TILE DRAINAGE, BEDS, AND FE-EDDHA APPLICATION EFFECT ON SOYBEAN PRODUCTION

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Tile Drainage, Beds, and Fe-EDDHA Application Effect on Soybean Productivity

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MASTER OF SCIENCE

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ABSTRACT

Eastern North Dakota has received excessive rainfall events since 1995, and soils are prone to waterlogging. This research evaluated the effects of subsurface tile drainage, raised beds, and iron-chelate (Fe-EDDHA) seed-application on iron-deficiency chlorosis (IDC) incidence in soybean [*Glycine max* (L.) Merr.], soybean growth, and yield, across six environments during 2013 and 2014. Tile drainage without beds increased soybean yield and reduced IDC by 11%. Beds resulted in more vigorous plants with 9% more biomass and increased soybean yield by 6%. There was no yield advantage to using both tile and raised beds within the same field. The Fe-EDDHA reduced plant population and IDC expression, increased plant biomass, but did not result in a yield increase. Farmers are encouraged to consider utilizing raised beds as a means to mitigate excess water. Additional research is needed to determine the cause of lower established plant density after seed application with Fe-EDDHA.

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INTRODUCTION

Soybean (*Glycine max,* L. Merr.) has been cultivated for centuries. Native to Southeast Asia, it was introduced into the United States around 1765. Initial usage of the soybean crop was for forage, but in the 1920s, soybean started to be used for food purposes, primarily oil and protein (Blessitt, 2008). Soybean, just like other crops, is subject to many stresses. Some of the abiotic stresses include water-logged soil, salinity, and iron-deficiency chlorosis (IDC) (Endres and Kandel, 2015). These stresses present a real problem, especially in the Upper Midwest where soybean is one of the major crops. Therefore, methods to mitigate yield losses due to stress need to be investigated. This research focused on different potential management practices for the abiotic stress iron deficiency, and the subsequent chlorosis in soybean.

The deleterious nutrient deficiency, IDC, may occur when soybean is planted in saturated, poorly drained, calcareous soils. It is characterized by interveinal yellowing (necrosis in severe cases) of the newest trifoliolate leaves. The symptoms appear during the V1 to V3 stages (Fehr et al., 1971) and may dissipate during the subsequent growth stages (Kandel, 2012). The result of IDC may be reduced yield at the end of the season due to decreased pod production, empty pods, and lower seed weight.

Research Objectives

The first objective of this research was to evaluate soybean growth and development, IDC incidence, and yield response with: 1) the presence of tile drainage, 2) planting on raised beds, 3) seed application of the iron-chelate compound labeled as 6% Fe in the form of ortho-ortho-Fe-EDDHA (iron ethylene diamine-N,N’-bis (hydroxy phenyl)acetic acid) utilizing the product Soygreen (West Central Inc., Willmar, MN; manufactured by Laboratorio Jaer, S.A, Barcelona, Spain), and 4) the combinations of these treatments. The second objective was to evaluate IDC, plant vigor and biomass response with and without the seed application of Fe-EDDHA.

Early detection of IDC with the eye is possible, but the yield damage may already have been done when the eye detects differences in leaf color. Earlier detection may allow corrective measures to prevent yield loss. Therefore, hypothetically, soybean receiving the Tile by Raised beds by Iron-Chelate treatment may yield significantly better than the control (no tile, no raised beds, and no iron-chelate) under environmental conditions where IDC may occur.

LITERATURE REVIEW

Tile Drainage Systems

One of the dominant soils in the Red River of the North Valley (RRNV) is the Fargo-Ryan series (USDA, 2013). This soil is 95 percent silty clay (about 45% silt, 50% clay) with the remaining 5% being sand and has a 0-1% slope. The natural internal drainage of a Fargo-Ryan is very poor and the soils need to be drained (surface and/or sub-surface) for crop production. The silty clay soil severely limits internal drainage, but with the installation of tile, water movement through the soil profile is greatly enhanced (Cihacek et al., 2012).

Since a silty clay soil is made up of fine to very fine soil particles, the pore spaces are very small. Water moves by capillary forces as well as gravity and the air-filled pore space may fill up very quickly as water moves through the soil profile. Once all pore spaces are filled by water, the soil is saturated (Sylvia et al., 1999). At this point, water may pond on the soil surface. However, when tile drainage is installed, the effects of poor internal drainage are considerably reduced, for the tile drainage provides an outlet for the water to move from the filled pore space to the unfilled tile pipe and in turn off-site (Brodshaug, 2011). As the RRNV is relatively flat, the subsurface drain needs to be at least 0.1% grade to properly function and have a high enough drainage coefficient in order to remove excess water from rainstorms within no more than 48 h (Orchs et al., 2001).

Soybean and Tile Drainage Systems

After soybean plants are established (i.e. post germination), short periods of excess water are less detrimental than if excess water occurs during germination, or during later growth stages such as R4 (full pod) versus V-stages such as V4 (fourth trifoliolate) (Oosterhuis et al., 1989). The only time when additional rainfall could be beneficial would be during critical cell differentiation periods, such as development of flowering structures, which in turn could lead to more pods and seeds, as well seed development characteristics such as protein and oil content (Nakagawa et al., 2018). During these periods, the soybean needs water to help translocate nutrients throughout the plant for the development of said complex structures (Salisbury and Ross, 1992).

Excess water and poor soil drainage can lead to ponding and with it, prolonged exposure of crops to flooding conditions. Excess water should be removed within 48 h to avoid injury. A longer period may result in yield loss, depending on flood tolerance of the cultivar (Sands, 2013). Brodshaug (2011) conducted soybean research at NDSU's Northwest 22 (NW22) research location during the 2009 and 2010 growing seasons. The focus of this research was subsurface tile drainage impacts on soybean development and soil properties in a clay soil. Half the treatments were tile drained, while the other half were not. The author concluded that even though characteristics such as yield, plant height, and vigor were not significantly higher and the IDC score not significantly lower (a lower IDC score means less IDC) on the drained portions of the field, the yield, plant height, and vigor were all nevertheless numerically higher and the IDC score lower. The reason why there was no *significant* difference was likely due to the weather in 2009 not being favorable for IDC incidence since there was little or no rainfall over long periods during the season. The author speculated that if conditions for IDC incidence had been ideal in 2009, significant differences would have been observed.

Tile Drainage Systems and Soil Properties

Tile drainage systems may benefit soybean development, but what is the impact on the environment? Whenever water moves offsite, it can move dissolved plant nutrients (i.e. Nitrogen (N)) offsite. These nutrients, in large amounts, may be toxic to aquatic life. One method to reduce outflow from subsurface tile drainage is the installment of controlled tile drainage systems utilizing a water control structure. With these structures, the water table can be kept at a certain depth, thereby controlling how much water leaves the field, and in turn reducing nutrient loss (Randall, et al., 1997).

Tile drainage can increase soil penetration resistance and improve trafficability. If the soil has greater penetration resistance (drier soil compared with saturated soil), heavy machinery will be able to drive on it easier, thereby increasing the ease of planting, spraying, and harvesting (Kornecki and Fouss, 2001). However, crops such as soybean have a soil penetration limit, which if crossed could lead to root injury and growth inhibition. This level was determined to be approximately 10 Mpa (Kirnak, et al., 2017). Brodshaug et al. found soil penetration resistance to be significantly higher with tile drainage, which means control structures should be in place to prevent the soil from becoming too dry and thereby increasing the soil penetration resistance above 10 Mpa.

Research conducted on soybean in a pot experiment using soils with high clay content observed significant decreases in chlorophyll content and significant increases in IDC in soybean in response to increase soil bulk density levels measured in terms of fraction of porosity and penetration resistance. The authors further concluded that these soil conditions kept pH at a high level (alkaline) and increased soil bicarbonate levels which indirectly lead to a decrease in Fe availability to the soybean plant (Inskeep and Bloom, 1986).

Raised Beds

Raised beds are a form of ridge tillage. Raised beds have a flatter and wider surface, thereby enabling one to plant directly on top. Utilizing raised beds is desired when the farmer wishes to reduce exposure of the crop’s roots to excess water. It is another water management practice that has been shown to be beneficial in areas with poorly drained soils (Benjamin et al., 1990; Bakker et. al, 2005). Although flooding may occur between the beds (in-furrow) this practice nevertheless may improve production since the flooding would occur in an area below the root zone during emergence and growth (Hatfield et al., 1998).

Raised Beds and Soil Temperature

Soil temperature early in the season is very important since crops require a certain temperature range to germinate. The growing point of crops such as soybean emerges with the cotyledons. Crops such as corn [*Zea Mays,* (L.)], on the other hand, have a growing point that remains under the soil surface until the fifth or sixth leaf stage (Hatfield et al., 1998). Raised beds have been shown to increase the soil temperature near the seed because of the lower water content in the top of the bed compared with flat land. A raised bed will provide a greater surface area for sunlight, and this can raise the soil temperature (Hoppe et al., 2017).

Raised Beds and Soil Moisture

Hoppe et al. (2017) conducted research with soybean in the RRNV studying raised beds effect. Iron-deficiency chlorosis was significantly reduced at one location with raised beds compared to flat land, and both plant population and yield were not significantly different. However, soil temperature and root mass were both observed to be significantly higher with raised beds. The authors concluded that raised beds could be utilized in soybean production under dry conditions, but determined that further research needs to be conducted in order to compare raised beds tillage with ideal or excess rainfall conditions.

Raised beds will shed the water off the bed into the furrows and potentially help relieve saturation stress caused by flooding. The preferable outcome after a rainstorm is for the soil water level to be at field capacity and excess water to drain away, ideally within 24-36 h, so that stress from excess water does not occur (Bakker et al., 2005). In a study conducted in Western Australia, Bakker et al. (2005) found that even though the water table rose at the same rate on both flat land and raised beds, the water table dropped more quickly in the raised beds. Furthermore, they found that surface water in-furrow drained away more quickly via run-off compared to the water on the beds. This was mainly due to wheel traffic in between the beds, as well as the bed-making equipment, which would compact the soil and increase bulk density. The bulk density of the soil is calculated by dividing the dry weight by the total volume (a component of which is pore space). Therefore, when the pore space is decreased by compaction, the bulk density increases (USDA NRCS, 2008). The authors concluded that due to this bulk density increase, the water moved off-site better in between the beds compared to the top of the beds. Additionally, waterlogging was reduced in the raised beds areas compared to the flat land area (Bakker et al., 2005).

Kirnak et al. (2017) conducted a soil compaction experiment studying various soil characteristics such as bulk density, soil saturation, and nutrient levels. Results showed bulk density to be higher with more compaction. Given the method used to make raised beds, one can assume that raised beds will naturally have a lower bulk density compared to flat, un-tilled land. Kirnak's study also demonstrated via different irrigation treatments that application of more irrigation water could lead to significantly higher bulk density within the upper soil profile. Given the high levels of soil saturation that readily occur in the RRNV, raised beds tillage may alleviate increased bulk density (i.e. compaction) in the main zone where plant roots will be developing.

Raised Beds and Plant Nutrition

When soybeans are in the early vegetative growth stages (primarily the V1-V2 stages), nutrients are available in the cotyledons, which are the nutrient source for the developing seedling. However, as the plant develops, more nutrients are required, due to the complex vegetative structure development. Large amounts of nutrients are also required when the soybean plants start to produce flowers, pods, and seeds (i.e. R1-R2, R3-R4, and R5-R6 respectively) (Kandel, 2012). Nitrogen is typically utilized by soybean from N-fixation in the root nodules, which are formed by interactions with soil rhizobacteria. These rhizobacteria are aerobic organisms, which means they require oxygen to survive. Soils that are poorly drained are typically low in rhizobacteria population, resulting in a lower nodulation formation on the soybean roots (Sylvia et al., 1999).

Availability of Fe can also be affected by water. When there is more water, Fe tends to be less available to plants. Two forms of Fe that can exist in the soil are Fe(II) and Fe(III). These are in two different oxidative states. Plants can take up Fe(II) but not Fe(III). Higher water content in the soil can lead to a change in the oxidative state of iron from Fe(II) to Fe(III) (Hansen et al., 2003).

Iron Chelate

The application of the iron-chelate compound ortho-ortho-Fe-EDDHA (henceforth, Fe-EDDHA) to soybean is a newer concept. The purpose of this product is to provide the plant with readily available Fe(II). Iron chelate has been used and sold, for instance, in a product called Soygreen, which can be applied in-furrow during planting. The recommended product rate is 3.36 kg ha^{-1}. This product should be applied either at planting or shortly after emergence of the soybean, since IDC typically affects soybean plants early in the growing season (V1-V3). If the roots grow with Fe-EDDHA present, IDC should be reduced. In a study conducted by Wiersma (2005) in Crookston, MN, on a soil known to cause IDC, Fe-EDDHA was used as a seed treatment (a method that is not commonly used). Compared to the 3.36 kg ha^{-1} rate, the conclusion was that the 4.5 kg ha^{-1} and 5.63 kg ha^{-1} rates of Fe-EDDHA resulted in longer periods of time that IDC tolerant crops were able to take up Fe(II), and that 11.25 kg ha^{-1} rate of Fe-EDDHA resulted in longer periods that IDC susceptible cultivars were able to take up Fe(II). Goos and Johnson (2000), after doing a similar study in the greenhouse as well as in the field, concluded that seed treatments with Fe work better in the greenhouse compared to field conditions.

Methods of Application

There are other methods of applying Fe-EDDHA such as foliar sprays or in-furrow applications. Gamble et al. (2014) conducted a soybean study in the Black Belt region in Alabama, testing different concentrations of Fe-EDDHA via foliar sprays and in-furrow applications. Visual chlorosis scores were lowered both for in-furrow application and foliar spray, but in-furrow application resulted in the most reduction. A high Fe-EDDHA in-furrow application rate (4.5 kg ha^{-1}) as well as the foliar spray resulted in significantly higher yield. The researchers concluded that both in-furrow as well as foliar sprays work to reduce detrimental effects of IDC.

Soil Properties and Fe(II) Availability

Soil type and properties can impact the availability of Fe(II) to plants. $CaCO_3$ levels tend to be higher in Eastern North Dakota soils with a high clay content and the resulting cation competition can negatively impact a plant’s ability to naturally chelate Fe. Schenkeveld et al. (2008) conducted an experiment using different forms of Fe-EDDHA in solution. Longer periods of IDC were observed on soils with a high clay content. Correlations were made between chlorophyll content and yield and yield was found to decrease significantly on soils with high clay content and $CaCO_3$ levels, and plants only seemed to respond to a Fe-EDDHA application when under iron deficiency stress. Speculative conclusions were made that, when plants did take up Fe-EDDHA and utilize the Fe, the EDDHA (chelating compound) would return to the soil/solution in which the plant was growing. If the soil had a high $CaCO_3$ content, cation competition as well as natural degradation would likely result in the EDDHA not being able to chelate more Fe (i.e. not make any more soil-Fe plant available). Overall, increases in yield in response to [Fe] level appeared to stop over time (eight weeks in this particular experiment), indicating Fe-EDDHA application rate has a limit, above which there is no further benefit.

MATERIALS AND METHODS

Tile by Raised Beds by Fe-EDDHA Experiment

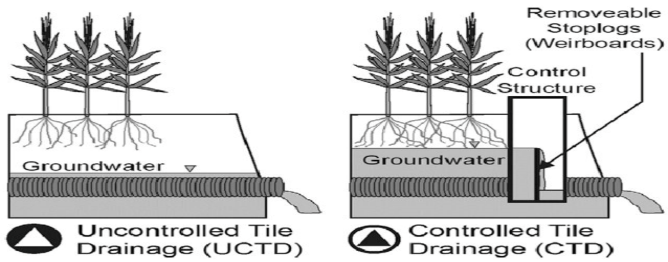
This experiment was established in two locations in 2013 and 2014: Northwest 22 (NW22) in Fargo, ND, with two environments (with open tile called "tile" or "drained" in this thesis and closed tile called "no tile" or "undrained"), and Casselton, ND (no tile installed at this location). These locations were selected based on soil type and raised bed construction feasibility. Soil type and fertility information are provided in Table 1.

Table 1. GPS location, soil type, and soil fertility for main plot locations at NW22, near Fargo, ND and Casselton, ND in 2013 and 2014.

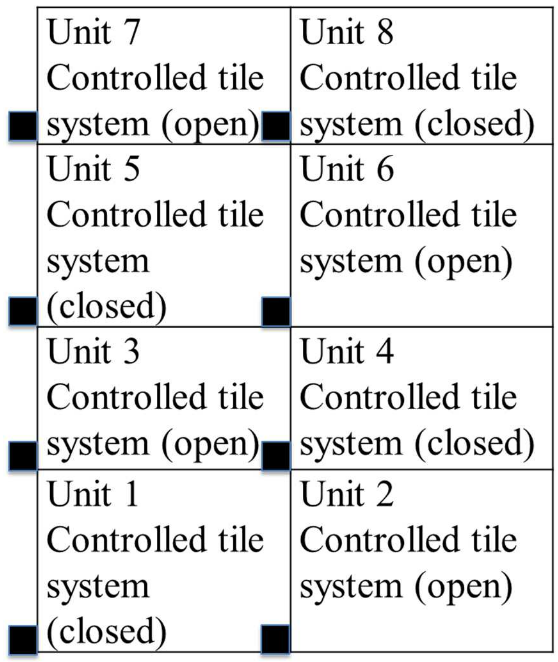
	GPS coordinates†	Soil type‡	N§	P	K
		0-150 cm	----- 0-15 cm -----		
			kg ha^{-1}	---- ppm ----	
NW22 near Fargo, ND (2013)	46°55'55.3"N; 96°51'32.2"W	Fargo-Ryan, silty clay	59.4	25	460
Casselton, ND (2013)	46°52'40.8"N; 97°15'04.0"W	Kindred-Bearden, silty clay loam	21.3	16	340
NW22 near Fargo, ND (2014)	46°55'55.3"N; 96°51'32.2"W	Fargo-Ryan, silty clay	53	25	460
Casselton, ND (2014)	46°52'40.8"N; 97°15'04.0"W	Kindred-Bearden, silty clay loam	19	16	340

†GPS location based on Google Maps.
‡Soil type information obtained from the Natural Resources Conservation Service Web Soil Survey.
§Fertility information based on soil test report from NDSU Soil Testing Lab.

The Fargo location, NW22, had tile drains installed in 2008. The tile drainage pipes were installed at a depth of 1 m and spaced 7.6 m apart. The pipe is 10 cm in diameter with an average drainage coefficient of 8 mm per 24 h. Eight experimental units were established at NW22, four of which simulate undrained and four drained. The four units that were designated drained in 2013 were also designated drained in 2014. Inline Water Level Control Structures (Agri Drain Corporation, Adair, IA) were used to control the water table (Fig. 1a).



a



b

Figure 1. a) Controlled tile drainage versus uncontrolled tile drainage (Sunohara et al., 2015) and b) Layout of controlled tile drainage system at NW22. Black squares represent control structure for each unit.

In the closed tile area, the water table would reflect a non-drained condition. Each experimental unit measured 0.3 ha and had its own control structure (Fig. 1b). The drained units of the field at NW22 had the stop blocks of the control structures pushed down (closed) during the winter and spring of 2013 to aid in flood reduction efforts, but then pulled up two weeks before planting to enhance trafficability during planting and also so that the drained treatment was present during germination. The control structures for the undrained units of the field were left closed. Planting did not commence until the field was dry enough to plant on both the drained and undrained portions of the field.

Five soybean cultivars were used at each location. Cultivars were selected based on IDC scores to ensure both IDC tolerant and susceptible cultivars were used and that maturity ratings were considered. Two of the providing companies did not have the same cultivars available for the 2014 season. However, the same characteristics (namely, IDC tolerance and maturity) were kept when selecting replacement cultivars to reduce variability across years and proxy numbers were assigned so that proxy (cultivar) data could be analyzed across years (Table 2).

This experiment was laid out in a randomized complete block design (RCBD) with a split-split-plot arrangement (NW22 only) and a split-plot arrangement at Casselton. At NW22 tile served as the main plot, raised beds as the sub-plot, and a 5 x 2 factorial arrangement of five cultivars by two Fe-EDDHA application rates as the sub-sub-plots. Casselton had raised beds as the main plot, with the same factorial arrangement as the sub-plot. There were four replications at each location. Since NW22 was the only location with tile drains installed, it was analyzed by year and combined across years to analyze the tile by no tile treatment effect. In this analysis, replication and environment were considered random effects and tile, raised beds, Fe-EDDHA application and cultivar were considered fixed effects.

When NW22 data were combined with the data from Casselton, the non-tile and tile areas were considered separate environments in order to analyze across Casselton and NW22 for both years. Therefore, this analysis was an RCBD with a split-plot arrangement, with raised beds being the main plot and the Fe-EDDHA by cultivar factorial being the sub-plot. In this analysis, the replication and environment were considered random effects and raised beds, Fe-EDDHA application and cultivar were considered fixed effects.

All of the data collected in this experiment was analyzed using the SAS 9.4 computer software (SAS Institute Inc., Cary, NC) at a probability level of 90% (α=0.10). Means were compared utilizing the Fisher's F-protected LSD at α=0.10 (i.e. LSD was calculated only if the F-test was significant).

The seeded experimental plot size used at both locations and during each year was 3 m wide x 7.6 m long with four rows per plot and 76 cm row spacing (22.8 m^2 total). Only the middle two rows were considered for data collection and the outside rows were considered buffer rows. The buffer rows were used for root data collection as this involved destructive sampling. A seeding rate of 531 050 seeds ha^{-1} was used with an anticipated established plant population of 454 048 plants ha^{-1}. This number is based on the expected germination rate of 95% and the expected plant loss of 10% (Kandel, 2013).

Table 2. Roundup Ready soybean cultivars, varying in maturity ratings and tolerance to iron-deficiency chlorosis, used in experiments in 2013 and 2014.

Company	Cultivar	Year(s) used	Maturity Group†	IDC Visual Score‡	Proxy§
DuPont Pioneer	90Y42	2013	0.4	2.6¶	1
DuPont Pioneer	90Y50	2014	0.5	2.8	1
DuPont Pioneer	90Y70	2013-2014	0.7	1.7	2
Dairyland Seeds Co.	DSR-0747/R2Y	2013-2014	0.7	2.6	3
Hyland Seed	HS 01RY02	2013-2014	0.1	1.7	4
NuTech	6088	2013	0.8	2.7	5
Channel Bio	0906R2	2014	0.9	2.7	5

†Maturity group numbers are provided by companies.
‡IDC visual chlorosis scores are based on the North Dakota Soybean Variety Trial Results for 2012/2013 and Selection Guide booklet (Kandel et al., 2012 and 2013). Scale: 1 = green; 5 = dead tissue.
§Proxy is a number system used to designate cultivars based on genetic qualities so that results can be analyzed across environments/years. In this case, maturity group and IDC score were taken into account when choosing/replacing cultivars. Cultivar 90Y50 replaced 90Y42 in 2014 and cultivar 0906R2 replaced 6088 in 2014.
¶IDC scores for 90Y42, 90Y50, and 90Y70 were obtained from DuPont Pioneer's scale (1 = worst; 9 = best) and are an estimate of what they might be on the NDSU scale.

A HR6 Hipper Roller (Pitonyak Machinery Corp., Carlisle, AR), with a 41 cm diameter drum to flatten the tops of the raised beds, was used to make raised beds 76 cm apart with a height of 30.5 cm in the fall of 2012 and 2013 on half of the planted area of each location (Fig.

2). The purpose of the raised beds was to dry out the soil and increase soil temperature. The row orientation was north-south at the Fargo and Casselton 2014. At Casselton in 2013, the rows were oriented east-west. The other treatment was only tilled conventionally and was called flat. Any needed repair of the raised beds was done in the spring using the same equipment.

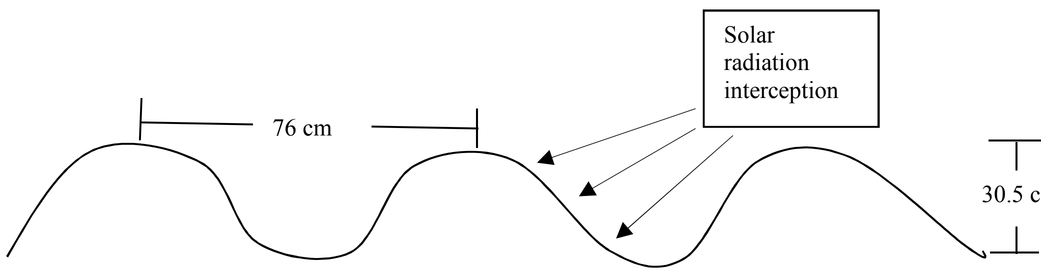


Figure 2. Raised beds.

Fe-EDDHA treatments consisted of 0 kg ha^{-1} and 3.36 kg ha^{-1} Soygreen. Soygreen is a soluble powder with 6% Fe in the ortho-ortho-Fe-EDDHA form. The 3.36 kg ha^{-1} rate was based on Soygreen distributor recommendations (Roger Strand, West Central, Inc., personal communication). This rate was the highest amount that could be seed-applied without the product falling off and is 454 g ha^{-1} less than the rate that produced acceptable results for IDC tolerant cultivars in research conducted by Wiersma (2005) in Crookston, MN. Susceptible cultivars, according to Wiersma (2005), need about 11.2 kg ha^{-1}. In this thesis, Soygreen will be called Fe-EDDHA. A mixture of the sticking agent Gum Arabic (Arabic Powder: *Acacia spp.*, Frontier, Natural Products CO-OP, Norway, IA), water, and Fe-EDDHA was used to treat the seed.

Three different germination tests were conducted: one with the full treatment (i.e. total mixture rate of 3.36 kg ha^{-1} of Fe-EDDHA) plus Gum Arabic and water, one with just Gum Arabic and water, and one with no treatment (control). Two germination observation dates were used (7 d and 14 d) using two different cultivars (90Y70 and 90Y42). Two different brands of

Gum Arabic (Arabic Powder: *Acacia spp.*) and TheDiet35 (The Fiber Diet35, Swanson Health Products, Fargo, ND) were used to determine if one brand was more effective at making the Fe-EDDHA stick to the seeds and whether germination was inhibited/delayed. The Ragdoll germination method was used and there were three replications of 100 seeds for each treatment. Germination percentages were calculated after each allotted timeframe. The Arabic Powder from Frontier was determined to be more effective/less inhibitory on germination rates and therefore was used (Table A4). Previous findings showed that a 40-23-37 grade mixture (i.e. 40% Fe-EDDHA, 23% Gum Arabic, 37% water, measured out by weight) at 3.36 kg Fe-EDDHA ha^{-1} produced germination levels above 90% (Wiersma, 2005), and therefore was the rate used in this study.

The following is an example of how the Fe-EDDHA was seed-applied. This example is taken from the procedure used to treat the necessary amount of seeds at NW22 (Eq. 1).

$$22.8\ m^2\ plot^{-1} * 80\ \text{Fe-EDDHA treated plots} = 1{,}824\ m^2\ (0.1824\ ha)\ \text{treated area}$$

$$0.1824\ \text{treated ha} * 531\ 050\ \text{seeds}\ ha^{-1} = 96\ 864\ \text{treated seeds}$$

$$96\ 864\ \text{treated seeds} / 5\ \text{cultivars} = 19\ 373\ (\approx 20\ 000)\ \text{treated seeds cultivar}^{-1}$$

(Equation 1)

Seeds were treated in bulk amounts (1 000 seeds at a time) and seed number was determined by seed weight. Seed weight was determined by weighing three samples (100 seeds each) per cultivar (since each cultivar had different seed sizes/weights) and taking the average weight. The following equation is the breakdown of how the total mixture was composed (Eq. 2).

3.36 kg (3 360 g) ha^{-1} of Fe-EDDHA at a rate of 40-23-37 of total mixture

3 360 g ha^{-1}/0.40 = 8 400 g ha^{-1} of total mixture (Fe-EDDHA + Gum Arabic + Water)

8 400 g of total mixture ha^{-1} ≈ 3 360 g Fe-EDDHA + 1 930 g Gum Arabic + 3 120 g Water

(Equation 2)

Equation 3 demonstrates how the product was applied. At NW22, for example, since the treated area is 0.1824 ha, only 1 530 g of total mixture is needed, therefore:

1 530 g of total mixture / 5 cultivars = 310 g per cultivar (i.e. per 20 000 seeds)/ 20 = 15.5 g of total mixture per 1 000 seeds

(Equation 3)

Each component (Fe-EDDHA powder, Gum Arabic powder, and water) was weighed separately and then combined. More total mixture was made than was needed to compensate for some of the mixture sticking to the insides of the container. After thoroughly mixing, 1 000 seeds were weighed out (according to each cultivar's respective 1 000-seed weight), and then 17.5 g (i.e. 15.5 g intended amount plus 2 g to compensate for expected product loss during drying and packaging) of product was applied and thoroughly mixed so as to completely coat the seeds. Seed were spread out on waxed paper (to avoid product sticking to paper) and allowed to dry before packaging.

In-Season Measurements

Cumulative Growing-Degree Days (GDD), beginning at planting date each year, were used to ensure that data were collected at relatively the same growth stage each year (Eq. 4).

$$[(T_{MAX} + T_{MIN})/2] - T_{BASE} \qquad \text{(Equation 4)}$$

where T_{MAX} is the daily maximum temperature, T_{MIN} is the daily minimum temperature, and T_{BASE} is the base temperature for the crop (the temperature at which physiological growth ceases). There are currently no GDD formulas established for soybean, therefore the formula for corn is used. The base temperature for corn is 10° C (NDAWN, NDSU). A complete list of field measurements/application dates for NW22 and Casselton are presented in Table 3.

Table 3. Dates of applications and measurements.

NW22			
2013 Measurement/Application		2014† Measurement/Application	
31-May	Planted	23-May	Planted
1-Jul	IDC score recorded	25-Jun	IDC score recorded
1,10 July	Vigor scores recorded	25 June and 3 July	Vigor scores recorded
19 June and 8 July	Applied Glyphosate	11-Jun	Glyphosate applied
20 Aug.	Dug plants for biomass	8 Aug.	Mustang Max applied for aphid control
2 Oct.	Plant heights recorded and Harvested trial	30 Aug.	Dug plants for biomass
		10 Sept.	Maturity notes
		26 Sept.	Plant heights recorded
		3 Oct.	Harvested trial
Casselton			
2013 Measurement/Application		2014† Measurement/Application	
13 and 14 June	Planted	23-May	Planted
8-Jul	IDC score recorded	8-Jul	IDC score recorded
8 and 19 July	Vigor scores recorded	2 and 8 July	Vigor scores recorded
13 Sept.	Dug plants for biomass	20 Aug.	Dug plants for biomass
3 Oct.	Plant heights recorded and Harvested trial	10 Sept.	Maturity notes
		26 Sept.	Plant heights recorded
		30 Sept.	Harvested trial

†Dates in 2014 were based on Growing-Degree Days (GDD) from 2013 so that measurements were done at similar growth stages.

A John Deere Model 71 planter (Moline, IA) was used with one planting unit per row. One packet of seed was poured into each planting cone. Seeds were planted at a depth of 2.5 cm. Two days after planting, plots were observed for any seed that was not planted properly and were manually re-planted. Planting dates were between mid-May and early June and both locations were planted within a few days from each other.

Throughout the growing season, Glyphosate [N-(phosphonomethyl) glycine] (Monsanto Co., St. Louis, MO) was used to control weeds. Manual weed control was done when needed.

Certain problem weeds included Roundup Ready canola [*Brassica napus*, (L.)] and wild buckwheat (*Polygonum convolvulus*, L.), which had to be managed via manual control methods. Weeds in the alleyways as well as any soybean plants were controlled using a sickle mower and/or cultivator.

Once soybeans reached the V1-V2 stage, plant population counts were taken. Of the inner two rows of each plot, 90 cm of plants in each row were counted and numbers averaged. If there were any missing rows, then one of the buffer rows was used. After emergence, a New Holland tractor (New Holland TT75A, Dublin, GA) with a rototiller attachment was used to make 1.5 m alleyways in between each experimental plot perpendicular to the row direction.

Visual scoring for IDC and vigor was done once IDC symptoms became visible. Visual IDC scoring was based on a scale of 1 to 5, with 1 being no IDC symptoms and 5 being severe IDC symptoms (i.e. necrosis and dead plants). Both the NDSU Plant Science Dept. and Soil Science Dept. (Goos and Johnson, 2000) use this IDC scale. Furthermore, this scale is used by soybean breeders to assess tolerance levels of soybean to IDC incidence, as well as growers to choose a tolerant cultivar when growing soybean on IDC-prone soils. Vigor scoring was based on a scale of 1 to 9 with 1 being very poor developed growth structures (i.e. leaves, stems, flowers, etc.) and 9 being well-developed growth structures. A vigor assessment is relative (i.e. dependent on the scientist scoring). Both the early vigor and IDC scores were recorded on the same day. Vigor was assessed once during the vegetative stages and once during the reproductive stages.

Water table readings were taken throughout the growing season to monitor the change in water table depth in the drained and undrained areas at NW22. In 2008, four observation wells, consisting of PVC pipe, were installed in each of the eight units at NW22. There were two replications of each depth (122 cm and 213 cm, respectively) per unit (one observation well of each depth at both ends of each unit). A water sensitive device, [(Water Level Meter Model 101, (Solinst Canada LTD, Toronto, Canada)] was used to take water table readings. Measurements were taken from all four observation wells in each unit. The frequency of readings was done in accordance with rainfall events and dry periods to measure changes in the water table over time. Air temperature and rainfall data was downloaded from North Dakota Agricultural Weather Network's (NDAWN) website. For NW22, the NDAWN station near Hector International Airport in Fargo, ND was used and the NDAWN station in Prosper, ND was used for Casselton.

Plants in a length of 90 cm of one of the buffer rows were dug up each year to measure biomass. Two replications at NW22 and Casselton were used. In order to ensure that only plant material was weighed, soil debris on the roots was rinsed off in the field. Plants were then placed in a dryer until dry plant biomass was reached (brittle leaves and stems). The whole plants were weighed, then the roots were cut off at the soil line and the above ground mass was weighed. The root mass was calculated by subtraction.

Before harvest, plant heights were measured by selecting three plants at random from one of the middle two rows of each plot. If middle rows were missing then a buffer row was used. Plant height was defined as the distance from the soil surface to the top-most node with a fully developed pod. Row lengths were measured after the soybean plants had reached harvest maturity. Harvest data was corrected to row length.

Once the soybean seed reached a moisture content of approximately 13%, plots were harvested using a Wintersteiger Classic combine (Winchester Ag, Reid, Austria). After harvest, each sample was cleaned of any plant debris left over from combining and then weighed for total seed weight. A small sample was taken out of each bag and analyzed using the Dickey John

(GAC-2100, Dickey John Corp., Auburn, IL) for test weight, moisture content, and 500-kernel weight. A Diode Array 7200 NIR Analyzer (Perten Instruments, Springfield, IL) at the Northern Crops Institute (NCI) was used to determine protein and oil content. Yield data was corrected to 13% seed moisture content.

Fe-EDDHA by Cultivar Mini Plot Experiment

Mini plots were planted to further observe the response of five cultivars to two levels of Fe-EDDHA application. Observations at each location were plant population, IDC score, vigor score, and biomass, and rate of germination to see if the delay in germination observed in the Ragdoll tests for Fe-EDDHA treated seed carried occurred in the field too (i.e. if the germination delay was what caused the lower plant populations recorded in the main experiment).

Each mini plot was 1.5 m in length with 76 cm row spacing and was comprised of two rows (both rows the same cultivar, one row treated with 3.36 kg ha^{-1} Fe-EDDHA and one control row with 0 kg ha^{-1} Fe-EDDHA). These plots were hand planted at a seeding rate of 531 050 seeds ha^{-1} in fields that were known to cause moderate to severe IDC symptoms in soybean (Dr. Ted Helms, soybean breeder, personal communication 2013 and 2014). Four locations were used (Arthur and Leonard, ND in 2013; Galchutt and Harwood, ND in 2014) (Table 4). All five cultivars (Table 2) were planted at each location and there were four replications.

Table 4. GPS coordinates, soil type, and fertility for mini-plot locations (Arthur and Leonard, ND, 2013; Galchutt and Harwood, ND, 2014).

Location	GPS coordinates†	Soil type‡	N§	P	K
		0-150 cm	---------- 0-15 cm ----------		
			kg ha^{-1}	------ ppm ------	
Arthur	47°10'00.1"N; 97°18'38.4"W	Barnes-Svea, loam (0-150 cm)	NA	NA	NA
Leonard	46°40'20.0"N; 97°14'38.3"W	Glyndon, loam (0-150 cm)	NA	NA	NA
Galchutt	46°20'25.7"N; 96°53'35.7"W	Arveson, loam (0-25 cm), fine sandy loam (25-80 cm), loamy fine sand (80-137 cm), silty clay loam (137-150 cm)	12.3	8	120
Harwood	46°58'40.7"N; 96°34'34.8"W	Augsburn, silty loam (0-27 cm), very find sandy loam (27-82.5 cm), and clay (82.5-150 cm)	19.1	10	160

†GPS location information based on Google maps.
‡Soil type information obtained from the Natural Resources Conservation Service Web Soil Survey.
§Fertility information based on soil test results (2014 only).

Plant population, visual IDC scoring, vigor scores, and aboveground biomass measurements were taken at all locations. Plant population counts were done by counting every plant in each row since each row was 1.5 m long. Visual IDC and vigor scores were recorded using the same scale as described previously. Based on cumulative GDDs, all the plants in each row were cut above ground at approximately R1 to R2, since the focus of this experiment was mainly the Fe-EDDHA effect on IDC expression (in addition to the effect on plant population establishment) and not yield. Once harvested, the plant samples were placed in dryers until leaves and stems were brittle and then weighed. Biomass was calculated for the whole harvested area as well as on a per plant basis.

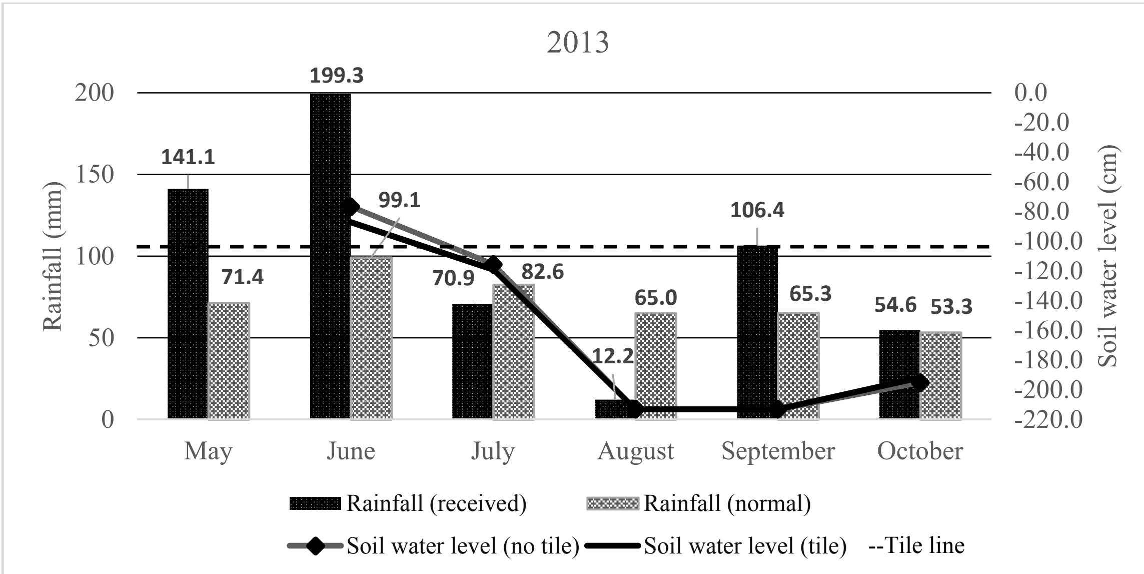
The experimental design of the mini plots was a RCBD with a split-plot arrangement of five cultivars by two Fe-EDDHA application levels. There were four replications at each location. Replication and environment were considered random effects and cultivar and Fe-EDDHA application rate were considered fixed effects. All of the data collected in this experiment were analyzed using the SAS 9.4 computer software (SAS Institute Inc., Cary, NC) at a probability level of 90% (α=0.10) for all observations. Means were compared utilizing the Fisher's F-protected LSD at α=0.10 (i.e. LSD was calculated only if the F-test was significant). Each year and location for the mini plots were analyzed separately due to significance being found within each year as well as variability in the results such as plant population.

RESULTS AND DISCUSSION

Rainfall and Water Table at NW22

In 2013, total monthly rainfall peaked at near 200 mm in June, was 130 mm in July, 12.2 mm in August, increased to slightly more than 100 mm in September, and dropped again to about 55 mm in October (Fig. 3a). This contrasted the expected 30-yr average rainfall, which predicted more rainfall to occur in July and August. In 2014, a similar pattern occurred with rainfall again peaking in June, largely decreasing (by about 100 mm) in July, increasing slightly August through September, and then decreasing again in October (only 7.7 mm) (Fig. 3b). The overall rainfall amount in 2014 was lower compared to 2013 (Fig. 3).

Water table levels were related to rainfall amounts as well as crop water use. In 2013, the water table level responded to limited rainfall by dropping below the tile line by the end of July and continuing to drop through September, but then increased by October. Since the water table level was below the depth of the deepest PVC pipe (i.e. greater than or equal to 213 cm below the soil surface) during August and September the Solinst Water Table Meter was unable to detect water and no data were recorded. In 2014, the water table did not drop below the tile line until August. Furthermore, differences between tile treatments were more noticeable with higher rainfall amounts (Fig. 3).



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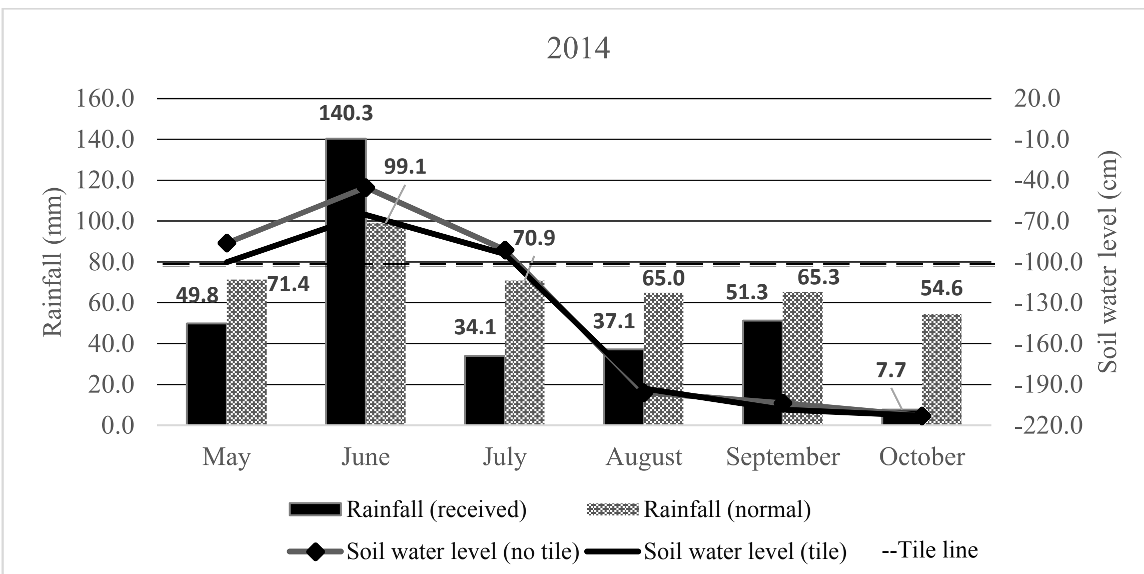

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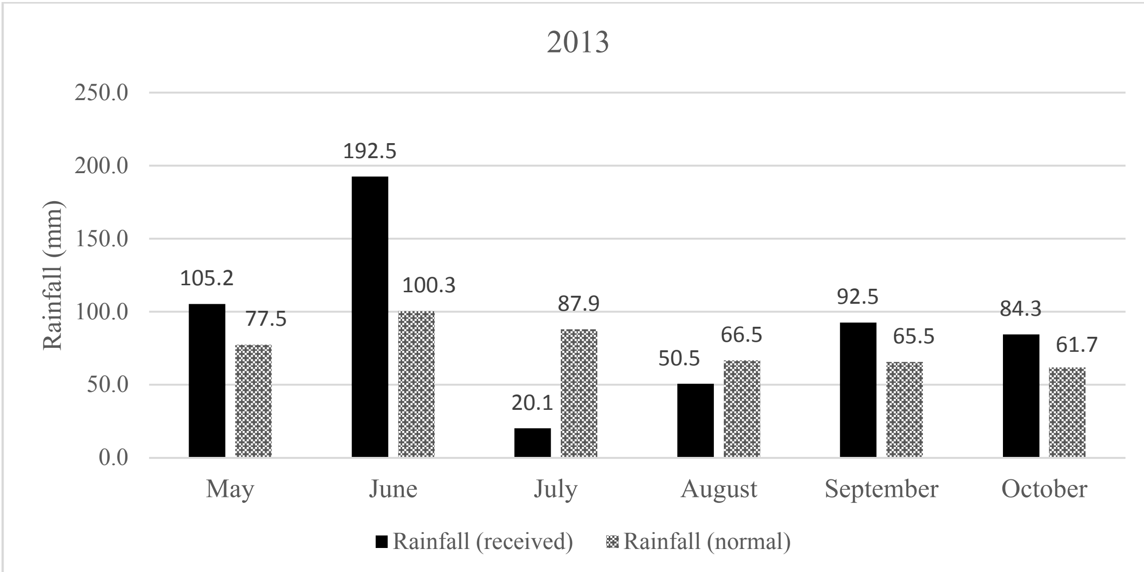
Figure 3. Monthly average water table level (tile versus no tile) and rainfall expected (30-yr 1981-2010 normal, NDAWN) versus received at NW22, near Fargo, ND, in (a) 2013 and (b) 2014. The water table readings for August and September (2013) and October (2014) are estimates since the water table was lower than 213 cm below the soil surface (i.e. below the deepest PVC pipe). The field was partially flooded in May (2013), so no data was collected.



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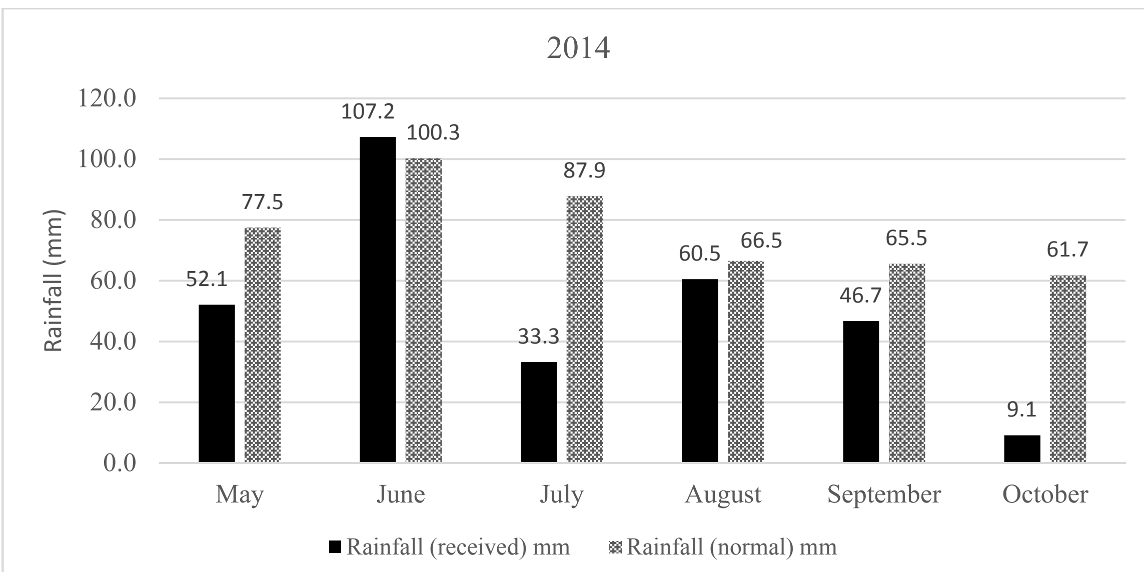

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Figure 4. Monthly average rainfall expected (30-yr 1981-2010 normal, NDAWN) versus received at Casselton, ND in (a) 2013 and (b) 2014.

Weather at Casselton

At Casselton, rainfall patterns were different compared to NW22, with 2013 precipitation peaking in June, dropping to a low amount in July and August, but then increasing slightly in

September and October (Fig. 4a). In 2014, rainfall peaked in June, dropped in July, increased in August, and dropped again September through October (Fig. 4b). Both years starkly contrasted the average 30-yr expected rainfall amount, which predicted more evenly distributed rainfall patterns.

Subsurface Tile Experiment

Tile Effect on General Agronomic Traits

The first question this experiment was designed to answer was the effect of tile drainage on general agronomic traits and if there is an interaction between tile drainage and using raised beds. The ANOVA with probability levels for the measured traits is summarized in Table 5.

The results for tile effect and tile by raised beds, raised beds, and Fe-EDDHA effects at NW22 combined across 2013 and 2014 are provided in Table 6. Only IDC was significantly different between drained (2.3) and undrained (2.6) (Tables 5 and 6). The lower, expected, IDC score for the drained treatment indicates that the plants had less chlorosis expression due to the excess moisture removal by the drain tile. Table 5 indicates that the tile main effect was not significantly different for other traits because raised beds were imposed on both treatments (drained and undrained) and raised beds, like tile drainage, alleviate the effect of excessive soil moisture in the root zone. Therefore, the interaction between tile and raised beds is the most important for answering questions about the effect of these two excess moisture alleviation methods.

Table 5. Probability levels for factors in the Analysis of Variance (ANOVA) for subsurface tile, raised beds, and Fe-EDDHA by proxy effects on general agronomic traits at NW22, near Fargo, ND in 2013 and 2014.

SOV	df	Plant population	EV†	IDC	PH	PC	OC	TW	1000 KWT	Yield
						Pr > F				
Tile	1	0.52	0.18	**0.04**‡	0.66	0.12	0.65	0.53	0.33	0.16
Raised beds	1	0.16	**0.004**	0.83	0.52	0.30	0.98	**0.07**	0.66	0.108
Tile*raised beds	1	**0.02**	0.28	0.72	0.23	**0.05**	0.41	0.47	0.72	**0.09**
Iron	1	0.19	0.28	0.17	0.93	0.97	0.30	0.65	0.86	0.23
Tile*iron	1	0.31	0.26	0.16	0.20	0.18	**0.01**	0.90	0.13	0.13
Raised beds*iron	1	0.33	**0.06**	0.50	0.90	**0.02**	0.52	0.84	0.22	0.63
Tile*raised beds*iron	1	0.38	0.18	**0.09**	**0.098**	0.48	**0.004**	**0.08**	0.97	0.86
Proxy§	4	0.34	0.53	**0.006**	0.65	0.20	**0.02**	0.22	**0.002**	0.52
Tile*proxy	4	0.16	0.36	0.62	0.86	0.48	0.76	0.35	0.26	0.90
Raised beds*proxy	4	**0.02**	0.46	0.35	0.19	0.65	0.38	0.18	0.91	0.78
Tile*raised beds*proxy	4	0.61	0.65	0.62	0.33	0.50	0.80	0.28	0.83	0.94
Iron*proxy	4	0.18	0.98	0.87	0.79	0.48	0.96	0.19	0.26	0.95
Tile*iron*proxy	4	0.63	0.73	0.11	0.71	0.49	0.29	0.55	0.48	0.45
Raised beds*iron*proxy	4	0.92	0.12	0.46	0.97	**0.006**	0.21	0.63	0.32	0.73
Tile*raised beds*iron*proxy	4	0.71	0.67	0.78	0.17	**0.05**	0.55	**0.07**	**0.03**	0.19

†EV = early vigor score, IDC = iron-deficiency chlorosis score, PH = plant height, PC = protein content, OC = oil content, TW = test weight, and KWT = kernel weight.
‡Bolded numbers are significant at $\alpha \leq 0.10$ level of confidence.
§Proxy is a number system used to designate cultivars based on genetic qualities so that results can be analyzed across environments/years in the event that a cultivar has to be changed from year to year. In this case, maturity group and IDC score were considered when choosing/replacing cultivars.

Tile by Raised Beds Effects on General Agronomic Traits

The interaction between tile and raised beds indicates significance for plant population, protein content, and yield (Tables 5 and 6). A higher plant population can be associated with a higher yield, or at least an expected higher yield (Taylor et al., 1982; Wiersma, 2007). On the undrained as well as the drained area the plant population for the raised beds was significantly higher indicating a positive effect of the raised beds on early season plant population regardless of drained treatment (Table 6).

Table 6. Means for subsurface tile, raised beds, tile by raised beds, and Fe-EDDHA by cultivar effects on general agronomic traits at NW22, near Fargo, ND in 2013 and 2014.

	Plant Population	IDC†	EV‡	PH§	PC	OC	TW	1000 KWT	Yield
	plants ha-1	1-5	1-9	cm	%	%	kg m^{-3}	g	kg ha^{-1}
				Tile effect					
No tile	301 216	**2.6**	4.6	53.5	32.4	18.5	736.0	164.5	2448
Tile	289 919	**2.3***	5.2	53.8	32.4	18.6	740.1	163.0	2478
				Raised beds effect					
Flat	262 662	2.5	**4.2**	52.8	32.4	18.5	**738.7**	163.7	2383
Raised beds	328 473	2.5	**5.6****	54.5	32.4	18.5	**737.4**‡‡	163.8	2543
				Tile x raised beds effect					
No tile x flat	269611b	2.6	3.7	51.4	32.5a	18.5	737.0	164.1	2289c
No tile x raised beds	332822a	2.6	5.5	55.5	32.4b	18.5	734.9	164.9	2607a
Tile x flat	255713b	2.4	4.8	54.2	32.3c	18.6	740.3	163.3	2477b
Tile x raised beds	324125a	2.3	5.6	53.4	32.4b	18.5	739.9	162.3	2478b
LSD 0.10	45290	ns¶	ns	ns	0.03	ns	ns	ns	77
				Tile x raised beds x Fe-EDDHA effect					
No tile x flat x no Fe-EDDHA	292654	2.8ab	3.8	51.6b	32.4	18.6a	735.5ab	161.9	2218
No tile x flat x Fe-EDDHA	246568	2.4ab	3.6	51.2b	32.6	18.4b	738.5ab	166.3	2360
No tile x raised beds x No Fe-EDDHA	353623	2.9a	5.4	54.9ab	32.4	18.5ab	735.8ab	165.0	2546
No tile x raised beds x Fe-EDDHA	312020	2.4ab	5.7	56.3a	32.4	18.6a	734.0b	164.9	2669
Tile x flat x no Fe-EDDHA	270059	2.6ab	4.9	54.2ab	32.4	18.6a	740.8a	163.0	2487
Tile x flat x Fe-EDDHA	241367	2.2b	4.7	54.3ab	32.3	18.6a	739.8ab	163.7	2468
Tile x raised beds x No Fe-EDDHA	344119	2.4ab	5.7	54.1ab	32.5	18.5ab	739.0ab	164.4	2511
Tile x raised beds x Fe-EDDHA	304130	2.2b	5.5	52.7b	32.4	18.5ab	740.8a	160.8	2446
LSD 0.10	ns	0.7	ns	2.5	ns	0.2	6.7	ns	ns
				Fe-EDDHA effect††					
No Fe-EDDHA	315114	2.7	5.0	53.7	32.4	18.6	737.8	163.6	2440
Fe-EDDHA	276021	2.3	4.8	53.6	32.4	18.5	738.3	163.9	2486

*,**,*** significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$) respectively. ‡‡ denotes significance at ($p \leq 0.10$).
†IDC Iron Deficiency Chlorosis score was based on a scale of 1-5 (1 = no IDC symptoms, 5 = severe IDC symptoms/plant death) established by Goos and Johnson (2008).
‡EV = early vigor score (July 1, 2013 and June 25, 2014). Vigor score was based on a scale of 1-9 (1 = very poor vigor, 9 = excellent vigor). Only early vigor scores are recorded due to IDC symptoms dissipating over time.
§PH = plant height, PC = protein content, OC = oil content, TW = test weight, and KWT = kernel weight.
¶ns = non-significant with Fisher's F-protected LSD. Numbers followed by the same letter, within the same column, within each respective interaction, are non-significantly different.
††Fe-EDDHA was seed-applied at a rate of 3.36 kg ha^{-1}.

This interaction also helps explain why a lower plant population was found on the drained treatment. The plant population was 269 611 plants ha^{-1} for the control (i.e. No tile-flat) and only 255 713 plants ha^{-1} for Tile-flat. Comparing the latter number with No tile-raised beds (332 822 plants ha^{-1}) might suggest that raised beds is a better treatment for early plant establishment compared to planting on flat land. The plant population for Tile-raised beds (324 125 plants ha^{-1}) was significantly higher compared to No tile-flat and Tile-flat. However, the plant population for Tile-raised beds was not as numerically different compared with to No tile-raised beds.

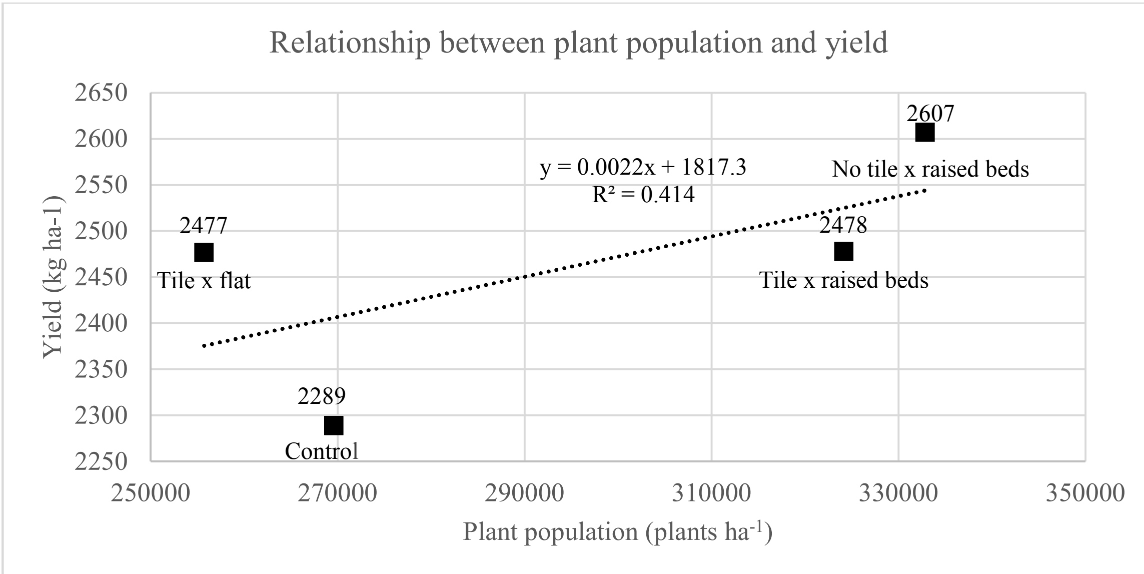
The yield for the control was significantly lower (2289 kg ha^{-1}) compared to Tile-flat (2477 kg ha^{-1}). This suggests that Tile, without the addition of raised beds is effectively able to remove the stress of excess water. However, No tile-raised beds resulted in a yield that was significantly higher (2607 kg ha^{-1}) compared to all other treatments: Tile-raised beds, Tile-flat and the control (2478 kg ha^{-1}, 2477 kg ha^{-1}, and 2289 kg ha^{-1}, respectively). The reason why No tile-raised beds was better than Tile-raised beds was likely due to over-alleviation of flood stress (i.e. too dry) combined with low rainfall during reproductive stages and suggests that in order to alleviate excess water stress, especially under the drier than normal environmental conditions observed in this experiment, one should select either subsurface tile drainage or raised beds tillage, but not both.

This is supported by the research conducted by Hoppe et al. (2017), which observed significantly higher soil temperatures along with significantly higher root mass in the raised beds under undrained conditions at NW22. However, plant population was found to be significantly lower on raised beds and there were no noticeable yield differences between the raised beds and control. The authors concluded that the raised beds were not having a negative effect on yield under the drier than normal conditions experienced in 2012.

Another solution to reducing waterlogging, but in a way that would not dry out the soil in drier than normal years, would be to closely monitor water table levels and adjust the stop-blocks in the drainage control structures accordingly. Given the high expense of tile drainage installation (Cihacek, et al., 2012) and the results of this research, raised beds would be a good option for alleviating excess water stress, but also a way to harvest water in dry years by using proper row orientation (i.e. perpendicular to slope of land).

A higher plant population with the same row spacing would result in plants being closer together, which could increase plant to plant competition. However, the main attribute that was used to score vigor was plant bushiness, a component of which is leaf-area index (LAI). Even though this experiment did not find a strong relationship between vigor and plant population, previous literature has. A study conducted on row spacing found LAI to be significantly higher for plants spaced further apart, but significantly higher light interception for plants that were closer together (i.e. more plants $unit^{-1}$ soil). As a result, significantly higher yield was found for the plants that were closer together (Taylor et al., 1982).

Another planting rate study conducted with soybean found that higher plant populations significantly increased yield. In some cases, these increased plant populations also significantly decreased IDC symptoms, although the results were not consistent (Naeve, 2006). A similar relationship (r^2=0.41) between plant population and yield can be observed in the Tile x Raised bed interaction (Fig. 5a). The IDC scores had little to no relationship (r^2=0.0012) to plant population (Fig. 5b). This lack of relationship as well as significance for IDC score was likely due to the tile and raised beds effects masking the effect of Fe-EDDHA application.



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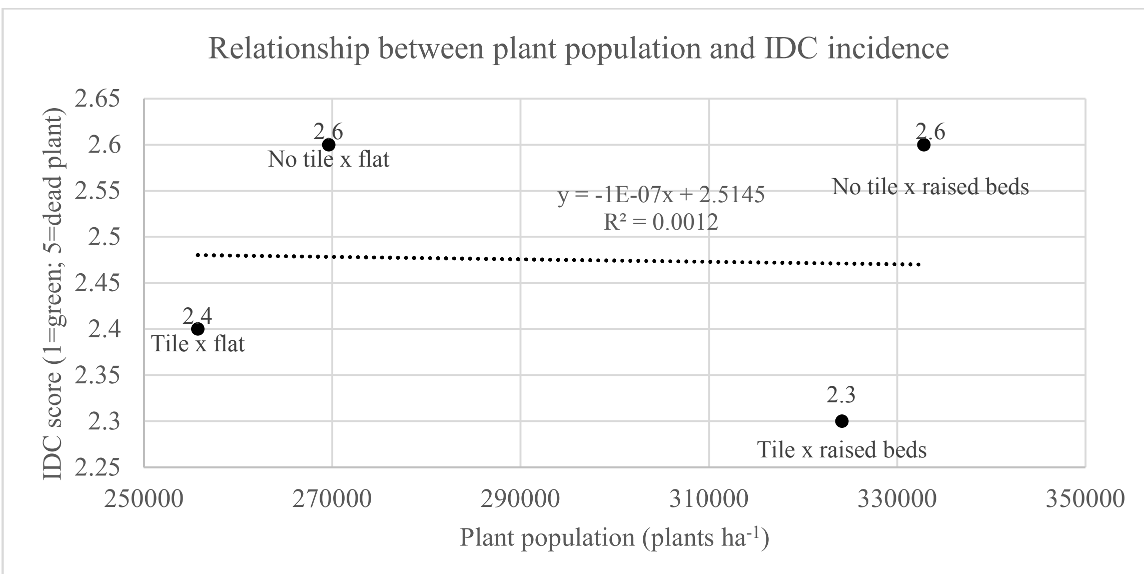

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Figure 5. Relationship between plant population and (a) yield and (b) IDC score for tile x raised beds interaction at NW22 in 2013 and 2014.

Protein content was also significantly different with the Tile x raised bed interaction. However, the differences were too small to imply any practical use for farmers. Given the rain patterns experienced both years and given both rainfall and the water table were low during pod fill each year, it can be speculated that the presence of both tile and raised beds made the soil too dry and in turn resulted in mild drought stress. Drought stress has been linked to a decrease in protein content in soybeans in other research (Nakagawa, 2018).

Table 5 shows the significant differences between the cultivars (proxy) for IDC score, oil content and 1000-kernel weight. This was expected as cultivars were selected based on IDC expression. The results were 2.6, 2.3, 2.7, 2.3, and 2.5 for proxy 1, 2, 3, 4, and 5, respectively. Aside from proxy 2 and 4, which were documented to be more tolerant to IDC, these results followed previous rankings (Table 2). Similar relationships between previously documented IDC scores and field scores were observed among cultivars used by Hoppe (2013) when averaging IDC scores across cultivars.

Tile x Raised Beds x Fe-EDDHA Effect on General Agronomic Traits

Significant differences were observed for IDC, plant height, oil content, and test weight for the Tile x raised beds x Fe-EDDHA interaction (Table 5). The interaction of all three methods, Tile-raised beds-Fe-EDDHA and Tile-flat-Fe-EDDHA, resulted in significantly lower IDC scores (both 2.2) compared to the control (2.8). However, the highest IDC score was for No tile-raised beds-no Fe-EDDHA (2.9). Although not all IDC score differences were significant, most of the interactions produced numerically lower IDC scores with Fe-EDDHA application versus without Fe-EDDHA (Table 6). For example, in the previously discussed Tile-raised beds interaction, No tile-raised beds was found to have the highest yield with no additional benefit of adding the Tile effect. Based on this information, one might expect an interaction such as Tile-raised beds-Fe-EDDHA to have a similar lack of benefit. On the contrary, Tile-raised beds-Fe-EDDHA resulted in one of the lowest IDC scores (mentioned above). This suggests a significant added benefit of Fe-EDDHA application.

Another added benefit of Fe-EDDHA is observed with plant height. No tile-raised beds-Fe-EDDHA produced the tallest plants (56.3 cm), which were significantly higher than the control (51.6 cm) and also significantly higher than Tile-raised beds-Fe-EDDHA (52.7 cm) (Table 7). One might expect a lower IDC score (2.2) to result in a taller plant, since IDC largely affects new growth structures on soybean plants. However, since plant height was measured immediately before harvest (compared to the IDC score, which was measured early season), the negative effect of over-alleviation of flood stress caused by both the tile and raised beds combined is likely what caused a lower plant height.

Furthermore, No tile-raised beds-Fe-EDDHA produced numerically taller plants compared to No tile-raised beds-no Fe-EDDHA, again suggesting an added benefit from Fe-EDDHA application. In addition to this, No tile-flat-Fe-EDDHA resulted in a numerically lower IDC score (2.4) but significantly shorter plants (51.2 cm) compared to No tile-raised beds-no Fe-EDDHA (2.9 and 54.9 cm, respectively). The longer-term benefit of raised beds compared to Fe-EDDHA application observed here further supports the finding of Goos and Johnson (2000 and 2001) that Fe-EDDHA is only a partial solution. Further research should be conducted to assess the potential benefit of mid- and late season Fe-EDDHA application.

Oil content was found to be significantly different between some treatments, but the differences were too small to be of any practical use to producers (Tables 5 and 6).

Tile Effect on Biomass Traits

Differences in biomass traits were observed in this experiment, namely plant and root biomass (referred to as aboveground and belowground biomass, respectively). The Analysis of Variance (ANOVA) and means are presented in Tables 7 and 8.

Table 7. Probability levels for factors in the Analysis of Variance (ANOVA) for subsurface tile, raised beds, and Fe-EDDHA by proxy effects on biomass traits at NW22, near Fargo, ND in 2013 and 2014.

SOV	df	TB†	PB	RB	TPB	IPB	IRB
				Pr > F			
Tile	1	0.76	0.70	0.93	0.53	0.49	0.86
Raised beds	1	0.14	0.24	0.15	0.47	0.47	0.54
Tile*raised beds	1	**0.07‡**	**0.06**	0.16	0.11	0.10	0.33
Fe-EDDHA	1	0.58	0.58	0.60	0.61	0.61	0.65
Tile*Fe-EDDHA	1	0.15	**0.048**	0.80	0.72	0.71	0.81
Raised beds*Fe-EDDHA	1	1.00	0.86	0.42	0.79	0.77	**0.08**
Tile*raised beds*Fe-EDDHA	1	**0.007**	**0.045**	0.31	0.42	0.39	0.91
Proxy§	4	0.23	0.23	0.32	0.30	0.27	0.53
Tile*proxy	4	0.25	0.21	0.38	0.55	0.56	0.33
Raised beds*proxy	4	0.77	0.78	0.31	0.53	0.55	0.28
Tile*raised beds*proxy	4	0.66	0.66	0.86	0.87	0.91	0.72
Fe-EDDHA *proxy	4	0.86	0.84	0.72	0.35	0.36	**0.058**
Tile*Fe-EDDHA *proxy	4	0.16	0.19	0.41	0.26	0.26	0.61
Raised beds*Fe-EDDHA *proxy	4	0.29	0.27	0.34	**0.020**	**0.02**	0.36
Tile*raised beds*Fe-EDDHA *proxy	4	0.72	0.73	0.63	0.78	0.82	0.33

†TB=total biomass per sample size (1 m of buffer row), PB = total aboveground biomass, RB = total root biomass, TPB = total biomass per plant, IPB = above ground biomass per plant, IRB = biomass per root.
‡Bolded numbers are significant at $\alpha \leq 0.10$ level of confidence.
§Proxy is a number system used to designate cultivars based on genetic qualities so that results can be analyzed across environments/years in the event that a cultivar has to be changed from year to year. In this case, maturity group and IDC score were considered when choosing/replacing cultivars.

Tile by Raised Beds Effect on Biomass

An interaction between tile and raised beds was observed for biomass traits (Table 7), with Tile-raised beds having a significantly higher total biomass and aboveground biomass (348.5 g and 315.9 g, respectively) compared to the control (316.6 g and 288.2 g) (Table 8). However, similar to what was observed for general agronomic traits, Tile-raised beds produced significantly lower total biomass and above ground biomass (348.5 g and 315.9 g, respectively) compared to No tile-raised beds (373.3 g and 338.3 g) as well compared to Tile-flat (367.3 g and 337.9 g). Tile-flat produced significantly higher total biomass and aboveground biomass compared to the control, but it was not as large of a difference as No tile-raised beds (373.3 g and 338.3 g) compared to the control (316.6 g and 288.2 g). This further supports the conclusion that raised beds are a better method of excess moisture alleviation compared to subsurface tile drainage under the field conditions observed in this experiment. The area with open tile (lower water table) combined with raised beds (elevated root zone) may have been too dry given the environmental conditions. Tile-raised beds may have provided too much alleviation of excess water stress.

Table 8. Means for subsurface tile, raised beds, tile by raised beds, and Fe-EDDHA by cultivar effects on biomass traits at NW22, near Fargo, ND in 2013 and 2014.

	TB†	PB	RB	TPB	IPB	IRB
	---g---					
			Tile effect			
No tile	345.0	313.3	31.7	19.2	17.5	1.7
Tile	357.9	326.9	31.0	21.2	19.5	1.8
			Raised beds effect			
Flat	342.0	313.1	28.9	22.6	20.8	1.8
Raised beds	360.9	327.1	33.8	17.9	16.2	1.6
			Tile by raised beds			
No tile x flat	316.6b	288.2c	28.4	20.3	18.7	1.7
No tile x raised beds	373.3a	338.3a	35.0	18.1	16.4	1.6
Tile x flat	367.3a	337.9a	29.4	24.8	22.9	1.9
Tile x raised beds	348.5b	315.9b	32.6	17.7	16.1	1.6
LSD 0.10	17.4	15.7	ns‡	ns	ns	ns
			Fe-EDDHA§			
No iron chelate	356.3	324.3	32.0	21.2	17.6	1.8
Iron chelate	346.6	315.9	30.7	19.2	19.5	1.6

†TB = total biomass per sample size (90 cm of buffer row), PB = plant biomass per sample, RB = root biomass per sample, TPB = total biomass per plant, IPB = biomass per plant, IRB = biomass per root.
‡ns=non-significant with Fisher's F-protected LSD. Numbers followed by the same letter, within each column, within each respective interaction, are non-significantly different.
§Fe-EDDHA was seed-applied at a rate of 3.36 kg ha^{-1}.

Raised Beds and Fe-EDDHA Application Experiment

For the combined environment analysis, NW22 was considered two environments within each year: environment one un-drained and environment two drained. The raised beds and Fe-EDDHA experiment was analyzed across six environments (NW22 drained and undrained 2013 and 2014 and Casselton 2013 and 2014). The second question this experiment was designed to answer was whether the use of raised beds and Fe-EDDHA seed application were effective IDC management methods and what the effect of these treatments was on seed yield. The ANOVA and means for the raised beds and Fe-EDDHA effects are presented in Tables 9 and 10.

Table 9. Probability levels for factors in the Analysis of Variance (ANOVA) for raised beds and Fe-EDDHA by proxy effects on general agronomic traits averaged over six environments at NW22, near Fargo, ND and Casselton in 2013 and 2014.

SOV	df	Plant population	EV†	IDC	PH	PC	OC	TW	500 KWT	Yield
						Pr > F				
Raised beds	1	**0.0006**‡	**0.002**	0.63	0.16	0.88	0.43	0.14	0.77	**0.07**
Fe-EDDHA	1	**0.01**	0.62	**0.056**	0.94	0.70	0.97	0.29	0.95	0.40
Raised beds x Fe-EDDHA	1	0.7208	0.65	0.77	0.50	0.58	0.20	0.40	0.16	0.95
Proxy	4	**0.004**	0.36	**0.007**	0.17	**0.0001**	**0.0001**	**0.04**	**0.0001**	**0.008**
Raised beds x proxy	4	0.4584	0.96	0.54	0.59	0.27	0.81	0.31	0.93	0.99
Fe-EDDHA x proxy	4	**0.02**	0.80	0.88	0.70	0.15	0.67	0.78	0.70	0.60
Raised beds x Fe-EDDHA x proxy	4	0.5387	0.91	0.47	0.93	0.32	0.47	0.45	0.79	0.61

†EV = early vigor score, IDC = iron-deficiency chlorosis score, PH = plant height, PC = protein content, OC = oil content, TW = test weight, and KWT = kernel weight.
‡Bolded numbers are significant at $\alpha \leq 0.10$ level of confidence.
§Proxy is a number system used to designate cultivars based on genetic qualities so that results can be analyzed across environments/years in the event that a cultivar has to be changed from year to year. In this case, maturity group and IDC score were considered when choosing/replacing cultivars.

Raised Beds Effect

When raised beds observations were averaged across iron treatments and cultivar (proxy), significance was found for plant population, early vigor score and yield (Table 9). The point of raised beds is to create an environment for the roots of a crop that is less compacted and saturated. Bulk density has been shown to be lower in raised bed tillage areas compared to flat land (Bakker et al., 2005; Kirnak et al., 2017). As a result, waterlogging potential was found to be lower on raised beds versus flat land and the water level in the flat land was observed to be closer to the soil surface more often than in raised beds areas (Bakker, et al., 2005). Similar observations were made at NW22 and Casselton, especially early in the season when rainfall amounts were higher (Figs. 3 and 4).

Table 10. Means for raised beds and Fe-EDDHA by cultivar effects on general agronomic traits averaged across six environments at NW22, near Fargo, ND and Casselton in 2013 and 2014.

Treatment	Plant Population	IDC†	EV‡	PH§	PC	OC	TW	1000 KWT	Yield
	plants ha^{-1}	1-5	1-9	cm	-------------%	--------------	kg m^{-3}	g	kg ha^{-1}
					Raised bed effect				
Flat	260674	2.3	4.4	56	32.5	18.2	741.1	167.0	2722
Raised beds	331058***	2.3	5.9**	58	32.5	18.2	738.9	167.1	2893‡‡
					Fe-EDDHA¶				
No Fe-EDDHA	310735	2.4	5.2	57	32.5	18.2	739.7	167.0	2787
Fe-EDDHA	280998**	2.2*	5.1	57	32.5	18.2	740.3	167.1	2828

*,**,*** significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$) respectively. ‡‡ denotes significance at ($0.10 > p > 0.05$).
†IDC Iron Deficiency Chlorosis score was based on a scale of 1-5 (1 = no IDC symptoms, 5 = severe IDC symptoms/plant death) established by Goos and Johnson (2008).
‡EV = early vigor score (July 1, 2013 and June 25, 2014). Vigor score was based on a scale of 1-9 (1 = very poor vigor, 9 = excellent vigor). Only early vigor scores are presented here due to IDC symptoms dissipating over time.
§PH = plant height, PC = protein content, OC = oil content, TW = test weight, and KWT = kernel weight.
¶Fe-EDDHA was seed-applied at a rate of 3.36 kg ha^{-1}.

Plant population was found to be significantly higher on raised beds (331 058 plants ha^{-1}) compared to the control (260 674 plants ha^{-1}) (Table 10). Hoppe (2013) conducted a field study in the RRNV with soybean and observed lower IDC symptoms with the raised beds treatment. When analyzing the raised beds effect, Hoppe documented a significant reduction in plant population, contrary to the findings of this study. However, one of the years in which Hoppe conducted his research was 2012, which had very low rainfall. Therefore, the author speculated that the lower plant population was due to raised beds being too dry (Hoppe, 2013 and 2017). One issue that can occur when clayey soil dries out is soil crusting. If this occurs during the VE soybean growth stage, plants may have a difficult time emerging properly.

Greater plant population would likely result in fuller rows. This aspect was observed when assessing vigor scores, which were observed to be significantly higher on raised beds versus flat land (5.9 and 4.4, respectively) (Table 10). Flood stress has been shown to negatively affect chlorophyll production. Oosterhuis et al. (1989) found that stomatal conductance significantly decreased in response to excess water stress. The physiology of stomatal conductance in relations to CO_2 absorption is that the plant can only absorb CO_2 in solution (i.e. the leaf surface must be wet at stomatal opening). This happens via transpiration. Roots will stop taking up water, however, to avoid over absorption of CO_2 (Salisbury and Ross, 1992). Oosterhuis et al. (1989) speculated that the root systems of the two soybean cultivars observed, resisted taking up water in response to flood stress, which in turn led to stomatal closure, increased water saturation of the stomatal area, and decreased photosynthesis.

Sammons et al. (1980) observed a correlation between plant height and stem dry-weight at maturity (SDWM). Whatever plant structure was being stressed (growth-stage dependent), that part would be reduced in development and thus increasing SDWM, which had a large impact on reducing yield. This relates to the finding of Zhang et al. (2017), where soybeans under iron-deficiency stress stopped producing biomass in order to increase antioxidant activity to cope with the iron stress. These two research articles further support the significantly lower vigor scores recorded on flat land, where excess water stress occurred.

Bakker et al. (2005) observed yields to be significantly higher on raised beds for all crops, but only in certain locations. These findings coincide with what was observed in this experiment. Yield was found to be significantly higher on raised beds (2893 kg ha^{-1}) compared to flat land (2722 kg ha^{-1}) when averaged across locations/years (Table 10), but was non-significant in certain individual locations/years, namely both years at Casselton (data not shown).

In the RRNV, soil temperatures are low during the springtime due to freeze and thaw cycles. Studies have shown soil temperature to have an impact on soybean production as well as the production of other crops. More water runoff and lower bulk density was observed on raised beds compared to flat land in a study conducted on a clayey soil in Western Australia (Bakker et al., 2005). Furthermore, temperatures can affect seed weight. The physics behind raised beds is that there is greater soil surface area after they are made and therefore there is more area for heat conductance from solar radiation. This would result in an increase in soil temperature, mainly during early season before any shading occurs due to canopy development (Fig. 2). Higher air temperatures were found to increase soybean seed weight and in turn yield in research conducted at Purdue University (Casteel, 2010). In this experiment, 1000-kernel weight was not-significantly different on raised beds (Tables 9 and 10).

Fe-EDDHA Application Effect

When Fe-EDDHA was applied, plant population and IDC were both found to be significantly lower (Table 10). Initially, the lower plant population was unexpected given that iron-chelate has been shown to improve soybean performance (Wiersma, 2005 and 2007; Goos and Johnson 2000 and 2001). However, after conducting germination tests, a delay in germination due to Fe-EDDHA seed treatment was found. Furthermore, when mini-plot trials were conducted to further study the effect of Fe-EDDHA application, variability in plant population response to Fe-EDDHA as well as the Cultivar x Fe-EDDHA interaction was found, although non-significant. Mini plots are discussed in more detail later.

The chemistry behind the Fe-EDDHA compound is that it reduces the iron in the soil that is not plant available, usually the trivalent oxidative state of Fe(III), to plant available form, usually the bivalent oxidative state of Fe(II) (Schenkeveld et al., 2008; Zhang et al., 2017). Adverse soil conditions, such as water-logging and a high $CaCO_3$ content (i.e. high pH) can lead to iron being plant unavailable (Schenkeveld et al., 2008; Hoppe, 2013; Gamble et al., 2014). Schenkeveld et al. (2008), after testing Fe-EDDHA soil application at different rates, concluded that higher rates of Fe-EDDHA in solution resulted in lower incidences of IDC. Significant differences in response to [Fe] were observed on soils that were more prone to IDC (i.e. more clayey soils). Furthermore, yield (measured in terms of aboveground dry-weight biomass) was observed to be higher with higher [Fe].

There was no significant Fe-EDDHA by cultivar interaction aside from plant population (Table 10). This lack of significance was expected given the variability observed on the mini-plots (discussed later). Furthermore, previous research has concluded that Fe-EDDHA effectiveness was based mostly on cultivar response to application (Goos and Johnson, 2000 and 2001; Schenkeveld et al., 2008).

Significance was observed for biomass traits on raised beds, namely total biomass, total root biomass, total biomass per plant, aboveground biomass per plant, and root biomass per plant. No significance was observed for Fe-EDDHA application (Table 11).

Raised Beds Effect on Biomass

Table 11. Probability levels for factors in the Analysis of Variance (ANOVA) for raised beds effect on biomass traits averaged over six environments NW22, near Fargo, ND and Casselton in 2013 and 2014.

SOV	df	TB†	PB	RB	TPB	IPB	IRB
		Pr > F					
Raised beds	1	**0.098**‡	0.13	**0.005**	**0.04**	**0.04**	**0.06**
Fe-EDDHA	1	0.71	0.84	0.29	0.57	0.58	0.40
Raised beds x Fe-EDDHA	1	0.56	0.48	0.69	0.57	0.54	0.46
Proxy§	4	**0.06**	**0.07**	**0.06**	**0.03**	**0.03**	**0.05**
Raised beds x proxy	4	0.55	0.62	**0.07**	**0.05**	**0.06**	**0.09**
Fe-EDDHA x proxy	4	0.95	0.96	0.79	**0.07**	**0.08**	**0.04**
Raised beds x Fe-EDDHA x proxy	4	0.48	0.40	0.79	0.12	0.11	0.49

†TB = total biomass per sample size (1 m of buffer row), PB = aboveground biomass per sample, RB = total root biomass per sample, TPB = biomass per plant, IPB = aboveground biomass per plant, IRB = biomass per root.
‡Bolded numbers are significant at $\alpha \leq 0.10$ level of confidence.
§Proxy is a number system used to designate cultivars based on genetic qualities so that results can be analyzed across environments/years in the event that a cultivar has to be changed from year to year. In this case, maturity group and IDC score were considered when choosing/replacing cultivars.

Table 12. Means for raised beds effect on biomass traits averaged over six environments NW22, Fargo, ND and Casselton in 2013 and 2014.

	TB†	PB	RB	TPB	IPB	IRB
	---			g---		
			Raised beds effect			
Flat	**357.7**	326.9	**30.8**	**24.5**	**22.5**	**2.0**
Raised beds	**392.6**‡‡	357.5	**35.1*** **	**19.3***	**17.6***	**1.7**‡‡
			Fe-EDDHA ‡			
No Fe-EDDHA	376.8	343.0	33.8	22.4	20.6	1.9
Fe-EDDHA	373.5	341.3	32.2	21.4	19.6	1.8

*,**,*** significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$) respectively. ‡‡ denotes significance at ($0.10 > p > 0.05$).
†TB = total biomass per sample (1 m of buffer row), PB = aboveground biomass per sample, RB = total root biomass per sample, TPB = biomass per plant, IPB = aboveground biomass per plant, IRB = biomass per root.
‡Iron chelate was seed-applied at a rate of 3.36 kg ha^{-1}.

Total biomass was 392.6 g and 357.7 g for raised beds and flat land, respectively. The total root biomass was 35.1 g and 30.8 g for raised beds and flat land, respectively (Table 12). The root mass data coincides with the findings of Hoppe et al. (2013 and 2017). Schendkeveld et al. (2007) measured yield in terms of aboveground dry-weight biomass. The authors speculated that better root development played a role in the soybean plant's ability to take up soil-applied Fe-EDDHA more efficiently, thus leading to the disappearance of IDC symptoms over time (Schenkeveld et al., 2007). A greater biomass would suggest more leaf and shoot area, thus more chlorophyll content. The significantly higher vigor score observed on raised beds (Table 12) supports two of the observations made on biomass.

The first is greater biomass would suggest more bushiness (i.e. plant/leaf tissue area), which was used to assess vigor. The second is the root mass was significantly higher on raised beds, thus leading to significantly higher total biomass overall. Since raised beds are known to alleviate excess water stress, this stress alleviation would be expected to result in plants with greater biomass. Greater biomass was observed in terms of plant height on less irrigated (i.e. less saturated) plots (Taylor et al., 1982). In the experiment reported here, plant height was also incorporated in the vigor scoring.

A cultivar trial studying the iron-efficiency of IDC tolerant soybean cultivars found that plants with high root mass were able to absorb iron more efficiently. The researchers speculated that this was due to greater root surface area and therefore more absorption area. The authors further concluded that iron efficient cultivars (i.e. IDC tolerant soybean) were better able to excrete reductase chemicals to reduce Fe(III) to Fe(II) (i.e. naturally chelate the Fe) than susceptible cultivars (Zhang et al., 2017).

Cultivar (proxy) response was found to be significant for all biomass traits (Table 11). Means for these biomass traits are presented in Table 13. As proxy 1 and 5 are means for two cultivars chosen specifically based on their respective IDC scores and maturity ratings, the observations for these proxies will not be discussed.

Table 13. Means for cultivar effect on biomass traits averaged across raised beds and Fe-EDDHA treatments at six environments at NW22 near Fargo, ND and Casselton in 2013 and 2014.

Cultivar	Proxy	Final plant population†	TB‡	PB	RB	TPB	IPB	IRB
			g					
90Y42/90Y50	1	303727 a	362.0 b	331.3 b	30.7 b	20.6 b	18.9 c	1.7 b
90Y70	2	295583 a	359.4 b	328.0 b	31.4 b	20.6 b	18.8 c	1.7 b
DSR-0747	3	319940 a	382.8 ab	349.3 ab	33.4 b	19.6 b	18.0 c	1.6 b
HS 01RY02	4	248286 b	363.7 b	332.5 b	31.2 b	24.4 a	22.4 a	2.0 a
6088/0906R2	5	311796 a	408.0 a	369.8 a	38.2 a	24.5 a	22.3 b	2.2 a
LSD 0.10		29391	30.2	26.5	4.6	3.1	2.8	0.3

†Final plant population data is an approximation based on initial plant population.
‡TB = total biomass per sample (1 m of buffer row), PB = aboveground biomass per sample, RB = total root biomass per sample, TPB = biomass per plant, IPB = aboveground biomass per plant, IRB = biomass per root.
§ns= non-significantly different at α=0.10 level of confidence.

The cultivar HS 01RY02 produced the most aboveground biomass per plant (22.4 g) of the cultivars that were available both years. Goos and Johnson (2001) observed a similar cultivar response to treatments when comparing the IDC scores and yields of three soybean cultivars. Overall, one of the cultivars was found to be the most dominant for both lowest IDC score and highest yield (Goos and Johnson, 2001).

Relating Goos and Johnson's (2001) findings to the biomass observations in both Tables 12 and 13, cultivar choice had an important impact on treatment effectiveness. In Table 12, the biomass observations for individual plants (total biomass per plant, aboveground biomass per plant, and root biomass per plant) were all found to be significantly lower on raised beds (19.3 g, 17.6 g, and 1.7 g) compared to flat land (24.5 g, 22.5 g, and 2.0 g). This initially unexpected difference could possibly be explained by the greater plant population observed on raised beds. If there is a greater plant population, then there is likely more plant light energy interception due to plants being closer together, as demonstrated by Taylor et al. (1982) in a row spacing study that observed higher light interception in narrow row spacing compared to wide row spacing. However, this same study also observed greater leaf-area index (i.e. larger trifoliolates) on plants spaced further apart (Taylor et al., 1982). Since foliage development would have a direct impact on biomass data, the higher plant population in the raised beds area compared to flat land may have had smaller trifoliolate leaves (not measured in this experiment). This is contrary to studies that have observed that plants such as soybean, when planted closer together, have benefited neighboring root systems due to increased rhizosphere activity in the intertwining root structures (Sylvia et al., 1999; Wiersma, 2007). Rhizobial activity was not studied in this experiment.

Fe-EDDHA by Cultivar Mini Plot Experiment

Two locations were selected each year to further assess the effect of Fe-EDDHA on IDC in soybean. The analysis of variance and means for each of these locations are presented in Tables 14 and 15. Mini-plot locations were analyzed individually because significant differences were found at each location as well as when results were averaged across locations/years (Table 14). Furthermore, contrasting observations were made across years (Table 15).

Table 14. Probability levels for factors in the Analysis of variance (ANOVA) for iron effect at all mini plot locations at Arthur, Leonard in 2013 and Galchutt and Harwood in 2014.

SOV	df	Plant population (initial)	IDC†	Early Vigor	Mid Vigor	Late Vigor	Biomass (total)	Biomass (per plant)	Plant population (final)
		Pr > F							
		Arthur							
Cultivar	1	**0.0007**‡	**0.0001**	**0.07**	**0.008**	**0.03**	**0.001**	**0.0001**	**0.003**
Fe-EDDHA	4	**0.006**	0.50	**0.001**	**0.0008**	**0.0001**	**0.0001**	**0.019**	**0.008**
Fe-EDDHA x cultivar	4	0.15	0.23	0.24	0.22	**0.002**	**0.002**	**0.002**	0.52
		Leonard							
Cultivar	1	**0.004**	**0.03**	0.20	**0.0007**	**0.0001**	**0.0005**	**0.0001**	**0.005**
Fe-EDDHA	4	0.20	**0.0001**	0.95	**0.007**	**0.02**	**0.0001**	**0.0001**	**0.02**
Fe-EDDHA x cultivar	4	0.49	0.24	0.59	0.90	0.43	0.59	0.40	0.99
		Galchutt							
Cultivar	1	0.76	**0.05**	0.99	0.70	0.87	0.90	0.49	0.76
Fe-EDDHA	4	**0.02**	**0.002**	**0.04**	**0.03**	0.38	**0.001**	0.28	**0.02**
Fe-EDDHA x cultivar	4	0.34	0.40	0.7719	0.44	0.39	0.6168	0.14	0.34
		Harwood							
Cultivar	1	**0.01**	**0.06**	**0.03**	0.51	0.30	0.28	**0.01**	**0.01**
Fe-EDDHA	4	0.19	**0.0007**	0.63	0.67	0.91	0.44	**0.02**	0.19
Fe-EDDHA x cultivar	4	0.38	0.57	0.64	0.34	0.73	0.68	0.63	0.38
		All locations							
Proxy§	1	0.14	**0.03**	0.15	0.53	0.13	0.54	**0.03**	0.15
Fe-EDDHA	4	0.65	**0.0001**	0.63	**0.03**	**0.03**	0.21	**0.002**	1.00
Fe-EDDHA x proxy	4	0.88	0.24	0.25	0.62	0.14	0.94	0.14	0.94

†IDC = iron-deficiency chlorosis score.
‡Bolded numbers are significant at $\alpha \leq 0.10$ level of confidence.
§Proxy is a number system used to designate cultivars based on genetic qualities so that results can be analyzed across environments/years in the event that a cultivar has to be changed from year to year. In this case, maturity group and IDC score were considered when choosing/replacing cultivars.

Table 15. Means for Fe-EDDHA effect on general agronomic traits and aboveground biomass at Arthur and Leonard, ND (2013) and Galchutt and Harwood, ND (2014).

	Plant population (initial)	IDC†	Early vigor	Mid vigor	Late vigor	Biomass (total)	Biomass (per plant)	Plant population (final)
	plants ha^{-1}	1-5	------------------1-9		------------------	---------- g	----------	plants ha^{-1}
				Arthur				
No Fe-EDDHA	250915	1.9	4.6	5.5	5.5	71.8	2.6	239287
Fe-EDDHA‡	289685**	1.8	5.4***	7.0***	6.6*	94.0***	2.8*	287489**
				Leonard				
No Fe-EDDHA	281408	2.4	5.0	4.4	4.3	58.2	2.0	256933
Fe-EDDHA	307109	1.7***	5.0	5.9**	5.2*	92.1***	2.8***	292223*
				Galchutt				
No Fe-EDDHA	248292	2.1	5.1	6.0	5.7	229.1	8.7	245312
Fe-EDDHA	199505*	1.8**	4.5*	4.9*	5.3	191.5***	9.6	197111*
				Harwood				
No Fe-EDDHA	350658	2.4	4.5	5.7	5.8	153.9	4.1	346450
Fe-EDDHA	314939	1.6***	4.7	5.9	5.8	163.6	5.4*	311159
				All locations				
No Fe-EDDHA	282818	2.2	4.8	5.4	5.3	128.3	4.4	271996
Fe-EDDHA	277809	1.7***	4.9	5.9*	5.7*	135.3	5.2**	271996

*,**,*** significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$) respectively. ‡‡ denotes significance at ($0.10 > p > 0.05$).
†IDC Iron Deficiency Chlorosis score was based on a scale of 1-5 (1 = no IDC symptoms, 5 = severe IDC symptoms/plant death) established by Goos and Johnson (2008).
‡Fe-EDDHA was seed-applied at a rate of 3.36 kg ha^{-1}.

For example, the initial plant population at Arthur (2013) was observed to be significantly higher with Fe-EDDHA, but at Galchutt (2014), initial plant population was significantly lower. This was expected due to the germination test results and partially explains the reduced plant population documented in the main trials. Final plant population was found to be significantly higher at two of the four locations. At Arthur, the initial plant population was significantly higher with the Fe-EDDHA versus the control (289 685 plants ha^{-1} and 250 915 plant ha^{-1}, respectively). The final plant population and biomass for Arthur was also significantly higher with Fe-EDDHA treatment (Table 15).

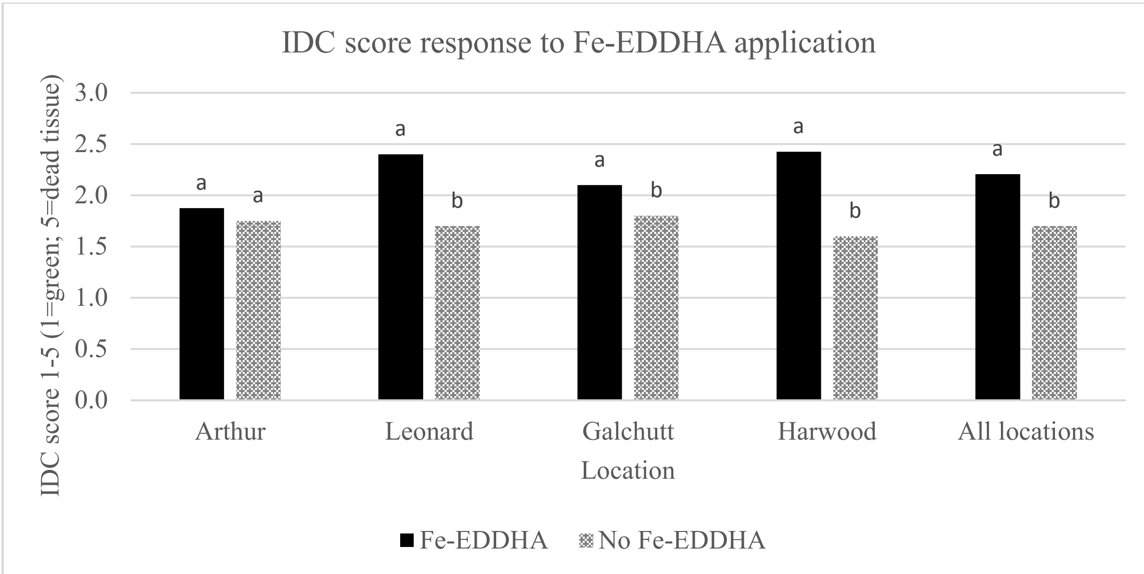
At Leonard, the initial plant population was non-significantly different between the two Fe-EDDHA treatments. However, the final plant population was significantly higher with Fe-EDDHA (292 223 plants ha^{-1}) compared to the control (256 993 plants ha^{-1}). The cause of this significance was a greater reduction in plant population on the control plots compared to the treated plots (a reduction of approximately 25 000 plants ha^{-1} and 15 000 plants ha^{-1}, respectively). Although yield measurements were not taken in the mini plots, previous research has measured yield in terms of biomass, which is largely influenced by chlorophyll content. Schenkeveld et al. (2008) observed a significant increase in dry weight biomass for soybean grown in aqueous solution with high [Fe] compared to low [Fe]. The soybean plants treated with Fe-EDDHA at Leonard produced significantly higher biomass compared to the control, both for total biomass (92.1 g and 58.2 g, respectively) and biomass per plant (2.8 g versus 2.0 g, respectively).

At Galchutt, the initial plant population response to the Fe-EDDHA treatment, reflected what was observed in the main trials, with the plant population being significantly lower with the Fe-EDDHA treatment compared to the control (199 505 plants ha^{-1} and 248 292 plants ha^{-1}, respectively). The germination delay/inhibition observed in the ragdoll tests carried over to the field. Furthermore, Wiersma (2007) observed no positive effect from Fe-EDDHA application if IDC-inducing conditions were not present. It is possible that at Galchutt the soil conditions were non-conducive for IDC symptoms and the seed treatment was a hinderance to water absorption. Soil [Fe] was not measured at the mini plot sites. Plant population, both initial and final, was non-significantly different at Harwood or averaged across all locations.

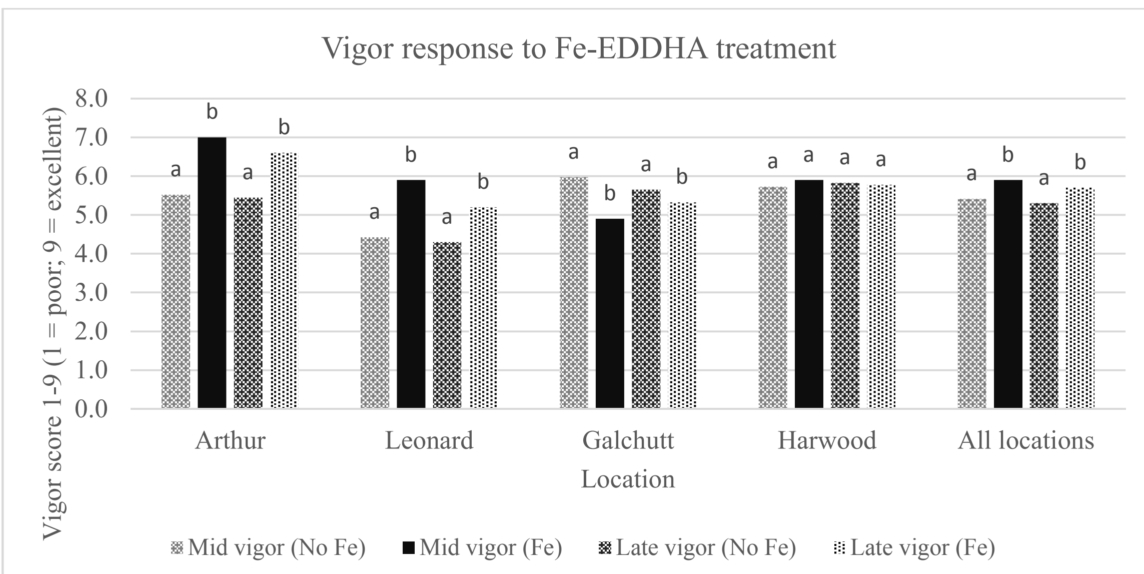
Both IDC and vigor scores were significantly different (Table 14). The Fe-EDDHA application significantly lowered IDC scores at three of the four mini plot locations, as well averaged across all locations (Table 15 and Fig. 6a). This was expected based on previous research. The dry matter accumulation rate in soybean can be influenced by stress factors. Certain signals such as those triggered by iron stress typically result in an increase in antioxidant activity and a decrease in biomass production (i.e. chlorophyll production). This in turn can lead to IDC symptoms (Zhang et al., 2017). Gamble et al. (2014) also documented improvements in

IDC scores (i.e. lower scores) with the application of Fe-EDDHA. Therefore, with the presence of Fe-EDDHA, iron-deficiency stress is reduced and therefore there is less need for the plant to divert biomass-producing energy to deal with said stress.

The results of the research conducted by Zhang et al. (2017) suggest that if more biomass is observed at the end of the growing season, then less stress occurred in-season. This supports higher yields (in terms of biomass and final plant population) that were associated with the significantly higher vigor scores observed at Arthur and Leonard (Table 15). The significantly lower early vigor scores and biomass at Galchutt are likely due to the significant reduction in plant population (Fig. 6b and Table 15).



a



b

Figure 6. Fe-EDDHA treatment effect on (a) IDC score and (b) mid- and late-season vigor scores at individual locations at Arthur and Leonard in 2013 and Galchutt and Harwood in 2014 and averaged across all locations/years. Columns with a different letter in graph (b), within mid and late vigor score, are significantly different at $\alpha = 0.10$.

Means for the interaction between cultivar and Fe-EDDHA application are presented in Table 16. Only the data for from Arthur are shown because this was the only location where significance was found for this interaction.

Table 16. Means for iron-chelate by cultivar interaction effect on general agronomic traits and above-ground biomass at Arthur, ND in 2013.

Fe-EDDHA‡	Cultivar	Plant population (initial)	IDC†	Early vigor	Mid vigor	Late vigor	Biomass (total)	Biomass (per plant)	Plant population (final)
		plants ha^{-1}	1-5	------------1-9	------------		----------g	----------	plants ha^{-1}
No Fe-EDDHA	90Y42	248301	2.5	4.6	7.1	6.6ab	90.0bc	3.3ab	236705
Fe-EDDHA	90Y42	252658	2.0	4.5	7.6	7.5a	94.4bc	3.1b	268983
No Fe-EDDHA	90Y70	224342	1.6	4.9	4.5	4.0bc	53.5cd	2.2c	213034
Fe-EDDHA	90Y70	326712	2.4	6	7.5	7.3ab	118.9a	3.3ab	316324
No Fe-EDDHA	DSR-0747	230877	1.6	3.4	3	3.8bc	44.1cd	1.7de	217338
Fe-EDDHA	DSR-0747	252657	1.3	4.6	4.9	4.4b	56.0c	2.0d	241009
No Fe-EDDHA	HS 01RY02	215630	1.5	4.3	7.1	6.5ab	87.2bc	3.5a	217338
Fe-EDDHA	HS 01RY02	259192	1.4	5	7.8	7.1ab	99.2b	3.2ab	266831
No Fe-EDDHA	6088	335424	2.1	5.9	5.9	6.4ab	84.4bc	2.3cd	312020
Fe-EDDHA	6088	357205	1.8	6.6	7.4	6.8ab	101.5ab	2.5c	344298
LSD 0.10		ns¶	ns	ns	ns	1.6	19.2	0.4	ns

†IDC = iron-deficiency chlorosis score.
‡Fe-EDDHA was seed-applied at a rate of 3.36 kg ha^{-1}.

Only the late vigor score, total biomass, and biomass per plant were found to be significantly different for the interaction between Fe-EDDHA and cultivar at Arthur (Table 16). All cultivars responded positively to the Fe-EDDHA application in terms of vigor, however only 90Y70 was observed to have a significantly higher late vigor score with Fe-EDDHA compared to the control (7.3 and 4.0, respectively). Cultivars 90Y42, 90Y70 and 6088 produced significantly higher total biomass with Fe-EDDHA application (94.4 g, 118.9 g, and 101.5 g, respectively) compared to the control (90.0 g, 53.5 g, and 84.4 g). Cultivar 90Y70 produced higher biomass per plant with Fe-EDDHA (3.3 g) compared to the control (2.2 g). Cultivar 90Y42 also produced higher biomass per plant, but without Fe-EDDHA. The initial plant population for 90Y42 was numerically higher with Fe-EDDHA. This might explain the lower biomass per plant since plants were closer together.

The mini plots were harvested on the same day. However, based on field observations, not all plots were at the same growth stage when they were harvested. The average growth stage at harvest time was R4 (beginning pod), but some plots were only at R3 and some at R5. Therefore, expected maturity differences (Table 2) may explain biomass differences in addition to treatment differences. For example, 6088 has a higher maturity rating than 90Y42 (0.8 and 0.4, respectively) (Table 2). Therefore, since 6088 would mature later, it is likely that 90Y42 had more developed reproductive structures when the mini plots were harvested.

Furthermore, the soybean plant tends to stop producing biomass (i.e. go dormant) in order to deal with a stress (Sammons et al., 1980). The rate of accumulation of dry weight has been shown to be largely influenced by a cultivar's ability to tolerate iron deficiency stress (Zhang et al., 2017).

SUMMARY OF RESULTS

This research demonstrated significantly reducing IDC symptoms with subsurface tile drainage, based on the analysis of tile alone at NW22. Yield for the Tile x Raised Beds interaction was significantly lower than the control as well as No tile-raised beds, but numerically similar to Tile-flat. Raised beds increased early vigor and plant population at NW22. Both raised beds tillage and subsurface tile drainage demonstrated a positive effect on plant biomass development in this experiment. When averaged across six environments, plant population, early vigor score, and yield were all significantly increased with raised beds compared to flat land. The application of Fe-EDDHA via seed treatment significantly reduced plant population. However, based on the delay in germination rates observed in the germination tests, this was an expected result. Iron-deficiency symptoms were significantly reduced when averaged across all locations. In this experiment, a significant increase in yield in response to Fe-EDDHA application was only observed at certain locations, namely NW22 (No tile) and Casselton in 2014 (data not shown). At the mini-plots, IDC symptoms were significantly reduced at three out of the four locations and plant vigor and biomass were both significantly increased in most cases.

CONCLUSIONS

Tile, Raised Beds, and Tile x Raised Beds Effect

Iron-deficiency chlorosis continues to be a problem in the RRNV. The environmental conditions in the RRNV (i.e. high soil saturation early in season, calcareous soil, etc.) are conducive to IDC. The fact that tile alone was shown to significantly reduce IDC suggests that tile is an effective IDC management method in the above-mentioned environmental conditions. Further research is needed on subsurface tile drainage to determine practicality of the cost/benefit of installation. Under certain environmental conditions, such as the ones observed in this experiment, there are no economic advantages of combining raised beds with tile and sometimes this combination can result in a lower yield compared to other methods. Raised beds tillage remains a cheaper method of controlling IDC in soybean fields, namely under field conditions that are drier than normal. Tile drainage can cost around \$2 400 ha^{-1} (Mahoney et al., 2011). However, in a year with continuous rainfall events where the rainfall exceeds the infiltration rate there might be a benefit of raised beds in addition to tile.

The positive effect of raised beds and tile drainage on soil properties was reflected in this experiment and demonstrates the continued benefit of both practices. However, as presented in this experiment and given the drier than normal environmental conditions that occurred in both 2013 and 2014, using both tile drainage and raised beds tillage in the same field would overcompensate and result in less water availability for the plant, which could lead to reduced yield or at least no added economic benefit. When a farmer considers installing tile drainage, it is recommended to also install the inline water level control structures, such as the ones used in this experiment to manage the water table in dry years.

Raised Beds Effect

Unlike the tile drainage effect, raised beds were present at both NW22 and Casselton. Conclusions can therefore be drawn that raised beds can have a significant impact on soybean production on a broad scale. Furthermore, contrary to the tile drainage effect only lasting as along as the water table is above the tile line and Fe-EDDHA application effect only lasting as a long as it is actively affecting soil chemistry, raised beds will have an ongoing effect and can be carried over from season to season via fall and spring maintenance. Raised beds can further be used to harvest water via proper row orientation.

Although soil penetration resistance data was not collected in this experiment, previous research has shown raised beds to be have lower bulk density (Hoppe, 2013). Continual raised beds in a field could therefore lead to less soil compaction, better drainage, and as a result, continual prevention of IDC conditions. Further research is needed to determine raised beds effect on plant development during mid- to late season compared to other mid- and late-season practices. Further research is also needed to assess the effect of row orientation.

Fe-EDDHA Application Effect

The application of Fe-EDDHA remains a partial solution and should therefore be combined with other methods to reduce IDC. Seed treatment may not be the best method of application given the plant population reduction observed in this experiment and other methods such as in-furrow application or foliar sprays should be considered first. No sound conclusions can be drawn about what the exact cause is of the germination delay/inhibition, but possibly the seed treatment delayed the time it took for water to be absorbed into the seed after planting. However, despite the reduction in plant population, seed-treatment of Fe-EDDHA is a practice that can help reduce IDC incidence and increase yield. No Fe-EDDHA treatment has been shown to completely eliminate IDC (Goos and Johnson, 2001; Gamble et al., 2014) and IDC-tolerant cultivar selection remains the best IDC management method based on other research (Goos and Johnson, 2000 and 2001) as well as the significant differences observed at all locations in this experiment due to cultivar differences.

Fe-EDDHA Application by Cultivar Mini Plot Experiment

The mini plot experiment was a better representation of the effect of Fe-EDDHA seed treatment, cultivar selection, and the interaction of these two methods on IDC reduction and a larger experiment (i.e. a main trial) should be done to further assess these effects. Main conclusions to be drawn from the mini plot experiment are that Fe-EDDHA applied via seed-application can delay germination rates during the ragdoll tests as well as reduce plant population in a field setting. However, the benefits, namely IDC reduction and an increase in biomass should continue to be investigated. This suggests that Fe-EDDHA application may benefit soybean production, but as mentioned above, other methods of application should be attempted first and other methods of seed-treatment should be studied to avoid the germination delay/inhibition.

REFERENCES

Bakker, D.M., G.J. Hamilton, D.J. Houlbrooke, and C. Spann. 2005. The effect of raised beds on soil structure, waterlogging, and productivity on duplex soils in Western Australia. Aust. J. Soil Res. 43:575-585.

Benjamin, J.G., A.D. Blaylock, H.J. Brown, and R.M. Cruse. 1990. Ridge tillage effects on simulated water and heat transport. Soil Tillage Res. 18:167-180.

Blessit, J. B. 2008. Productivity of raised seedbeds for soybean production on clayey soils of the Mississippi delta. M.S. Thesis. Mississippi State Univ., Starkville.

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

Brodshaug, J.A. 2011. Subsurface tile drainage in clay soils in a northern climate and its effect on various soybean cultivars and soil properties. M.Sc. Thesis, North Dakota State Univ., Fargo.

Casteel, S., presenter. 2010. Soybean physiology: How well do you know your soybean? Indiana CCA Conference. West Lafayette, IN. 15 Dec. 2010.

Cihacek, L.J., D. Franzen, X. Jia, R. Johnson, and T. Scherer. 2012. Evaluation of soils for suitability for tile drainage performance. NDSU Ext. Bul. SF-1617.

Endres. G., and H. Kandel (eds.) 2015. Soybean Growth and Management: Quick Guide. A1174 Revised. North Dakota State Univ. Ext. Service.

Fehr, W.R., C.E. Caviness, D.T. Burmood, and J.S. Pennington. 1971. Stage of development descriptions for soybeans, Glycine max (L.) Merrill. Crop Sci. 11:929-931.

Gamble, A.V., J.A. Howe, D. Delaney, E. van Stanten, and R. Yates. 2014. Iron chelates alleviate iron chlorosis in soybean on high pH soils. Agron. J. 106:4:1251-1257. doi:10.2134/agronj13.0474.

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

Goos, R.J., and B.E. Johnson. 2001. Seed treatment, seeding rate, and cultivar effects on iron deficiency chlorosis of soybean. J. Plant Nutr. 24:8:1255-1268. doi: 10.1081/PLN-100106980.

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

Hatfield, J.L., R.R. Allmaras, G.W. Rehm, and B. Lowery. 1998. Ridge tillage for corn and soybean production: environmental quality impacts. Soil Tillage Res. 48:145-154.

Hoppe, A.R. 2013. Soybean productivity as affected by raised seedbeds and subsurface drainage. M.Sc.Thesis, North Dakota State Univ., Fargo.

Hoppe, A.R., G.H. Mehring, H. Kandel. 2017. Soybean productivity as affected by raised beds under dry environments. Open Agric. J. 11:46-59.

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

Kandel, H. 2012. Soybean Growth and Development. NDSU PLSC Ext. 6/7/12 http://www.ag.ndsu.edu/cpr/plant-science/soybean-growth-and-development-6-7-12 (accessed 19 Sept 2018)

Kandel, H. (ed). 2012. North Dakota soybean variety trial results for 2012 and selection guide. North Dakota State Univ. Ext. Ser. Pub. A843-12.

Kandel, H. (ed). 2013. North Dakota soybean variety trial results for 2012 and selection guide. North Dakota State Univ. Ext. Ser. Pub. A843-13.

Kirnak, H., Z. Gokalp, E. Dogan, and O. Copur. 2017. Soil characteristics of soybean fields as effected by compaction, irrigation, and fertilization. Legume Res. 40:4:691-697.

Kornecki, T.S., and J.L., Fouss. 2001. Quantifying soil trafficability improvements provided by subsurface drainage for field crop preperations in Louisiana. Appl. Eng. In Agron. 17:777-781.

Mahoney, S., J. Lawrence, Q. Ketterings, K. Czymmek, E. Young, and L. Geohring. 2011. Subsurface (tile) drainage benefits and installation guidance. Agron. Fact Sheet Serv. Cornell Univ. Coop. Ext. 57. http://nmsp.cals.cornell.edu/publications/factsheets/factsheet57.pdf (accessed 19 Sept 2018)

Naeve, S.L. 2006. Iron deficiency chlorosis in soybean: Soybean seeding rates and companion crop effects. Agron. J. 98:1575-1581.

Nakagawa, A.C.S, H. Itoyama, Y. Ariyoshi, N. Ario, Y. Tomita, Y. Kondo, M. Iwaya-Inoue, and Y. Ishibashi. 2018. Drought stress during soybean seed filling affects storage compounds through regulation of lipid and protein metabolism. Acta Physiol. Plant. 40:6:111.

Oosterhuis, D.M., H.D. Scott, R.E. Hampton, and S.D. Wullschleger. 1989. Physiological responses of two soybean [*Glycine max*, (L.) Merr] cultivars to short-term flooding. Environ. Exp. Bot. 30:1:85-92.

Orchs, W.J., R.D. Wenberg, and B.G. Bishay. 2001. Water management (drainage). *In:* Marlow, R.L. and P.H. Willey. Part 650 Engineering field handbook: national engineering handbook. USDA – NRCS.

Randall, G.W., D.R. Huggins, M.P. Russelle, D.J. Fuchs, W.W. Nelson, and J.L. Anderson. 1997. Nitrate losses through subsurface tile drainage in conservation reserve program, alfalfa, and row crop systems. J. Environ. Qual. 26:1240-1247.

Salisbury, F.B., and C.W. Ross. 1992. Plant physiology. 4th ed. Wadsworth, Inc. Belmont, CA.

Sammons, D.J., D.B. Peters, and T. Hymowitz. 1980. Screening soybeans for tolerance to moisture stress: A field procedure. Field Crops Res. 3:321-335.

Sands, G. 2013. Agriculture drainage design and water management workshop. Moorhead, MN. 12-13 Feb. 2013.

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

Singh, A., J.N. Singh, and S.K. Tripathi.1971. Effect of soil compaction on the growth of soybean (*Glycine max* L. Merr.). Indian J. Agric. Sci. 41:422-426.

Sunohara, M.D., N. Gottschall, G. Wilkes, E. Craiovan, E. Topp, Z. Que, O Seidou, S.K. Frey, and D.R. Lapen. 2015. Long term observations of nitrogen and phosphorus export in paired-agriculture watersheds under controlled and conventional tile drainage. J. Environ. Qual. Vol. 44 No. 5, p. 1589-1604, doi: 10.2134/jeq2015.01.0008.

Sylvia, D.M., J.J. Fuhrmann, P.G. Hartel, and D.A. Zuberer. 1999. Principles and Applications of Soil Microbiology. Prentice Hall, Inc. Upper Saddle River, NJ.

Taylor, H.M., W.K. Mason, A.T.P. Bennie, and H.R. Rowse. 1982. Responses of soybeans to two row spacings and two soil water levels. I. An analysis of biomass accumulation, canopy development, solar radiation interception and components of seed yield. Field Crops Res. 5:1:1-14.

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

Wiersma, J. 2007. Iron acquisition of three soybean varieties grown at five seeding densities and five rates of Fe-EDDHA. Agron. J. 99:4:1018-1028.

USDA NRCS. 2008. Soil Water Quality Inidcators: Bulk Density http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_051591.pdf (accessed 19 Sept 2018).

USDA NRCS. 2013. Web Soil Survey. http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx (accessed 5 Sept 2018).

Zhang, M., Z. Yi, Q. Qiu, F. Meng, D. Rao, J. Zhao, W. Zhang, and X. Yan. 2017. Relationship between low iron stress and iron efficiency of soybean varieties. As. Agric. Res. 9:12:37-42.

APPENDIX A

Table A1. Means for iron-chelate by variety interaction on general agronomic traits and above-ground biomass at Leonard, ND in 2013.

		Plant population (initial)	IDC†	Early vigor	Mid vigor	Late vigor	Biomass (total)	Biomass (per plant)	Plant population (final)
Fe-EDDHA‡	Proxy§	plants ha^{-1}					-----------------g-----------------		plants ha^{-1}
Fe-EDDHA	1	276616	1.9	4.4	6.5	5.9	93.7	3.1	260376
No Fe-EDDHA	1	217808	2.4	5.5	4.6	4.1	54.0	2.1	221642
Fe-EDDHA	2	352849	1.6	5.5	7.1	7.4	128.2	3.2	350754
No Fe-EDDHA	2	326712	2.0	5.5	6.3	6.5	97.0	2.6	322780
Fe-EDDHA	3	368095	1.9	5.0	4.5	2.0	64.7	1.8	307717
No Fe-EDDHA	3	315822	2.5	4.4	2.9	2.4	33.6	1.0	279742
Fe-EDDHA	4	263548	1.3	4.3	7.6	7.8	115.8	4.2	241009
No Fe-EDDHA	4	228698	2.3	4.0	5.8	6.4	70.0	3.0	204427
Fe-EDDHA	5	274438	1.6	5.9	3.5	3.1	58.2	1.7	301261
No Fe-EDDHA	5	318000	2.9	5.5	2.6	2.1	36.3	1.2	256072

†IDC = iron-deficiency chlorosis score, V1 = vigor score 1, V2 = vigor score 2, and V3 = vigor score 3.
‡Fe-EDDHA was seed-applied at a rate of 3.36 kg ha^{-1}.
§Proxy numbers are based on IDC score and maturity group ratings. 1=90Y24 and 90Y50; 2=90Y70; 3=DSR 0747/R2Y; 4=HS 01RY02; and 5=6088 and 0906R2.

Table A2. Means for iron-chelate by variety interaction on general agronomic traits and above-ground biomass at Galchutt and Harwood, ND in 2014.

Fe-EDDHA‡	Proxy§	Plant population (initial) plants ha^{-1}	IDC†	Early vigor	Mid vigor	Late vigor	Biomass (total)	Biomass (per plant)	Plant population (final) plants ha^{-1}
							---------------g--------------		
				Galchutt					
Fe-EDDHA	1	257004	2.3	4.8	5.4	6.4	213.7	8.1	253920
No Fe-EDDHA	1	239580	2.3	5.1	5.5	5.6	217.4	8.3	236705
Fe-EDDHA	2	185130	2.0	4.3	5.3	5.5	184.3	9.4	182908
No Fe-EDDHA	2	285318	2.3	5.4	7.3	6.0	250.3	7.8	281894
Fe-EDDHA	3	193842	1.8	4.6	4.5	5.3	192.9	9.2	191516
No Fe-EDDHA	3	243936	2.3	5.0	5.5	5.8	242.5	9.4	241008
Fe-EDDHA	4	167706	1.3	4.3	4.3	4.3	175.2	12.3	165693
No Fe-EDDHA	4	257004	1.8	5.4	6.4	5.8	210.9	7.4	253920
Fe-EDDHA	5	193842	1.6	4.4	5.1	5.3	191.6	9.2	191516
No Fe-EDDHA	5	215622	2.1	4.6	5.3	5.1	224.7	10.9	213035
				Harwood					
Fe-EDDHA	1	315810	1.6	4.8	5.3	5.9	157.5	5.5	312020
No Fe-EDDHA	1	426888	2.4	5.1	5.4	5.1	143.1	2.9	421765
Fe-EDDHA	2	289674	1.3	3.9	6.1	6.6	159.5	5.2	286198
No Fe-EDDHA	2	365904	2.4	4.5	6.0	6.8	160.3	3.8	361513
Fe-EDDHA	3	405108	2.1	5.9	6.6	3.9	157.6	3.4	400247
No Fe-EDDHA	3	355014	2.4	5.5	6.3	4.5	140.9	3.4	350754
Fe-EDDHA	4	163350	1.3	2.5	3.8	5.3	114.2	7.9	161390
No Fe-EDDHA	4	189486	2.1	1.9	4.9	6.1	133.0	6.3	187212
Fe-EDDHA	5	400752	1.6	6.5	7.8	7.3	229.3	5.1	395943
No Fe-EDDHA	5	415998	2.9	5.6	6.1	6.6	192.1	4.2	411006

†IDC = iron-deficiency chlorosis score, V1 = vigor score 1, V2 = vigor score 2, and V3 = vigor score 3.
‡Fe-EDDHA was seed-applied at a rate of 3.36 kg ha^{-1}.
§Proxy numbers are based on IDC score and maturity group ratings. 1=90Y24 and 90Y50; 2=90Y70; 3=DSR 0747/R2Y; 4=HS 01RY02; and 5=6088 and 0906R2.

Table A3. Means for iron-chelate by variety interaction on general agronomic traits and above-ground biomass at all mini plot locations in 2013 and 2014.

Fe-EDDHA‡	Proxy§	Plant population (initial) plants ha^{-1}	IDC†	Early vigor	Mid vigor	Late vigor	Biomass (total)	Biomass (per plant)	Plant population (final) plants ha^{-1}
							----------------g----------------		
Fe-EDDHA	1	275522	1.9	4.6	6.2	6.4	139.8	4.9	273825
No Fe-EDDHA	1	283144	2.4	5.1	5.7	5.4	126.1	4.2	279204
Fe-EDDHA	2	288591	1.8	4.9	6.5	6.7	147.8	5.3	284046
No Fe-EDDHA	2	300569	2.1	5.1	6.0	5.8	140.3	4.1	294805
Fe-EDDHA	3	304926	1.8	5.0	5.1	3.9	117.8	4.1	285122
No Fe-EDDHA	3	286412	2.2	4.6	4.4	4.1	115.3	3.9	272211
Fe-EDDHA	4	213449	1.3	4.0	5.8	6.1	126.1	6.9	208731
No Fe-EDDHA	4	222704	1.9	3.9	6.0	6.2	125.3	5.0	215724
Fe-EDDHA	5	306559	1.7	5.8	5.9	5.6	145.1	4.6	308255
No Fe-EDDHA	5	321261	2.5	5.4	5.0	5.1	134.4	4.6	298033

†IDC = iron-deficiency chlorosis score, V1 = vigor score 1, V2 = vigor score 2, and V3 = vigor score 3.
‡Fe-EDDHA was seed-applied at a rate of 3.36 kg ha^{-1}.
§Proxy numbers are based on IDC score and maturity group ratings. 1=90Y24 and 90Y50; 2=90Y70; 3=DSR 0747/R2Y; 4=HS 01RY02; and 5=6088 and 0906R2.

Table A4. Germination test results† for two cultivars (90Y70 and 90Y42) at 21º C for 7 and 14 days.

	-----7 days-----		-----14 days-----	
Treatment	90Y70	90Y42	90Y70	90Y42
	--------------------------%	-------------------------		
Control	99.0a	96.7a	99.0a	96.7
Frontier‡	94.3b	92.7b	97.0b	96.0
Diet 35§	94.3b	94.3ab	97.0b	96.0
Fe + Frontier	88.7c	87.3c	95.0c	97.0
Fe + Diet 35	92.7b	92.7b	96.7b	96.0
AVG	93.8	92.7	96.9	96.3
LSD 0.10	4	3.7	1.4	ns

†One hundred seeds were treated in each observation. A rate of 3.36 kg Fe-EDDHA ha^{-1} was applied.
‡Frontier brand of gum Arabic (Natural Products CO-OP, Norway, IA).
§TheDiet35 brand of gum Arabic (Swanson Health Products, Fargo, ND).

APPENDIX B

105A V2	105B V2
104A V5	104B V5
103A V4	103B V4
102A V3	102B V3
101A V1	101B V1

Figure B1. Example of one replicate of a set of five mini plots (grey indicates Fe-EDDHA Treatment; V indicates cultivar number; A and B indicate row).