Managing Iron Deficiency Chlorosis with Agronomics and Economics

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Abstract

Iron Deficiency Chlorosis (IDC) is a major yield-limiting stress for soybean [*Glycine max* (L.) Merr.] grown on the calcareous soils of the U.S. upper Midwest. During the 2021 growing season, six on-farm research trials were established at three locations in Western Minnesota (Graceville, Foxhome, and Danvers) on two IDC-prone field areas (hotspot vs. non-hotspot) to evaluate the effectiveness of three of the most often used management strategies for IDC from a system's approach: variety selection, seeding rates, and iron chelates. Three Fe-EDDHA rates (0, 2 and 4 lbs. Soygreen® acre^{-1}) were applied in-furrow at the time of planting either a highly tolerant (AG13XF0) or a moderately tolerant (AG12XF1) variety at 125,000 and 175,000 plants acre^{-1}. Nitrogen was supplied to create a range of IDC symptomology. Visual chlorosis scores (VCS), drone imagery, and ground based NDVI were assessed as methods for measuring the severity of symptoms. Our preliminary results suggest different management strategies to be recommended depending on the field location and the intensity of IDC symptoms. At Graceville non-hotspot, the application of Soygreen® increased soybean yield by 54 to 60% in the susceptible variety where IDC was amplified by N addition. At Graceville hotspot, regardless of variety, Soygreen® application increased yield by at least 35 bushels when N was applied. Where N was not applied and no Soygreen® was added, the highly tolerant variety yielded 72% more than the moderately tolerant one. At Foxhome non-hotspot, the yield of the moderately tolerant variety was decreased by 25% when N was added. At Foxhome hotspot, the highly tolerant variety produced 28% more than the moderately tolerant variety when IDC intensity was not increased by N application. At Danvers non-hotspot, there were no differences in grain yield between treatments. Differently, at Danvers hotspot, three main things were found. At higher seeding rate treatments, the application of 4 lbs. Soygreen® acre^{-1} significantly increased the yield of the moderately tolerant variety. Without Soygreen® applied, increasing the seeding rate of the highly tolerant variety from 125,000 to 175,000 plants acre^{-1} resulted in a yield increase of 36 bushels. At increased seeding rates and without Soygreen® application, the highly tolerant variety produced 52% more than the moderately tolerant one. Overall, variety selection, increased seeding rate, and in-furrow application of iron chelates were effective in controlling IDC and minimizing yield losses. Further research will evaluate the impact of these three management strategies individually and collectively on return on investment. Coupled with an economic analysis, this work will provide producers with more refined recommendations for managing IDC and will support them in their efforts to achieve the greatest economic return on every farm.

1. Introduction

Iron Deficiency Chlorosis (IDC) is a major and economically important abiotic stress for soybean [*Glycine max* (L.)] production in the calcareous soils of the U.S. Upper Midwest (Inskeep and Bloom, 1987; Franzen and Richardson, 2000). In soybean, IDC symptoms are characterized by interveinal chlorosis of the leaves (yellow leaves with dark green veins) and stunting of the growth, both of which lead to significant end-of-season yield reductions (Hansen et al., 2003; Inskeep and Bloom, 1987). In the North Central U.S., the total area of soybean grown on IDC-prone soils was reported to be approximately 4.45 million acres, with associated yield losses averaging 12 bushels acre^{-1}. In addition, it has been estimated that the impact of IDC on soybean yield costs soybean growers approximately $260 million annually in the U.S. (Hansen et al., 2004; Peiffer et al., 2012).

Iron (Fe) is the fourth most abundant mineral element in the Earth's crust, and therefore, IDC is not related to an absence of Fe in the soil (Stucki et al., 1988; Hansen et al., 2003). The type of iron present in the soil solution is what determines availability for plant uptake and, consequently, the incidence of IDC (Inskeep and Bloom, 1987; Marschner and Römheld, 1994). Soybean plants prefer to take up the soluble form of iron [Fe(II)] (Kaiser et al., 2011). When iron becomes less soluble in the soil, i.e., is present in its ferric [Fe(III)] form, plants cannot take it up (Naeve, 2004). Without a sufficient and continuous supply of this micronutrient, the formation of chlorophyll, necessary for photosynthesis and several other plant processes that require it, is restricted, resulting in IDC development, a common symptom of iron deficiency (Goos and Johnson, 2000; Gambel et al., 2014).

There are two major mechanisms that plants have developed to increase their ability to take up iron when growing in iron-limiting conditions: strategy I and strategy II (Marschner et al., 1986). As a strategy I plant, soybean relies on the acidification of the rhizosphere via proton and chelating substance (such as organic acid) extrusion, which then allows less soluble ferric iron (Fe^{3+}) to be reduced to more soluble ferrous iron (Fe^{2+}) by a ferric reductase enzyme located at the root's cell wall (Romheld 1987). This soluble form of iron is then transported into the root via a ferrous transporter encoded by IRT1 (iron-regulated transporter 1) located in the plasma membrane (Kim and Guerinot, 2007). Alternatively, strategy II plants such as corn (*Zea mays*) secrete a phytosiderophore into the rhizosphere that binds with insoluble ferric iron. This entire ironsiderophore complex [Fe(III)-PS] is then transported into the root zone where the iron is converted to Fe^{2+} in order to be used by the plant (Marschner and Romheld, 1994).

However, several soil physical and chemical properties and their interactions can change the solubility of iron in the soil, resulting in a difficult environment for plant-mediated Fe reduction mechanisms (Inskeep and Bloom, 1987; Hansen et al., 2003). Factors such as levels of calcium carbonates, soil pH, soluble salt concentration, soil moisture content, and residual soil nitrate can significantly influence the incidence of IDC, making it a very complex stress to manage (Morris et al., 1990; Hansen et al., 2003; Rogovska et al., 2007; Liesch et al., 2012; Bloom et al., 2011). Soils with pH in the range of 7.4 – 8.5 precipitate iron as Fe hydroxides [$Fe(OH)_x$], lowering its solubility (Lucena and Hernandez-Apaolaza, 2017). Calcium carbonates in the soil act as a strong base, raising the pH and oxidizing ferrous iron [Fe(II)] to ferric iron [Fe(III)]. In addition, calcium carbonates also have a buffering effect, neutralizing the H^+ protons and organic acids excreted from the roots, thus inhibiting the reduction of Fe^{3+} to Fe^{2+} (Kaiser et al., 2011; Hansen et al., 2003). High soil moisture and high NO^{3-} concentrations affect IDC intensity because both factors lead to a buildup of bicarbonates in the soil. These bicarbonates neutralize the acidity around the roots and limit the conversion of unavailable ferric iron to readily available ferrous iron, a necessary step in Fe uptake by soybean plants (Aktas and van Egmond, 1979; Inskeep and Bloom, 1987).

Due to the large impact of IDC on soybean production, farmers adopted several agronomic practices to mitigate yield losses (Hansen et al., 2003). Among the most widely used management strategies are variety selection, seeding management (including seeding rates and row spacing), and fertilizer application (including foliar and in-furrow applications of iron chelates) (Hansen et al., 2003). However, because different management strategies can come at significant expense to soybean growers, the tradeoffs in cost and yield associated with their utilization may not maximize economic returns. Therefore, in order to overcome the problem and ensure profitability, practical and economical solutions are needed.

Planting varieties with improved tolerance has long been suggested as the most important and most practical strategy to manage IDC and adoption of this strategy is considered fundamental for profitable production in IDC-prone soils (Niebur and Fehr, 1981; Goos and Johnson, 2000; Wiersma, 2005; Naeve and Rehm, 2006; Helms et al., 2010; Kaiser et al., 2014). The exception has been proposed for field areas where IDC is not present. Previous studies have shown that in the absence of IDC, susceptible varieties yield significantly more than tolerant varieties (Helms et al., 2010; Kaiser et al., 2014). For that reason, the practice of planting a higher yielding but susceptible variety where there is low risk of IDC, and a tolerant variety in areas affected by moderate to severe IDC, is still supported. Furthermore, even the most tolerant varieties can experience yield reductions when severe IDC conditions prevail (Froelich and Fehr, 1981; Kaiser et al., 2014). Overall, the best way to manage severe IDC is to pair tolerant varieties with other management strategies reported to be effective at minimizing losses.

Increasing seeding rate has been proposed as another viable option to minimize the effects of IDC in soybean fields (Lingenfelser et al., 2005; Naeve, 2006; Wiersma, 2007). In 1999, Goos and Johnson (2001) planted a susceptible variety at three different seeding rates: 75,000 (half of the recommended plant population for soybean grown in 30-inches rows in North Dakota), 150,000 (recommended plant population), and 300,000 (twice the recommended plant population) plants acre^{-1}, and found that doubling the seeding rate significantly increased grain yield and reduced IDC symptoms. They found that an 8.7 to 14 bushel acre^{-1} yield gain was realized by planting at a higher seeding rate compared to the recommended rate for North Dakota in chlorosis-producing soils. From a farmer's standpoint, adjusting seeding rates in affected vs. non-affected soybean fields can be efficiently done by mapping the hotspot and non-hotspot areas in the tractor's GPS and varying the plant density with variable-rate seeding equipment (Gaspar, 2010). However, it is important to note that soybean seed has become increasingly expensive, which could be a limiting factor when it comes to ensuring economic feasibility when using increased plant populations (Henke, 2021).

Application of iron chelates has also been effective in controlling IDC and thus contributing to yield increases in soybean grown under severe IDC conditions (Ferreira et al., 2019b; Wiersma, 2007; Schenkeveld et al., 2008; Gamble et al., 2014; Kaiser et al., 2014). An iron chelate is a synthetic or naturally derived product that binds iron at multiple sites, keeping soluble iron readily available in the soil solution for plant uptake (Wittwer et al., 1965; Lucena-Rodriguez and Apaolaza-Hernandez; 2010). Wiersma (2007) obtained increases in grain yield of 15% with seed-applied Fe-EDDHA. Kaiser et al. (2014) reported significant yield increases with in-furrow application of Fe-EDDHA at a rate of 3 lbs. acre^{-1} for a susceptible variety grown under moderate to severe IDC conditions. Foliar applications of iron have also been investigated but have shown varying levels of success (Liesch et al., 2011; Chatterjee et al., 2017). Nevertheless, while providing soybean plants with a chelated form of iron may help overcome the effects of IDC, the cost associated with the product could make its usage questionable (Dobbles, 2020). Previous studies have shown that if an iron chelate is used at a rate greater than that needed to maximize yield, it could become not cost-effective (Wiersma, 2005; Wiersma, 2007; Kaiser et al., 2014). Without considering the cost of the iron fertilizer, applying this type of product as a liquid suspension in-furrow at planting is considered a viable option to soybean growers in Central and Western Minnesota because many of them possess planters equipped with such technology for similar applications on other crops (Kaiser et al., 2014).

The objectives of this research are (1) to evaluate the effectiveness of three of the most commonly used management strategies for IDC in soybean from a system's approach: variety selection, seeding rate, and iron chelates; and (2) given that there is a trade-off in cost and yield relative to the adoption of each of these management strategies, this study also aims to evaluate the impact of variety selection, seeding rate, and iron chelate rates individually and collectively on return of investment.

2. Materials and methods

2.1 Research environments and experimental design

During the 2021 growing season, six on-farm paired factorial research trials were established at three locations in Western Minnesota, on soils where IDC has historically produced mild to severe iron deficiencies. The three locations for this experiment included a farm near Danvers, MN (45.256131° N, -95.706498° W) in Swift County, a farm near Graceville, MN (45.574702° N, -96.408939° W) in Big Stone County, and a farm near Foxhome, MN in Wilkin County (46.208768° N, -96.414094 ° W). At each location (farm), the same trial was planted in two different sites, one site was planted in the "hotspot" IDC part in the field where IDC is severe, and the other site was planted in the "non-hotspot" IDC part in the field where there can be some IDC, but symptoms are not as severe as in the hotspot area (Figure 1); the non-hotspot IDC sites will be referred to as neutral spots to facilitate interpretation. Therefore, field sites are herein referred to as Danvers Neutral, Danvers Hotspot, Graceville Neutral, Graceville Hotspot, Foxhome Neutral, and Foxhome Hotspot. All field sites were chosen based on soybean IDC history provided by the farmer cooperators.

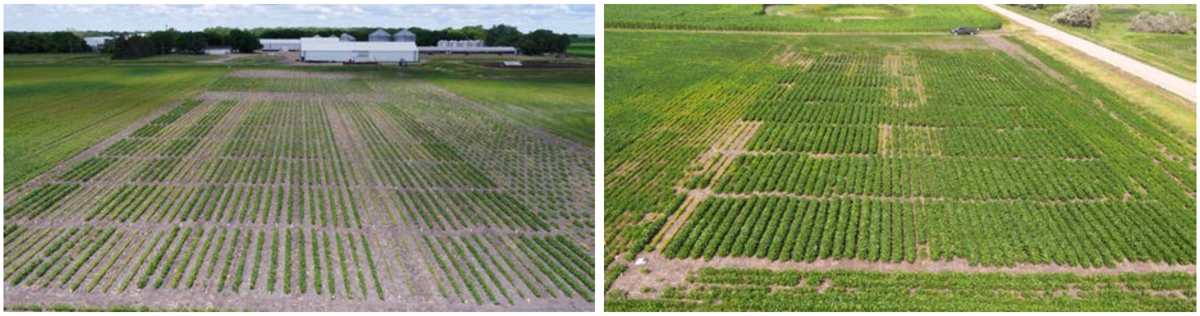

Figure 1. Hotspot (left) and Neutral (right) field sites in Graceville, MN.

The experiment was arranged to test 24 treatments, corresponding to the factorial combination of four main factors and their levels: Two varieties (AG12XF1 = moderately tolerant and AG13XF0 = highly tolerant), two seeding rates (125,000 and 175,000 plants acre^{-1}), three rates of iron chelate (0, 2, and 4 lbs. Soygreen AST® acre^{-1}), and two levels of IDC severity by supplemental nitrogen application (69 lbs. N acre^{-1} and no N). The application of supplemental N is an effective protocol developed to create a range of IDC symptoms, and it has been successfully used in previous IDC studies (Dobbles, 2020). In our work, nitrogen fertilizer was manually applied on the furrow on May 18th as granular urea CH_4N_2O (46–0-0) (Loveland Products, Inc., CO) at 150 lbs. urea acre^{-1}. A full table of the 24 treatments can be found in Table 1.

Figure 2. Granular urea manually applied on the furrow 7 days after planting at Danvers and 8 days after planting at Graceville and Foxhome. The date of application was determined based on weather conditions to minimize N losses.

Table 1. The full set of 24 treatment arranged by the factorial combination of four factors and their levels. The IDC tolerant (AG13XF0) and IDC susceptible (AG12XF1) varieties are both extensively grown in Western Minnesota (DEKALB Asgrow/Bayer Crop Science, MO, USA).

Treatment	**Soygreen® (lbs. acre^{-1})**	**Nitrogen**	**Population (plants acre^{-1})**	**Variety**
1	0	Nitrogen	125,000	Tolerant
2	0	Nitrogen	125,000	Susceptible
3	0	Nitrogen	175,000	Tolerant
4	0	Nitrogen	175,000	Susceptible
5	0	No Nitrogen	125,000	Tolerant
6	0	No Nitrogen	125,000	Susceptible
7	0	No Nitrogen	175,000	Tolerant
8	0	No Nitrogen	175,000	Susceptible
9	2	Nitrogen	125,000	Tolerant
10	2	Nitrogen	125,000	Susceptible
11	2	Nitrogen	175,000	Tolerant
12	2	Nitrogen	175,000	Susceptible
13	2	No Nitrogen	125,000	Tolerant
14	2	No Nitrogen	125,000	Susceptible
15	2	No Nitrogen	175,000	Tolerant
16	2	No Nitrogen	175,000	Susceptible
17	4	Nitrogen	125,000	Tolerant
18	4	Nitrogen	125,000	Susceptible
19	4	Nitrogen	175,000	Tolerant
20	4	Nitrogen	175,000	Susceptible
21	4	No Nitrogen	125,000	Tolerant
22	4	No Nitrogen	125,000	Susceptible
23	4	No Nitrogen	175,000	Tolerant
24	4	No Nitrogen	175,000	Susceptible

All six field sites were planted at a ground speed of 1.9 mph with a four-row precision research planter (Almaco Seed Pro 360) pulled by a John Deere 6130R. Plots at Foxhome and Graceville were planted on May 10th, 2021, and plots at Danvers were planted on May 11th, 2021.

Figure 3. John Deere 6130R and Almaco Seed Pro 360 used to sow the 2021 IDC trials.

Treatment combinations were organized in a randomized complete block with split-plot treatment design and replicated four times (Figure 4). Iron chelate levels were whole plots (3 whole plots per replication) and the combination of varieties, seeding rates, and N the subplots (8 subplots per whole plot). All 96 plots at each field site were 30 ft long x 10 ft wide with 4 rows spaced 2.5 ft apart. This experimental design was selected to facilitate the logistics involved in the application of iron chelate in-furrow as a liquid suspension while sowing the research trials.

Whole Plot

SubPlot

Rep 1			Rep 2		
0 lbs	**2 lbs**	**4 lbs**	**2 lbs**	**4 lbs**	**0 lbs**
0 Soygreen Susceptible 125,000 No N	**2 Soygreen** Tolerant 125,000 N	**4 Soygreen** Tolerant 125,000 N	**2 Soygreen** Tolerant 125,000 N	**4 Soygreen** Tolerant 125,000 No N	**0 Soygreen** Tolerant 175,000 N
0 Soygreen Tolerant 175,000 N	**2 Soygreen** Tolerant 175,000 N	**4 Soygreen** Susceptible 125,000 N	**2 Soygreen** Susceptible 175,000 N	**4 Soygreen** Susceptible 125,000 No N	**0 Soygreen** Susceptible 125,000 N
0 Soygreen Tolerant 125,000 No N	**2 Soygreen** Susceptible 175,000 No N	**4 Soygreen** Tolerant 175,000 No N	**2 Soygreen** Tolerant 175,000 N	**4 Soygreen** Tolerant 175,000 N	**0 Soygreen** Susceptible 125,000 No N
0 Soygreen Susceptible 175,000 No N	**2 Soygreen** Tolerant 175,000 No N	**4 Soygreen** Tolerant 125,000 No N	**2 Soygreen** Susceptible 175,000 No N	**4 Soygreen** Susceptible 175,000 N	**0 Soygreen** Susceptible 175,000 N
0 Soygreen Tolerant 175,000 No N	**2 Soygreen** Tolerant 125,000 No N	**4 Soygreen** Susceptible 175,000 N	**2 Soygreen** Tolerant 125,000 No N	**4 Soygreen** Susceptible 175,000 No N	**0 Soygreen** Tolerant 125,000 No N
0 Soygreen Susceptible 125,000 N	**2 Soygreen** Susceptible 175,000 N	**4 Soygreen** Tolerant 175,000 N	**2 Soygreen** Tolerant 175,000 No N	**4 Soygreen** Tolerant 175,000 No N	**0 Soygreen** Tolerant 125,000 N
0 Soygreen Tolerant 125,000 N	**2 Soygreen** Susceptible 125,000 N	**4 Soygreen** Susceptible 125,000 No N	**2 Soygreen** Susceptible 125,000 N	**4 Soygreen** Susceptible 125,000 N	**0 Soygreen** Tolerant 175,000 No N
0 Soygreen Susceptible 175,000 N	**2 Soygreen** Susceptible 125,000 No N	**4 Soygreen** Susceptible 175,000 No N	**2 Soygreen** Susceptible 125,000 No N	**4 Soygreen** Tolerant 125,000 N	**0 Soygreen** Susceptible 175,000 No N

Figure 4. Layout of the IDC experiment in a randomized complete block with split-plot treatment design. Only two reps are shown in this figure. Each whole plot (levels of iron chelate) consists of eight subplots (combination of seeding rate vs. variety vs. N). In total, 576 plots were evaluated across all locations and sites. The dimensions of each field trial were 240 feet long and 120 feet wide.

The chelated form of iron used in this study was a commercial form of *ortho-ortho* Fe EDDHA labeled Soygreen® AST with 1.8% of iron (CHS Inc., Inver Grove Heights, MN). Soygreen® AST contains 3 lbs. of Soygreen® per gallon of product. All three iron chelate rates (0, 2, and 4 lbs. Soygreen® acre^{-1}) were mixed with water resulting in 0-, 10-, and 20-gallons acre^{-1} mixture rates, respectively, and supplied as an in-furrow liquid suspension at planting with an in-furrow tubular delivery system attached to the planter behind the seed tube that drizzles the product directly on the seed.

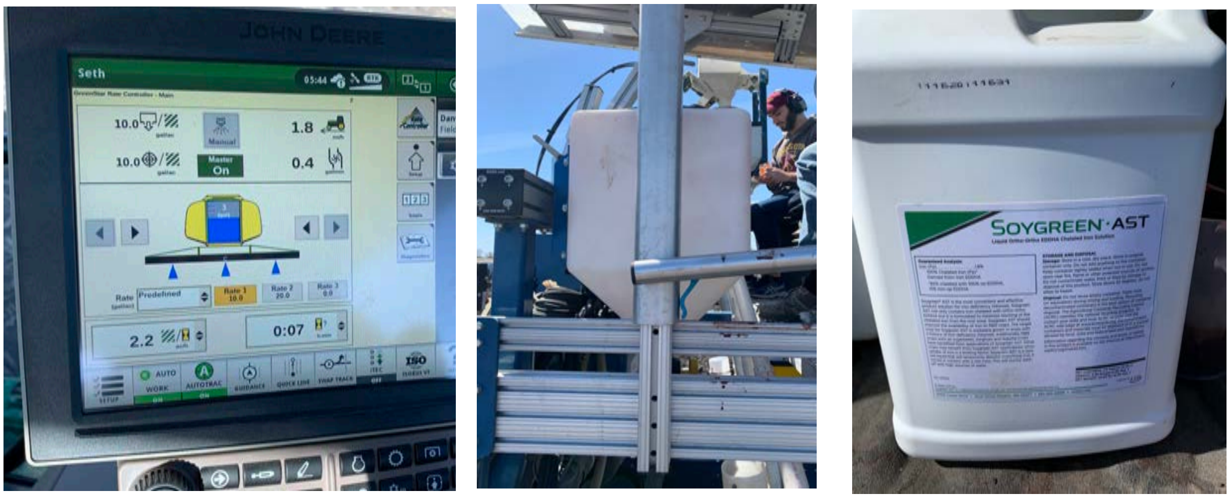

Figure 5. In-furrow application of Soygreen® AST. 0, 10, and 20 gallons of mixture (Soygreen® AST plus water mixed in 5-gallons tank) were applied at planting.

The two soybean varieties, one rated as highly tolerant to IDC (AG13XF0) and the other rated as moderately tolerant to IDC (AG12XF1), were planted at two different seeding rates (125,000 plants acre^{-1} or 7 plants foot^{-1} and 175,000 plants acre^{-1} or 10 plants foot^{-1}). The AG13XF0 is a group 1.3 relative maturity and the AG12XF1 is a group 1.2 relative maturity. Based on their maturity groups, the two seeding rates utilized were 25,000 plants below and 25,000 plants above the recommendation (150,000 plants acre^{-1}) for these two varieties in Western MN. The Almaco Seed Pro 360 includes a high-precision single plate metering unit along with a FieldMap Software that allows for automatic adjustment of seed counts by the planter. This system enabled the two different plant populations to be accurately planted. Thus, plant population maps corresponding to each treatment's seeding rate were created in Almaco FieldMap (version 2.1) and loaded into the planter's system for use at the time of planting.

Both varieties are XtendFlex®, with tolerance to dicamba, glyphosate, and glufosinate. The AG13XF0 is rated as resistant to soybean cyst nematode (*Heterodera glycines)* (SCN) and AG12XF1 is rated as susceptible to SCN. For this reason, multiple soil samples were collected within each site to assess SCN egg counts. This nematode causes chlorosis of the leaves, stunting of the growth, and can cause necrosis if severe. Therefore, SCN egg counts were determined to avoid any misinterpretation of symptoms.

Each of the six sites was thoroughly characterized for soil and weather conditions. Weather stations were installed at each location prior to planting to collect average daily temperature and accumulated rainfall data. Multiple composite soil samples (8 cores per sample at 0 – 6 inches depth) were collected per replication immediately after planting and were sent for laboratorial analysis for common soil chemical factors, micronutrients series, and calcium and magnesium contents. Samples were oven-dried at 40 °C and analyzed at the Research Analytical Laboratory of the University of Minnesota for available nutrients (K, Mn, Zn, Cu, Fe), soil electrical conductivity (EC), pH, calcium carbonate equivalent (CCE), Olsen-P, and organic matter (OM) according to the Recommended Chemical Soil Test Procedures for the North Central Region (1988).

Figure 6. Weather stations were installed at each field site and soil cores were collected for soil characterization.

Weeds were controlled following standard agronomic practices for soybean production in Western Minnesota, based on the specific weed species and densities. Escapes were removed by hand weeding. Insect pests and diseases were either absent or considered inconsequential. Growth stages were recorded following the soybean stage descriptions in Caviness and Fehr (1977). Initial plant populations were recorded for each plot at V3 by manually counting the number of plants in 1 m of each of the two center rows.

2.2 IDC symptoms assessment

After emergence, each plot was evaluated weekly for IDC symptoms using a 1 to 5 visual rating system, ground-based NDVI and NDRE using a portable crop canopy sensor, and high-resolution drone imagery. Ground-based NDVI and NDRE and visual scores were used to evaluate if UAV-acquired vegetation index values have at least the same capability as ground-based methods to rate IDC symptoms. Growth stages were recorded on the same days as IDC symptom evaluation. Thus, data were collected at the following growth stages: V1, V2, V3, V4, R1, R2, R3, R4, R5, and R5.5.

The 1 to 5 visual severity scoring has been traditionally used to evaluate IDC symptoms in soybean. In this system, a rating of "1" indicates a plot that is 100% green (no IDC present); a score of "2" indicates slight yellowing most likely in the upper part of the canopy where IDC initiates; a score of "3" indicates chlorosis throughout the canopy with most plants in the plot turning yellow; a score of "4" indicates severe chlorosis where all plants are yellow and some are becoming stunted and necrotic; and a score of "5" indicates the most severe IDC symptoms where the entire plot is damaged and dying or completely dead (severe necrosis plus plant death). IDC severity scoring was completed by an experienced rater who understands IDC conditions and only the center two rows of every plot were rated to avoid competition effects between rows of different plots.

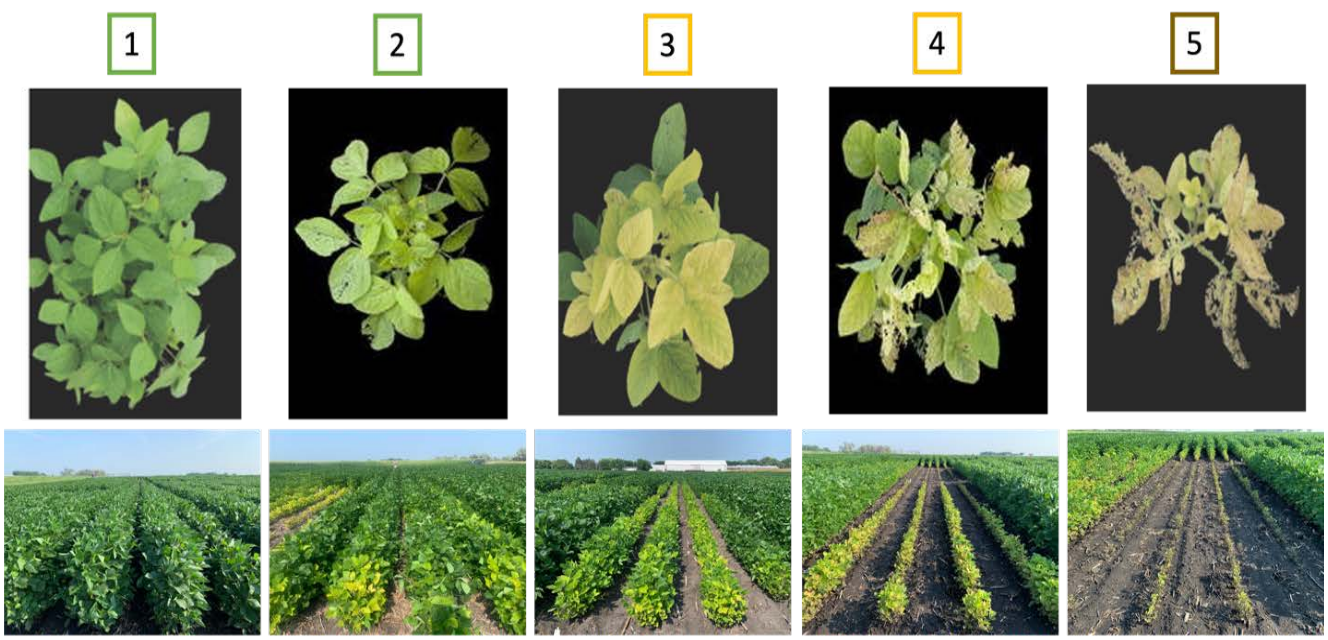

Figure 7. Plots showing 1 - 5 IDC severity ratings.

Portable crop canopy sensors such as the Crop Circle™ (Holland Scientific Inc., NE, USA) represent another tool to collect crop health information. The Crop Circle™ is an active crop canopy sensor that provides NDVI and NDRE vegetation index data as well as basic reflectance information from plant canopies and soil. Ground-based vegetation indices were collected from the second row of each plot. The Crop Circle was run approximately one meter above the canopy to collect approximately 50 spectral measurements per plot. The individual spectral measurements were averaged for further analysis.

Figure 8. Ground-based crop reflectance data being collected with an active crop canopy sensor.

Drone imagery was collected on the same day as visual ratings. Drone imagery was acquired with a RedEdge-MX sensor (MicaSense Inc., Seattle, WA, USA) mounted on a DJI Inspire 2 (DJI Technology Co. Ltd., Shen Zhen, China). This sensor captures the five following spectral bands almost at the same time: blue (475 nm x 32 nm), green (560 nm x 27 nm), red (668 nm x 14 nm), red-edge (717 nm x 12 nm) and near-infrared (842 nm x 57 nm). The drone was programmed to fly each field autonomously with the assistance of Pix4D Capture (Pix4D, SA), which remotely controls UAV flight path, desired flight altitude, and image overlap. The UAV was flown at a ground speed of 1 meter per second and at 23 meters above ground level. This altitude was used to obtain proper ground sample distance of 1.6 cm/pixel, and consequently, high spatial resolution. All images were captured from nadir view with 75% front and 75% side overlap. On average, about 200 images were captured per flight.

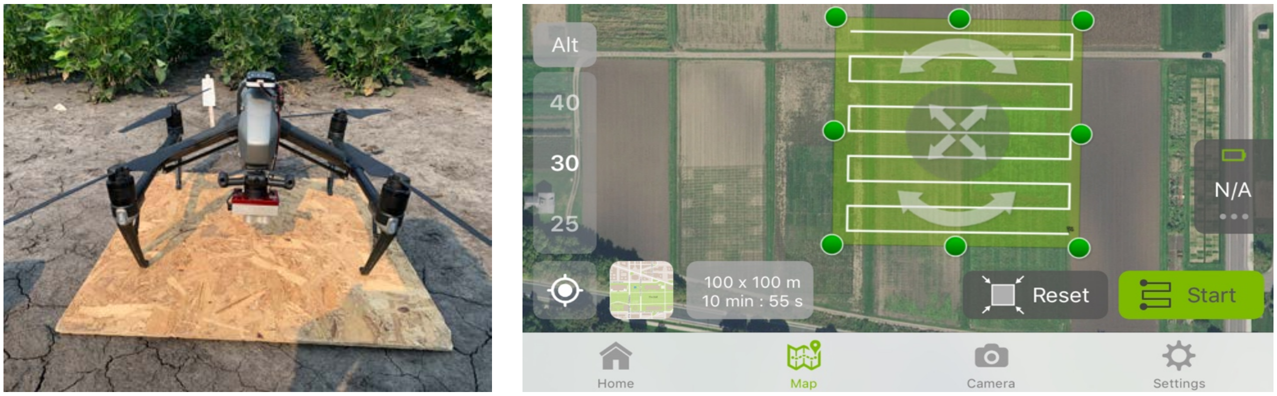

Figure 9. Multispectral drone imagery acquisition with a Micasense RedEdge MX sensor mounted on a DJI Inspire 2 drone and Pix4D Capture application utilized to perform each mission.

Before and after each mission, images of the RedEdge-MX calibrated reflectance panel (CRP) were taken for calibration purposes to provide consistent(?) representation of light conditions during each flight. The CRP has unique known reflectance values across the visible and near-infrared light spectrum and are provided by Micasense to convert the raw pixel values of an orthomosaic image into reflectance. Additionally, a downwelling light sensor (DLS) was integrated with the camera on the drone, measuring the solar irradiance. This DLS is a global shutter for distortion-free images.

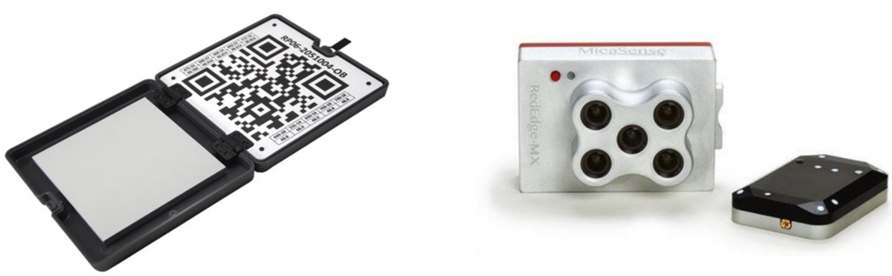

Figure 10. A CRP and DLS (right side of second image) used for radiometric calibration and reflectance map generation.

All flights were conducted within two hours of solar noon (between 10:00 am and 2:00 pm) to limit shadow effects. Four ground control points (40 cm x 20 cm cement pavement blocks painted red) were placed at the corners of each field site and remained there for the duration of the growing season. Their coordinates were collected at one time point during the summer (before the first flight) using a Trimble Handheld GPS unit (Garmin eTrex® 20x, Garmin, Lenexa, KS) and were used for geometric calibration.

2.3 Grain yield data and statistical analysis

Final yields were recorded for every plot at every location. Yield data were collected by harvesting the center two rows of each plot with a small plot combine (Almaco SPC40, Almaco, IA, USA) at R8 maturity stage The first and fourth rows were considered border rows and were not harvested to avoid competition effect between rows of different plots. The weight of each sample (total grain mass plot^{-1}) was converted to yield (bu acre^{1}) and adjusted to a 13% moisture basis (130 g kg^{-1}).

All statistical analyses were done with R Studio (version 4.1.2 "Bird Hippie") software and differences considered significant at $p < 0.05$. Visual IDC scores were compared to final grain yields using a Spearman correlation analysis. Mixed effects models were fitted using restricted maximum likelihood with the nlme function from the *nlme* package (version 1.0.+) for every location separately. All four main factors (Soygreen® rate, variety, seeding rate, and N) and site were considered as fixed effects, while block (replication) and block by Soygreen® rate were random effects. In other words, a full factorial analysis of variance (ANOVA) was used to test the fixed effects of the factorial arrangements of Soygreen®, variety, seeding rate, and N, and all six sites and all their corresponding interactions for the response variable grain yield. All models (for each location) included the random effect of block (replication) and block by Soygreen® rate. Location was first considered a fixed effect in the model to test for variation among locations. Due to a significant treatment and site by location interaction and variation across locations, a separate analysis was performed within each location to test for treatment and site effects.

Model assumptions of homogeneous variance and normal distribution of the residuals were checked by examining the residual plots. Site often produced heterogenous variance at Danvers and Graceville (plots at hotspot sites were more variable), which was accounted for by allowing the estimation of different variances for each level of site (hotspot vs. neutral) with the *varIdent* function of the *nlme* package. For the models in which the five-way interaction term was not significant, the non-significant effects were excluded from the model to help evaluate the hypothesis of other fixed effects. Pairwise mean comparisons for the response variable grain yield were performed by calculating the Tukey Honest Significant Difference (HSD) post-hoc test with the cld emmeans function from the *emmeans* package.

3. Preliminary results and discussion

3.1 Soil properties and IDC symptom severity

Soil sampling was conducted right after planting to measure soil chemical properties at each of the six field sites and to assess the presence and prevalence of SCN. Table 2 summarizes each field site in terms of soil chemical properties and nematode egg counts.

Table 2. Soil average Olsen soil test P, ammonium acetate K, soil/water pH, DTPA-extractable Fe, electrical conductivity (EC), and calcium carbonate equivalency (CCE). Soil samples were composed of 6 soil cores per replication (4 replications = 24 cores per site) taken at a depth of 0 to 6 inches.

Location/Site	P (mg/kg soil)	K (mg/kg soil)	pH	Fe (mg/kg soil)	EC (S/m)	CCE (g/kg)
Foxhome Neutral	9.25	173	8.1	3.94	0.08	49
Foxhome Hotspot	10.75	173	8.15	3.25	0.12	72
Graceville Neutral	80.5	394	7.9	8.25	0.23	60.75
Graceville Hotspot	67	346.5	8	7.48	0.11	115.75
Danvers Neutral	28.75	290	8	8.97	0.06	94
Danvers Hotspot	29.5	325.5	7.9	7.22	0.13	118.75

Three factors that are generally considered to be determinant in identifying areas of high IDC risk are soil pH higher than 7.5, CCE higher than 5% (CCE > 50 g kg^{-1}), and soluble salts concentration (measured by EC) greater than 1 mmhos cm^{-1} (Hansen et al., 2003; Naeve, 2004). Across all six research sites, soil pH averaged 8 (range 7.9 to 8.15), CCE averaged 85 g kg^{-1} (range 49 – 118.75 g kg^{-1}), EC averaged 1.2 mmhos cm^{-1} (range 0.8 to 2.3 mmhos cm^{-1}), and DTPA-extractable iron averaged 6.52 mg kg^{-1} soil (range 3.25 to 8.97 mg kg^{-1} soil).

Based on the soil analysis results, a method developed by Naeve and Rehm (2006) was utilized to estimate IDC severity across research sites. Naeve and Rehm (2006) proposed an index using the following equation:

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

where EC is in S m^{-1}, CCE is in g kg^{-1}, and Fe-DTPA is in mg kg^{-1}. An index value greater than 1 would indicate that the location would not likely be impacted by IDC, and a value less than 0.05 would indicate that the location is likely to elicit strong IDC symptoms. The IDC index values for all six field sites are shown in the table below:

Location	IDC Index Value
Foxhome Neutral	0.55
Foxhome Hotspot	0.28
Graceville Neutral	0.41
Graceville Hotspot	0.32
Danvers Neutral	0.64
Danvers Hotspot	0.24

The index above was utilized to provide a better measurement of IDC severity at each research site instead of only calling it Neutral and Hotspot. As indicated by the index values above, we predict more IDC symptoms (higher severity) at the hotspot sites and less IDC symptoms (lower severity) at the neutral sites. This was confirmed by visual ratings as shown in Figures 11, 12, and 13.

3.2 Effects on soybean grain yield at Danvers

Visual IDC scores taken at R1 growth stage were used to classify symptoms into three severity categories (low, moderate, and severe IDC symptoms), following the approach used by Kaiser et al. (2014). A visual IDC score less than 1.5 was considered no or low IDC, a score between 1.5 and 2.5 was considered moderate, and a score greater than 2.5 was severe. Given that most previous IDC studies did not separate research sites into hotspot and non-hotspot areas, this was done to allow for a more standardized comparison of results.

At Danvers, a four-way interaction was found between site, Soygreen®, variety, and seeding rate. Since site conditions interacted with treatments, indicating differences in response to IDC management strategies for differing levels of IDC severity, a separate analysis was performed within each site to test for treatment effects (Figure 11).

At Danvers Neutral, there were no differences in grain yield between treatments. The lowest average yield (58 bu ac^{-1}) was found for the moderately tolerant variety with no Soygreen® application and 125,000 plants $acre^{-1}$, but it was not statistically different from the highest average yield (66 bu ac^{-1}) achieved with two other treatments (moderately tolerant variety with 2 lbs. Soygreen® $acre^{-1}$ and low seeding rate, and tolerant variety with 2 lbs. Soygreen® $acre^{-1}$ and high seeding rate). In addition, our results suggest that there are no substantial yield differences between moderately tolerant and highly tolerant varieties when grown under low IDC severity conditions (neutral sites), which is different than what was found by Helms et al. (2010) and Kaiser et al. (2014). This divergence in findings could be due to the utilization of different varieties in our study. In our research, the highly tolerant variety (AG13XF0) may possess a yield potential equal or greater than of the moderately tolerant variety (AG12XF1), due to breeding programs developing new higher yielding IDC tolerant soybean varieties. Furthermore, these findings support the those of Helms et al. (2010) in that there is evidence that some varieties have high yield on both the IDC and non-IDC areas of a field.

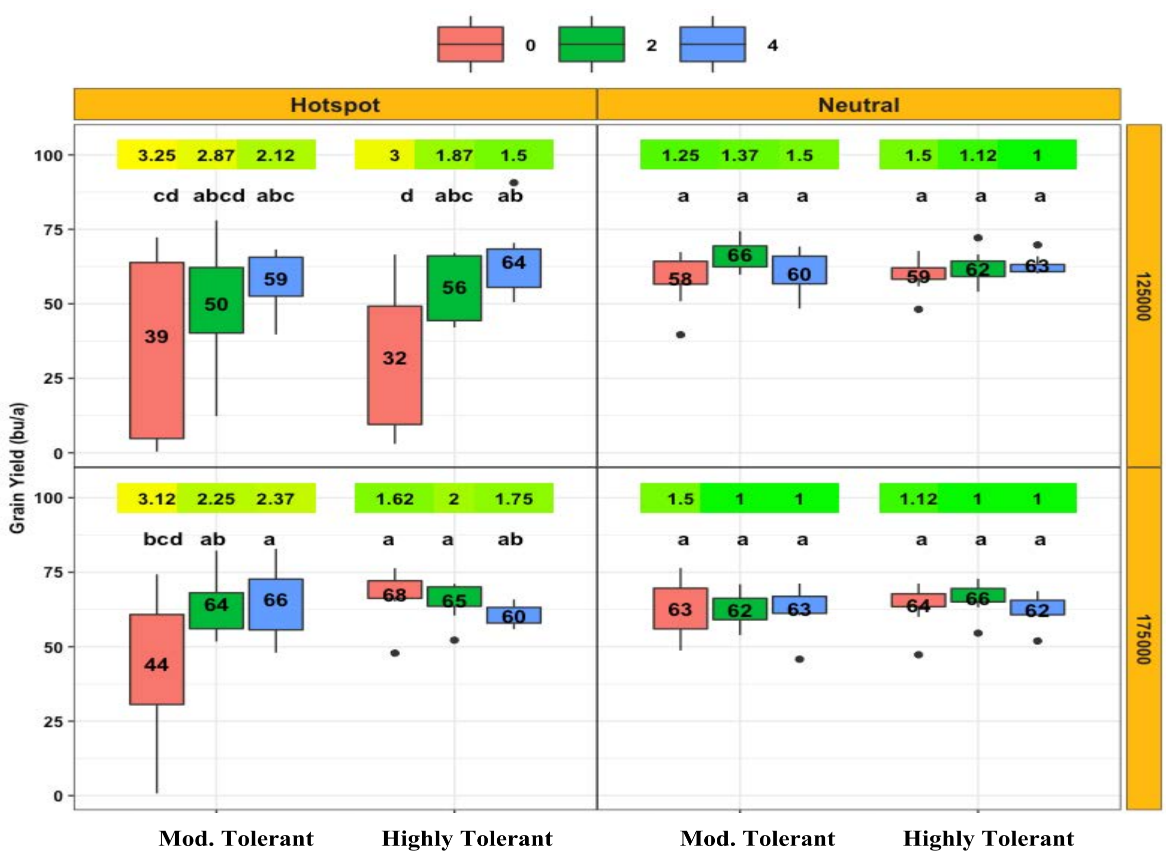

Figure 11. Soybean yield (bu ac^{-1}) by variety, seeding rate, and iron chelate rates for Danvers, MN. Treatment means shown with letters not connected by the same color were not significantly different at $P<0.05$ using Tukey's HSD. IDC severity scores at R1 are shown as green and yellow boxes above significance letters. The non-hotspot area is referred as Neutral.

At Danvers Hotspot, three main results were found. First, without Soygreen® application, the higher seeding rate significantly increased the yield of the highly tolerant variety. A yield increase of 36 bushels was found by increasing the plant population of the highly tolerant variety from 125,000 plants $acre^{-1}$ to 175,000 plants $acre^{-1}$. These results support previous research by Penas et al. (1990), Lingenfelser et al. (2005) and Wiersma (2007). Wiersma (2007) denotes, however, that the less tolerant (or more susceptible) cultivars showed a greater response to higher plant populations than the tolerant one, which is different than what we found. In our study, at an increased seeding rate and without Soygreen® application, the less tolerant variety yielded 44 bushels $acre^{-1}$ while the most tolerant one yielded 68 bushels $acre^{-1}$, which was statistically different at $P < 0.05$. Soybean varieties with high tolerance to IDC are described as having an increased capacity to reduce Fe^{3+} to Fe^{2+}, enhancing iron uptake (O'Rourke et al., 2007). The mechanism behind the population effect is associated with the adaptive components that strategy I plants have developed to increase iron uptake when growing in Fe-limiting conditions (Naeve, 2004). When the seeding rate is increased, there are a greater number of plants per unit of row acting to acidify the rhizosphere to enhance iron availability and acquisition (Rengel and Marschner, 2005). Therefore, our results may be explained by an increased reductive capacity and acidification of the region immediately surrounding the roots as the result of growing a variety better able to reduce Fe^{3+} to Fe^{2+} at a higher plant density.

Second, the only treatment that significantly reduced the yield (by at least 43%) of the highly tolerant variety was a lower seeding rate without Soygreen® application. This indicates that genetic tolerance alone is not enough for IDC management in areas where IDC is severe. The adoption of an additional management strategy (Soygreen® application and/or increased seeding rate) is needed to allow for average yields similar to those of the highly tolerant variety when no or low IDC symptoms are present. These findings corroborate those of Kaiser et al. (2014), who reported that soybean yield was greatly reduced for both highly tolerant and less tolerant varieties grown without any other management treatments under severe IDC conditions. Froelich and Fehr

(1981) also reported that even the most tolerant IDC varieties can experience yield reductions when severe IDC conditions prevail. An explanation for such yield reductions may be that no varieties exist with complete tolerance to IDC (Fehr, 1982).

Lastly, under severe IDC conditions, the yield of the moderately tolerant variety was maximized with the use of an increased seeding rate and with the application of Soygreen®. Significant differences were not found between Soygreen® rates and plant populations, but average yields were increased from 50 and 59 bushels acre^{-1} with 2 and 4 lbs. Soygreen®, respectively, at the low seeding rate to 64 and 66 bushels acre^{-1} with 2 and 4 lbs. Soygreen®, respectively, at the high seeding rate, compared to no Soygreen®. The only statistical difference in yields was found between 0 and 4 lbs. Soygreen® acre^{-1} for the susceptible variety at a high seeding rate (from 44 to 66 bushels acre^{-1}).

3.2 Effects on soybean grain yield at Graceville

At Graceville, a separate analysis was performed for the hotspot and neutral sites to test for treatment effects, because a four-way interaction was found among site, Soygreen®, variety, and N. Previous studies have shown that residual nitrate (NO_3^-) in the soil exacerbates the severity of IDC (Silva and Uchida, 2000; Bloom et al., 2011a; Kaiser et al., 2011). When an increased amount of NO_3- is present in the soil, soybean plants preferentially take up NO_3^- and extrude bicarbonate (HCO_3^-) to balance intracellular charge (Aktas and van Egmond, 1979; Merry et al., 2021). In the soil, bicarbonate also has a buffering effect, neutralizing the protons released from the roots that were meant to acidify the rhizosphere and reduce ferric iron to ferrous iron (Inskeep and Bloom, 1987). Therefore, by adding urea on the furrow right after planting, severe IDC conditions were promoted, resulting in worsening of IDC symptoms and in significant yield reductions.

As shown in Figure 12, the lowest average yield in the Graceville Neutral site (48 bushels acre^{-1}) was found for the moderately tolerant variety when nitrogen was applied and no Soygreen® was added. Even though no statistical differences were found, the moderately tolerant variety yielded 21 bushels more when nitrogen was not applied. This indicates that even at neutral spots, where lower IDC intensities are expected, excessive nitrate levels in the soil can induce severe IDC conditions and have a substantial effect on soybean yield. For this reason, if planting a less tolerant or susceptible variety, soybean growers should strongly consider the adoption of a management strategy that minimizes losses, such as the application of Soygreen® in-furrow at planting. In our study, the application of 2 and 4 lbs. Soygreen® acre^{-1} increased the yield of the moderately tolerant variety by 29 and 26 bushels, respectively, which was statistically significant at P<0.05. Results from the current study agree with past research regarding the potential benefits of Fe-EDDHA applied to the seed. Kaiser et al. (2014) found that in-furrow Fe-EDDHA application at a rate of 3 lbs. Soygreen® acre^{-1} significantly increased the yield of a susceptible variety under moderate to severe IDC conditions. Soil-applied Fe-EDDHA treatments were also found to prevent IDC and increase grain yield in other soybean studies (Penas et al.,1990; Wiersma, 2005; Schenkeveld et al., 2008; and Gamble et al., 2014).

Figure 12. Soybean yield (bushels acre^{-1}) by variety, iron chelate, and nitrogen application for Graceville, MN. Letter not connected by the same color were significant at P<0.05 using Tukey's HSD. IDC severity scores at R1 are shown as green and yellow boxes above significance letters. The non-hotspot area is referred as Neutral.

Furthermore, even though these findings indicate an effect for grain yield response to in-furrow application of Soygreen® for the moderately tolerant variety, the yields achieved are no better than those for the highly tolerant variety with or without Soygreen® application. When N was added at the Neutral site and no Soygreen® was supplied, the highly tolerant variety out yielded the moderately tolerant variety by 13 bushels acre^{-1} and its average yield (61 bu ac^{-1}) was not statistically different than those of the moderately tolerant variety with Soygreen® application. Therefore, if the application of Soygreen® is not a viable option, farmers can grow a highly tolerant variety instead of a moderately tolerant variety and still get some yield boost, even though this difference (from 48 to 61 bu ac^{-1}) was not statistically different in this study.

At the Graceville Hotspot site, treatments showed different effects on grain yield. When N was added, Soygreen® application significantly increased soybean yield regardless of variety. Yields of 38 and 45 bushels were found for the moderately tolerant variety, and 35 and 36 bushels for the highly tolerant variety with the application of 2 and 4 lbs. Soygreen®, respectively. These findings comport with what was observed at Danvers, MN. When severe IDC conditions prevail, in-furrow application of Soygreen® is an effective alternative for managing iron chlorosis (Gamble et al., 2014; Kaiser et al., 2014).

In contrast to what was found at the Graceville Neutral site, where the yield of the highly tolerant variety was not significantly reduced when N was added, N application at the Hotspot site worsened IDC symptoms and significantly reduced grain yield (~33%) of the highly tolerant variety. Although this variation could be explained by the use of different varieties, our results differ from those of Wiersma (2010), who reported that iron-efficient varieties were unaffected by increasing nitrogen rates. In their study, varieties were classified as iron-efficient based on the author's personal observations over several years. In the current study, we rely on characterization by the breeding program that developed the variety.

Beyond that, it is important to note that the moderately tolerant variety without any Soygreen® treatment provided the lowest final yields regardless of N application (37 and 35 bushels acre^{-1} with and without N application, respectively). Thus, growing a less tolerant or susceptible variety in hotspot areas without any other management solution should never be recommended. Also, when N was not added, the highly tolerant variety alone yielded less than with Soygreen® treatments (from 61 bu ac^{-1} without Soygreen® to 75 and 76 bu ac^{-1} with 2 and 4 lbs. Soygreen® acre^{-1}, respectively), but differences were not statistically significant. This suggests that growing a highly tolerant variety alone may be an option in hotspot areas that are not worsened by excessive soil nitrate levels if the application of Soygreen® is not feasible. It is important to reinforce, however, that grain yield is not maximized in this case as with the application of Soygreen®, but the genetic resistance appears to be enough to avoid significant losses. These research findings disagree with what was found for Danvers Hotspot site. While average chlorosis symptoms were similar for highly tolerant varieties in Hotspot areas at Danvers and Graceville, as shown in Table 2, soil properties associated with high IDC risk were more prevalent at Danvers, which could partially explain why the highly tolerant variety did not perform as well as in the Hotspot site at Graceville.

3.3 Effects on soybean grain yield at Foxhome

At Foxhome, a three-way interaction was found between site, variety, and nitrogen (Figure 13). Therefore, a separate analysis was performed within sites (Neutral and Hotspot) to test for significance of treatment effects.

At the Foxhome Neutral site, nitrogen application increased IDC symptoms and significantly reduced the yield (by 25%) of the moderately tolerant variety. As described previously, excessive nitrate in the soil can exacerbate IDC severity (Wiersma, 2007). Therefore, as recommended for the neutral site in Graceville, when IDC severity may be increased by increased levels of NO_3- due to N carry-over from a previous crop or by N mineralization, soybean growers should utilize highly tolerant varieties as an alternative to less tolerant or susceptible varieties. In the present study, not enough evidence was found to state that the highly tolerant variety produced significantly more than the less tolerant one when IDC intensity was increased by N application, but a yield difference of 9 bushels acre^{-1} was found (from 36 bu ac^{-1} with the moderately tolerant variety to 45 bu ac^{-1} with the highly tolerant variety). As indicated by several previous studies, genetic resistance is the most practical and effective strategy for managing IDC and should always be considered for profitable production in moderate to severe chlorosis conditions (Goos and Johnson, 2000; Kaiser et al., 2014). Alternatively, when N was not added, both varieties yielded similarly (48 and 47 bushels acre^{-1} for the moderately tolerant and highly tolerant varieties, respectively), indicating that either a less tolerant or highly tolerant variety could be used at neutral spots.

At Foxhome Hotspot, the highly tolerant variety produced 28% more than the moderately tolerant variety when N was not added, a statistically significant difference at $P<0.05$. When N was added, the yield of the highly tolerant variety was reduced by 9%, but that difference was not statistically significant.

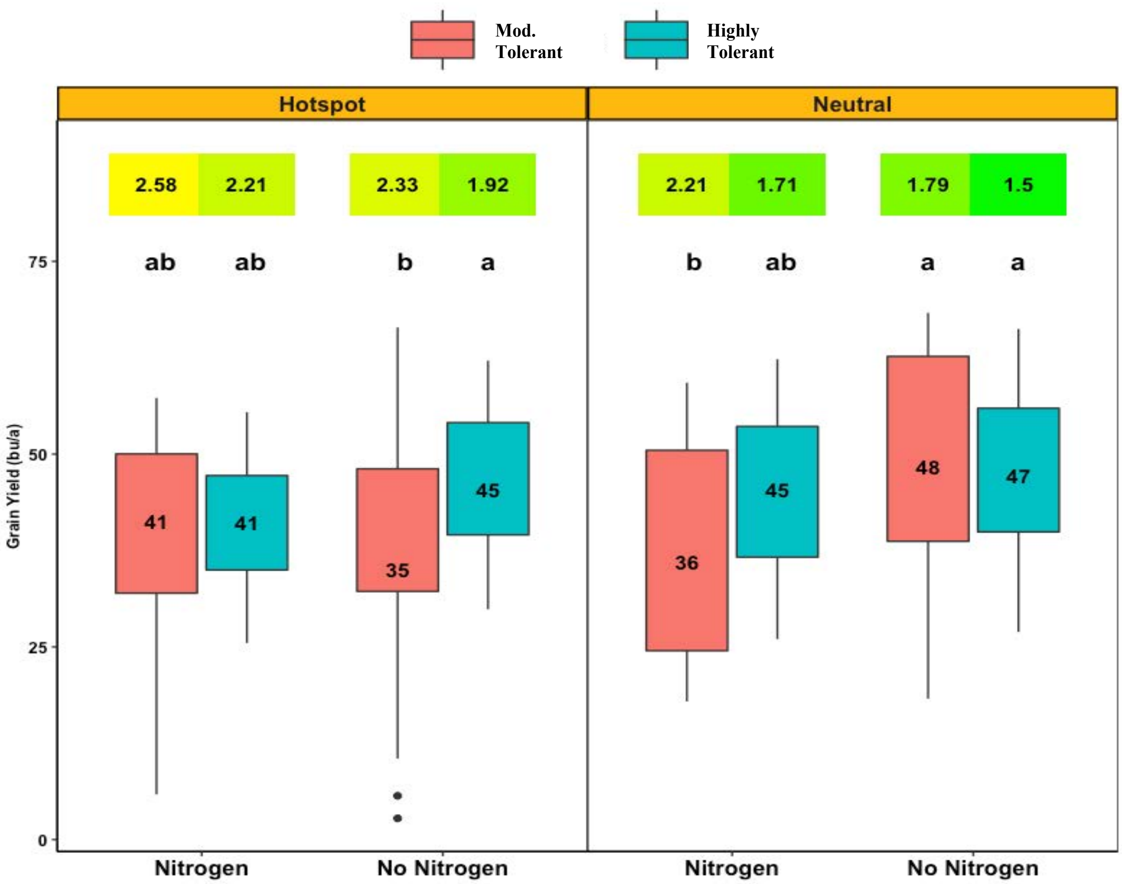

Figure 13. Soybean yield (bushels acre^{-1}) by site, variety, and nitrogen for Foxhome, MN. Letter not connected by the same color were significant at $P<0.05$ using Tukey's HSD. IDC severity scores at R1 are shown as green and yellow boxes above significance letters. The non-hotspot area is referred as Neutral.

4. Preliminary conclusions

Our preliminary results suggest that different management strategies should be recommended depending on the field location and the intensity of IDC. In neutral spots, where lower intensities of IDC are found, treatments have less effect on soybean yield. In hotspots, where IDC is severe, treatments varied in their effect on soybean yield.

5. Further steps

As mentioned previously, this study aims to evaluate the impact of variety selection, seeding rate, and iron chelate rates individually and collectively on return on investment. Therefore, yield response data will be further utilized, along with grain prices and input costs, to define the optimum combinations of these three management strategies in terms of profitability. Coupled with an economic analysis, this work will provide producers with more refined recommendations for managing IDC and will support them in their efforts to achieve the greatest economic return on every farm.

Recent advances in High-Throughput Phenotyping have enabled time-series measurements that monitor the development of soybeans affected by IDC through physiological stages and responses to the environment. However, it has been used only with the categorical classification of IDC in a 1 to 5 rating system. Assessment of IDC in the field with a more quantitative approach, which includes final yields in the analysis, is needed to provide a deeper physiological understanding of IDC and how it affects grain yield. By utilizing yield response data against vegetation index values generated from drone imagery at several time points during the 2021 growing season, we aim to study the temporal dynamics of IDC symptoms in soybeans and to develop a model that can estimate/predict grain yield across fields. Ground-based NDVI and Visual Scores will be utilized for model validation.

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