goos and Johnson, 2000) but is in contrast to the results reported by Karkosh et al. (1988) where tolerant varieties alone responded to Fe-EDDHA. There was still some evidence that a yield response from IF-Fe was possible for a more IDC tolerant variety but its use was best suited when IDC was severe.

Although the use of Fe-EDDHA had some positive results, the Fe-EDDHA rate used in this work may be greater than needed to maximize yields. Wiersma (2005, 2007) used differing rates of Fe-EDDHA chelate and concluded that the use of Fe-EDDHA on soybean was not cost-effective. At the time of the current research, the cost of 3.36 kg Fe-EDDHA ha^{-1} used was US\$12.20. A soybean value of US\$367 Mg^{-1} would require a 33 kg ha^{-1} increase in grain yield for Fe-EDDHA to be cost-effective. Since all grain yield increases were 0.1 Mg ha^{-1} or greater (data not shown), the Fe-EDDHA used in this study would be profitable, in contrast to the conclusions presented by Wiersma (2005). In-furrow Fe-EDDHA presents a relatively low-cost solution to mitigate the effects of moderate to severe IDC even when a tolerant variety is planted.

Trifoliate Nitrate-Nitrogen and Iron Concentration

The main effect of oat and variety significantly ($P \leq 0.10$) differed for soybean trifoliate NO_3–N concentration across most sites (Table 5). The oat treatment alone resulted in the greatest reduction in NO_3–N concentration (Table 6). A significant interaction between variety and oat occurred as a result of significant differences between the varieties but only when oat was not present. When oat was present, the concentration of trifoliate NO_3–N did not differ among varieties. As a result, the susceptible variety had a greater reduction in trifoliate NO_3–N concentration as a result of oat compared to the tolerant variety regardless of IDC severity. In fact, the relative level of trifoliate NO_3–N concentration in the susceptible variety was 2.66 times greater than that in the tolerant when grown under severe IDC with no IDC treatment, whereas the concentration was 1.6 times greater for the susceptible variety when the severity of IDC was low.

A reduction in trifoliate NO_3–N concentration would be expected if the oat crop was reducing NO_3–N in the soil before soybean commenced uptake. The total amount of N taken up by the oat crop varied by site and is summarized in Table 7. There was no correlation among oat N uptake, soil test NO_3–N (0–15 cm), and either trifoliate NO_3–N concentration for the no-oat treatments or the reduction of NO_3–N under the oat treatment (data not shown). It should be mentioned that since only trifoliate NO_3–N concentration was measured, the potential for dilution effects due to differences in plant growth could not be determined. Although the main effect of IF-Fe was not significant, there was a 48% reduction in trifoliate NO_3–N concentration for the susceptible variety under severe IDC stress when oat was not seeded. Again, this reduction could be a result of a dilution of NO_3–N resulting from an increase in growth, since grain yield was more consistently affected by IF-Fe for the susceptible variety and an accompanying increase in plant growth would also be expected. The data indicate that, overall, the oat companion crop does have an effect on trifoliate NO_3–N concentration. However, trifoliate NO_3–N concentration cannot be easily predicted through routine soil tests or when measuring N uptake by a companion crop and cannot be used as a predictor of soybean grain yield.

There has been some debate as to the specific mechanism of the increased severity of IDC from greater uptake of NO_3–N by soybean. At the root surface, the uptake of NO_3–N can result in an increase in pH near the root surface that neutralizes protons released by the plant to reduce Fe^{3+} to Fe^{2+}, which is the form taken up by the plant (Lucena, 2000). Other researchers have theorized that excess uptake of NO_3–N in the leaves is responsible for increases in IDC. Kosegarten et al. (1999, 2001) found that nitrate accumulation in the leaves of sunflower (*Helianthus annus* L.) resulted in an increase of the apoplastic pH. This increase in leaf apoplastic pH lowers the plant's ability to reduce Fe^{3+} to Fe^{2+}, which increases the severity of IDC in the plant, since Fe^{3+} cannot be transported through the plasma membrane. Although both are plausible explanations of the effects of NO_3–N on the severity of IDC, which of them presents the greatest effect is still of some debate.

The main effect of variety, oat, and IF-Fe significantly affected trifoliate Fe concentration across sites (Table 5). Even though the main effects were significant, there were two three-way interactions that bear greater consideration. These included (i) the interaction of severity rating × variety × oat, and (ii) the interaction of severity rating × IF-Fe × oat. For the severity rating × variety × oat interaction, a severity rating × variety interaction occurred only when oat was not seeded. In fact, there was no difference in the relative concentration of trifoliate Fe when oat was seeded in the three severity rating classes among either the tolerant or the susceptible varieties (Table 6). In the absence of oat, there was no difference in the trifoliate Fe concentration of the tolerant variety among the three severity classes. Trifoliate Fe concentration increased linearly with the severity class of the field areas. In fact, the relative concentration of trifoliate Fe was similar in the susceptible variety under no IDC and in the tolerant variety grown under severe IDC. Trifoliate Fe concentration was slightly higher for the susceptible variety grown without oat than for the tolerant variety when no IDC was present.

The severity rating × IF-Fe × oat interaction indicated a change in the effect of IF-Fe based on severity rating, but only when oat was not seeded. In-furrow Fe reduced the trifoliate Fe concentration of the tolerant variety under moderate and severe IDC and that of the susceptible variety under severe IDC to similar levels to those attained when IF-Fe was not applied in areas with field areas with no IDC. Lucena (2000) indicated that a higher Fe concentration in the leaves of chlorotic plants is possible as a result of Fe^{3+} building up within the leaf apoplast. The reduction in trifoliate Fe concentrations following treatment with IF-Fe could indicate the potential for more available Fe being supplied to the plant and hence a reduction as a result of the dilution of Fe caused by increased vegetative growth. The inconsistent effect of oat on soybean grain yield and the large reduction in trifoliate Fe concentration did not support the dilution of Fe in the plant as being the full cause for the reduction in trifoliate Fe concentration. Inskeep and Bloom (1987) and Bloom et al. (2011) noted that trifoliate samples taken from fields had a much higher in Fe concentration than those grown in greenhouse pot studies. They noted that fine clays high in Fe-rich smectite may be adhering to the leaves following splash contamination from raindrop impacts on the soil surface, which would increase the concentration of Fe. The reduction in trifoliate Fe concentration in treatments with the oat companion crop may be due to a reduction in the amount of Fe contamination caused by the oat companion crop intercepting raindrops.

Zuo et al. (2000) studied the impact of intercropping corn (a Strategy 2 plant) with peanut [*Arachis hypogaea* L. (a Strategy 1 plant)] and also noted an increase in leaf Fe content under the intercropped system. It was theorized that phytosiderophores released by the root of corn were aiding peanut in uptake of Fe, thereby mitigating IDC. Since the concentration of Fe was greater without oat for the current soybean study, the effect of phytosiderophores released by the oat companion crop for increasing the uptake of Fe does not appear plausible. Due to the potential for contamination of Fe, a more controlled environment may be required to determine if Fe uptake is greater with the oat companion crop as a result of phytosiderophores being released from the oat. If the amount of Fe contamination can be reduced a potential benefit from phytosiderophores released by oat may be established.

There were significant correlations between trifoliate NO_3–N and Fe concentration when no oat companion crop was planted at Sites 2 ($r = 0.19$), 3 ($r = 0.41$), and 5 ($r = 0.43$) (data not shown). Similar correlations occurred when the oat was seeded with soybean at the same three locations. The impact of oat on NO_3–N and Fe concentration appears to serve as a poor indicator of grain yield response. The relationship between trifoliate NO_3–N concentration and the severity of chlorosis may confirm the effects of nitrate on the reduction of Fe^{3+} in leaves, as proposed by Kosegarten et al. (1999, 2001).

The differences among varieties may be indicative of a tolerance mechanism for IDC whereby the tolerant variety may take up less NO_3–N from the soil or may be more efficient in the reduction of Fe^{3+} in the presence of high NO_3–N levels. If varieties differ consistently in NO_3–N concentration, taking early trifoliate samples may be a way to screen varieties for IDC severity early in the season. However, the lack of significant variety differences at the one location where IDC severity was low calls this practice into question. Other methods such as determination of seed Fe concentration (Wiersma, 2005) may be better suited to screen varieties across multiple locations.

The decrease in trifoliate Fe concentration for the IF-Fe treatment may provide some evidence of increased Fe^{2+}

Table 8. Relative response of soybean variety [susceptible (S) and tolerant (T)] and iron deficiency chlorosis (IDC) severity rating on soybean grain protein and oil concentration. Calculations are relative to the tolerant variety grown on soils with no or low IDC severity with no IDC management treatment.

IDC severity	Relative grain protein†		Relative grain oil	
	S	T	S	T
	%			
No or low	97.0d	100.5a	103.7a	100.0b
Moderate	97.1d	99.7b	103.3a	100.3b
Severe	97.1d	98.6c	103.2a	99.8b
Mean‡	97.0B	99.6A	103.4A	100.0B

† Numbers followed by the same letter are not significantly different for the analysis of the interaction between soybean variety and IDC severity.
‡ Mean of variety across all IDC severity levels. Uppercase letters represent significance for the variety main effect.

availability in response to Fe-EDDHA. This decrease could be easily explained by dilution of Fe in the plant tissue through greater plant growth. Since the oat effect was highly significant regardless of the severity of IDC, trifoliate Fe concentration would not be a good predictor of final yield. The high levels could give an indication of the potential severity of the chlorosis to differentiate among sites, but a specific critical value may be difficult to determine, as there was no correlation between trifoliate Fe concentration and final grain yield (data not shown). Although many factors that contribute to IDC are understood, there are still many complex interactions that still need to be studied to better understand when and where IDC will develop within a field.

Soybean Grain Quality

Seed protein and oil concentration were significantly ($P \leq 0.10$) affected by variety at five locations. A significant interaction between severity rating and variety occurred only for seed protein concentration. The interaction was due to a decrease in seed protein concentration with increasing severity of IDC for the tolerant variety (Table 8). The resulting decrease was only 1.9% between areas of low and severe IDC. Seed protein concentration was 3% lower on average for the susceptible variety compared to the tolerant one and always differed regardless of IDC severity. Seed oil concentration was 3% greater for the susceptible variety and there was no significant rating × variety interaction. Use of an oat companion crop or IF-Fe did not affect seed protein or oil concentration.

Seed protein and oil concentration were affected by the factors that affected yield and not by IDC alone. Varietal differences dominate grain protein and oil concentration responses, which agrees with the findings of Naeve and Rehm (2006). Although the oat treatment did affect seed protein concentration at individual locations (data not shown), any increase in protein was a direct result of decreased grain yield. It is likely that any NO_3–N taken up was used for the production of protein rather than the development of yield due to the over-competition of oat. Soybean farmers in Minnesota have been concerned about increasing grain protein but factors affecting IDC are not likely to contribute to significant decreases in grain protein.

Influence of Soil Test Variables on IDC Severity

Soil test data and site temperature and precipitation data were analyzed using multiple regression with stepwise selection to determine which variables could be used to determine the severity of IDC. Relative yield was calculated by dividing the grain yield of the susceptible variety by the grain yield of the tolerant in each grid cell for the control treatment only. Since IDC was not present in all grid cells, the effect of high or low soil test values may differ based on whether IDC may be present. For instance, high soil NO_3–N has been previously shown to increase the severity of IDC in calcareous Mollisols in western Minnesota (Bloom et al., 2011). However, high NO_3–N levels in soils that are not prone to IDC may have a different effect. Since there may be some variation in how soils respond, two different scenarios were considered for determining which field areas would be used for the analysis. First, all locations were considered. Second, only field areas where pH was greater than 7.0 were considered. Factors considered to be significant among all locations are given in Eq. [1]; Eq. [2] summarizes data from the field areas with a soil pH greater than 7.0. In these equations, SOM is in g kg^{-1}, EC is in S m^{-1}, DNT_{May} (departure from normal for the average May temperature) is in °C, Fe-DTPA is in mg kg^{-1}, and T_{Aug} (average temperature in August) is in °C.

$$\text{Severity} = (11.9 \times \text{pH}) - (0.49 \times \text{SOM}) - (96.9 \times \text{EC}) + (3.18 \times \text{DN}T_{\text{May}}) - 171.5 \quad [1]$$

$$\text{Severity} = (51.0 \times \text{pH}) - (0.49 \times \text{SOM}) + (2.28 \times \text{Fe-DTPA}) + (2.64 \times T_{\text{Aug}}) - 334.3 \quad [2]$$

Soil pH and SOM were the only significant factors that occurred within both models. Soil EC was significant for the model that included data from all locations (Eq. [1]), whereas Fe-DTPA was significant when only zones with a pH greater than 7.0 were considered (Eq. [2]). Higher grain yield in field areas with a higher Fe-DTPA concentration is consistent with the findings of Naeve and Rehm (2006). However, the positive impact of pH in both models is surprising, since pH is typically negatively associated with soybean yield in IDC-affected soils (Hansen et al., 2003). Knowing the potential risk for IDC based on a few key factors such as EC and CCE may be important. However, they do not guarantee that a yield reduction due to IDC will occur. There needs to be a better understanding of the variability of IDC within the same area of a field on a year-to-year basis if any prediction model is to be developed. The reason that models such as those proposed by Rogovska et al. (2007) and Naeve and Rehm (2006) may better correlate to yield could be due to smaller inference spaces and a more controlled environment.

Past work by Inskeep and Bloom (1986) identified soil moisture as being highly critical in determining if IDC will occur even on soils prone to the problem. Although soil moisture was not directly measured, temperature and rainfall patterns could prove useful in modeling IDC severity. In the case of the modeled data, only the departure from normal for the average May temperature was significant (Eq. [1]), whereas the average August temperature was significant for the data considering field areas with a pH greater than 7.0 only (Eq. [2]).

Although it was significant, the total amount of variation that could be explained by the models was relatively low. The R^2 values were 0.14 for the full data model (Eq. [1]) and 0.20 for the high pH (>7.0) model (Eq. [2]). For Eq. [1], SOM could explain the most variation, followed by soil pH, soil EC, and the departure from normal for the May temperature (R^2 = 0.07, 0.03, 0.02, and 0.02, respectively). For Eq. [2], soil pH explained the most total variation, followed by SOM, Fe-DTPA, and average temperature in August (R^2 = 0.10, 0.06, 0.03, and 0.01, respectively). Soil moisture measurements from the individual grid cells may have been more helpful in determining IDC severity. When soil moisture measurements should be taken is a very important question. If measurements were taken when IDC was present then it is doubtful that any treatment could be used as a corrective measure. If mid-season precipitation is more important, as was determined in this study, then the prediction of where severe chlorosis will occur would not be possible, as the data would not be available before soybean planting. The low degree of correlation for each model indicates that strip trials may not be better suited for determining soil factors that would indicate where IDC will be most severe. Development of a model to predict IDC is needed but may be difficult due to the complexity of factors that cause IDC in soybean.

CONCLUSIONS

The effect of an oat companion crop and Fe-EDDHA applied on the soybean seed for the mitigation IDC were assessed on two soybean varieties that varied in tolerance to IDC using a strip trial methodology. Soybean grain yield was consistently increased by IF-Fe when chlorosis severity was moderate to severe. Yield increases occurred more often for the susceptible variety, but the grain yield level achieved for the susceptible variety with IF-Fe was no greater than the yield of the tolerant variety with or without IF Fe or an oat companion crop. The oat companion crop lowered the amount of NO_3–N in soybean trifoliate leaves regardless of IDC severity. However, yield was typically reduced by the oat companion crop when severity was low or when the oat crop was terminated too late in the growing season. The oat companion crop increased yield of the susceptible variety but only in areas of severe IDC. The combination of IF-Fe and oat resulted in the highest yield for the susceptible variety grown in areas of severe IDC, but did not achieve a higher yield than the tolerant variety. There was no positive benefit of the oat crop alone on the yield of the tolerant variety. Selecting a tolerant variety was still the best management strategy for managing moderate to severe levels of IDC. In-furrow application of Fe-EDDHA would be recommended, but optimal rates should be identified based on the severity of IDC to ensure that application is economically feasible.

Several soil factors were considered in the development of a prediction model for determining IDC severity. The relative yield of the susceptible variety was found to correlate to SOM at 0 to 15 cm, pH, Fe-DTPA and soil EC. The influence of two climatic factors did not significantly aid in the prediction of yield, as the best model could only predict 20% of the total variation in the relative yield of the susceptible variety. To develop better prediction models, field areas affected by IDC should be studied over multiple years to determine how the variability of IDC severity changes across years. However, it may be difficult to develop a single model to predict the severity of IDC based on routinely measured variables accurately.

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