THE RELATIVE NITROGEN FIXATION RATE AND COLONIZATION OF ARBUSCULAR MYCORRHIZAL FUNGI OF IRON DEFICIENT SOYBEANS

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The Relative Nitrogen Fixation Rate and Colonization of Arbuscular Mycorrhizal Fungi of Iron Deficient Soybeans

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Podrebarac, Frances Ann, M.S., Department of Soil Science, College of Agriculture, Food Systems, and Natural Resources, North Dakota State University, August 2011. Relative Nitrogen Fixation Rate and Colonization of Arbuscular Mycorrhizal Fungi of Iron Deficient Soybeans. Major Professor: Dr. R. Jay Goos.

Soybeans (*Glycine max* L. Merr.) are a symbiont of two beneficial associations: biological nitrogen fixation (BNF) with *Bradyrhizobium japonicum*, and arbuscular mycorrhizal fungi (AMF). Within the Northern Great Plains of the USA, iron deficiency chlorosis (IDC) of soybean is a yield-limiting factor. The effects of IDC on BNF and AMF are not well defined. This study was conducted to determine the effects of IDC on BNF and AMF. A laboratory study was performed to compare three methods of measuring ureide-N, a product of BNF in soybeans. Field studies in soybean were performed at three locations at eastern North Dakota. The experimental design was a factorial combination of three cultivars and three treatments. The three cultivars, in order of decreasing chlorosis susceptibility, were NuTech NT-0886, Roughrider Genetics RG 607, and Syngenta S01-C9 RR. The three treatments were control, *Sorghum bicolor* L. companion crop planted with the soybean seed, and FeEDDHA applied with the soybean seed. Chlorosis severity was the greatest and least for the NuTech and Syngenta cultivars, respectively. The FeEDDHA treatment decreased chlorosis severity. Ureide levels were abnormally high in plants severely stunted by IDC. The excess accumulation of ureides in IDC-stunted plants suggests that plant growth was reduced more than the rate of nitrogen fixation. The AMF population was at an adequate level at all locations and not affected by cultivar or treatment, in general. In the laboratory study, the Patterson et al. method had greater ureide concentrations due to the non-specific measuring of ammonium compounds compared to the Vogels and Van der Drift and Goos methods.

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DEDICATION

This thesis is dedicated to my family who supports me in all my endeavors.

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GENERAL INTRODUCTION

Organization of Thesis

The General Introduction includes a Literature Review covering: Soybeans in North Dakota, Overview of the Soybean Life Cycle, Iron in Plants, Iron Deficiency Chlorosis in Soybean, The "Chlorosis Paradox," Biological Nitrogen Fixation, Arbuscular Mycorrhizal Fungi, Symbiont Interactions, Study Objectives, and References Cited. The General Conclusions and References Cited follow the Papers 1 and 2. Paper 1 entitled, "A Comparative Study of Ureide Analyses", compares two published methods and one unpublished method of ureide analysis. This paper provides background for Paper 2, which utilized the unpublished ureide analysis method. Paper 2, "Relative Nitrogen Fixation Rate and Arbuscular Mycorrhizal Fungi Colonization of Iron Deficient Soybeans", examines the relationship among iron deficiency chlorosis and the symbionts of soybeans.

Literature Review

Soybeans in North Dakota.

The soybean is an important North Dakota crop, particularly in the eastern third of North Dakota. Soybeans are a valuable food source for oil (20-30% lipids), protein (40-50%), and carbohydrates (26-30%) (Gibbs et al., 2004). The interest of incorporating soybean into the daily diet has increased in the Western hemisphere due to the potential health benefits, such as potential anti-carcinogens, decreasing cholesterol, and reduced risk of coronary heart disease (Bethlenfalvay et al., 1985; Messina et al., 1994; Franke et al., 1995; Lucas et al., 2001). The acres of soybeans harvested have been steadily increasing since 1946 in North Dakota (Berglund and Helms, 2003). Soybean yield has

approximately doubled from 1,009 kg ha^{-1} in 1954 to 2,287 kg ha^{-1} in 2010 (USDA-National Agricultural Statistics Service, 2010). It is projected that North Dakota farmers will plant 1.6 million hectares in 2011, an increase of 40,470 hectares compared to 2010 (Knutson, 2010).

Overview of the Soybean Life Cycle.

The soybean is a legume classified as an oilseed with its center of origin in East Asia. An understanding of the vegetative (V) and reproductive (R) stages of the soybean assists soybean growers' crop management decisions, such as fertilization, and pesticide applications (McWilliams et al., 1999). Soybean germination and emergence is influenced by soil, water, and temperature (Helms et al., 1996; Helms et al., 1997). The soybean begins germination when the seed absorbs about 50% of its weight in water. The emergence (VE) stage begins when the radical emerges from the seed (Hübel and Beck, 1993). Lateral roots develop from the primary root and then the hypocotyl (stem) emerges and grows toward the soil surface. The VE stage occurs when the cotyledons (seed leaves) are above the soil surface. Following the VE stage, the unifoliate leaves fully expand in the cotyledon (VC) stage. After the VC stage, the V stages are counted and numbered by the upper most fully developed leaf node on the main stem. The first trifoliolate (V1) stage occurs when the first trifoliolate off the main stem is fully expanded (Fehr and Caviness, 1977; McWillams et al., 1999). At the V2 stage, biological nitrogen fixation (BNF) by *Bradyrhizobium japonicum* has been established and is active (Keyser and Li, 1992). The plant axillary buds toward the top of the stem develop at the V5 stage. At the V6 stage plants are approximately 30 to 35 cm tall with seven nodes and by this time the unifoliolate leaves and the cotyledons may have senesced. The axillary buds will develop into the

racemes (flower clusters) at approximately one week during the R1 stage (beginning bloom). The R1 stage is reached when the plants are approximately 38 to 46 cm tall between the V7 to V10 stage. The first flower is always initiated on the third to sixth node and the full bloom (R2), beginning pod (R3), full pod (R4), beginning seed (R5), and full seed (R6) developmental stages follow. Stress, such as temperature or moisture, experienced in the R3 stage may reduce total pod number, bean number per pod, or seed size which decreases yield. The soybean can partial compensate with temporary stressor, but the ability to compensate decreases as the plant develops. In favorable conditions, approximately 60-75% of flowers are aborted on the soybean. Out of the flowers aborted, 30-38% are aborted before pod development, and 30-38% are aborted due to pod abortion. Thus, additional stresses increases flower abortion and pod abortion, which will decrease yield. The R4-R6 stages are critical for seed yield in that stress experienced during the R4-R6 stages results in a greater yield reduction compared to stress experienced at other vegetative and reproductive stages. Biological nitrogen fixation and root growth are complete at the R5 and R6 stages, respectively. The beginning maturity (R7) occurs when one pod on the main stem turns brown (mature color). Full maturity (R8) follows with approximately 95% of the pods reaching mature color. Harvest should occur after five to ten days of drying weather after the R8 stage, as the soybean seed water content dries to less than 15% (McWilliams et al., 1999).

Iron in Plants.

Iron is a micronutrient of plants, necessary for chlorophyll development, energy transfer, plant respiration, plant metabolism, and soybean root nodule formation (Hell and Stephan, 2003; Lemanceau et al., 2009). The uptake, and distribution of Fe is a highly

regulated process by the plant due to the insolubility or toxicity of Fe (Hell and Stephan, 2003; Lemanceau et al., 2009). Iron is a highly reactive metal, and it can be found as two reversible redox species: Fe (II) (ferrous) and Fe (III) (ferric). The high reactivity enables Fe to be a catalyst in reduction-oxidation (redox) reactions, and sometimes acts as a toxin (Hell and Stephan, 2003). Iron can function as a toxin when Fe in contact with O_2, generates $OH^{\bullet}$, a non-selective, highly reactive radical. In soils, Fe is predominantly Fe (III) with the Fe (II) occurring more commonly in anoxic soil environments (Brown, 1978; Lemanceau et al., 2009). Iron is not limited by quantity in soils, as Fe is the fourth most abundant element on the earth's crust (Lemanceau et al., 2009). The limiting factor of Fe is its solubility. The solubility of Fe, and thus availability for plant utilization, is pH dependent (Lindsay, 1979; Marschner, 1995). Iron solubility decreases in alkaline soil ($pH > 7$) compared to an acidic soil ($pH < 7$) (Lindsay, 1979).

Plants have developed two strategies in order to obtain Fe by roots (Römheld and Marschner, 1986). Soybeans and other higher plants except *Gramineae* species utilize Strategy I. Strategy I is subdivided into three mechanisms: (a) acidification of soil solution by excretion of H^+ or organic acids, (b) reduction of Fe (III) by reductases and reducing compounds to Fe (II), (c) transport of Fe (II) by iron transporters through plasmalemma transport (Hell and Stephan, 2003; Lemanceau et al., 2009). *Gramineae* plants utilize Strategy II, which release phytosiderophores to chelate Fe (III) in the rhizosphere (Hell and Stephan, 2003).

Iron is a highly reactive transitional metal that when regulated serves as an efficient cofactor and catalyst and a potential toxin (Hell and Stephan, 2003). At the cellular level, plants utilize molecular oxygen as an electron acceptor generating $O_2^{\bullet -}$ or H_2O_2. The

production of $O_2^{\bullet-}$ or H_2O_2 is not by itself harmful, but contributes to $OH^{\bullet}$ (Hell and Stephan, 2003). The $OH^{\bullet}$ is a non-selective molecule that reacts with most molecules within living cells, such as deoxyribonucleic acid (Hell and Stephan, 2003). Iron catalyzes the formation of $OH^{\bullet-}$ under the reactions termed Fenton chemistry (Fe (III) $O_2^{\bullet-} \rightarrow$ Fe (III) + O_2; Fe (II) + $H_2O_2 \rightarrow$ Fe (III) + OH^- + $OH^{\bullet}$)(Briat, 2002). The accumulation of O_2, H_2O_2, and the generation of $OH^{\bullet}$ may be a factor influencing the physiology of IDC.

Iron Deficiency Chlorosis in Soybean.

Iron deficiency chlorosis (IDC) is a nutritional disorder characterized by yellowing leaflets, interveinal chlorosis, stunting, and a reduced yield in quality and quantity (Goos and Johnson, 2000; Lucena, 2000). Symptoms of IDC are often observed within the first few weeks after emergence (Franzen and Richardson, 2000). Soybean growers in the west central and southwest areas of Minnesota estimate 24% yield loss is due to IDC, which has been estimated at 120 million dollars annually (Hansen et al., 2003). Soybean cultivars are ranked by IDC susceptiblity by the public and private soybean industry (Inskeep and Bloom, 1984; Franzen and Richardson, 2000; Goos and Johnson, 2000; Hansen et al., 2003). Planting soybean cultivars less susceptible to IDC is critical to alleviate chlorosis symptoms in IDC prone soils, as the ability to obtain Fe during Fe deficiency stressors differs by cultivars (Froehlich and Fehr, 1981; Fehr, 1984; Jolley et al., 1986; Goos and Johnson, 2000; Hansen et al., 2004). Regardless of soybean cultivar, IDC remains a yield-limiting factor in soybean production in the Northern Great Plains (Hansen et al., 2004).

Soybean cultivars are either considered Fe efficient or Fe inefficient (Terry et al., 1991). Iron inefficient cultivars are unable to elicit responses to obtain Fe by utilizing Strategy I (Terry et al., 1991). Soybeans unable to obtain an adequate concentration of Fe

will exhibit symptoms of IDC. Soybean cultivars less susceptible to IDC obtain soil Fe via Strategy I, thus developing chlorophyll and maturing with minimal yield losses compared to IDC susceptible cultivars.

Iron deficiency chlorosis is a unique nutrient deficiency compared to other plant nutrient deficiencies, as the majority of nutrient deficiencies develop from a simple lack of the nutrient in the soil. However, Fe deficiency develops from a combination of stresses (Hansen et al., 2004). These stresses may differ by location, as the intensity and presence of IDC varies seasonally and spatially (Franzen and Richardson, 2000; Hansen et al., 2003; Naeve and Rehm, 2006).

Calcareous and alkaline soils are associated with Fe deficiency, as high HCO_3^- concentrations and alkaline soil pH decreases the solubility of Fe (Franzen and Richardson, 2000; Schenkeveld et al., 2008). It is known that Fe compounds are very insoluble (Lindsay, 1979). In the pH range of most soils, an increase of 1 pH unit results in a 1000-fold decrease of Fe (III) solubility (Lindsay, 1979; Lucena, 2000). Soil water content, reactive soil carbonates, exchangeable bases, and the concentration of CO_2 influences the concentration of HCO_3^- (Hansen et al., 2004). Severe IDC has been observed in soils with high HCO_3^- concentrations (Inskeep and Bloom, 1986; Inskeep and Bloom, 1987; Morris et al., 1990; Franzen and Richardson, 2000; Hansen et al., 2003).

Soil salinity, soil Fe forms, and micronutrient concentrations are also soil properties associated with IDC. Soils with greater soluble salt concentrations (EC) generally form more severe IDC (Inskeep and Bloom, 1987; Morris et al., 1990; Loeppert et al., 1994; Franzen and Richardson, 2000). The availability of iron in exchangeable, available, and reducible forms decreases with an increase in salinity (Dahiya and Singh, 1979). In

addition iron availability, root growth decreases as soil salinity increases (Hansen et al., 2007). Some studies have found that the concentration and form of soil Fe influences the severity of IDC (McKeague and Day, 1966; Morris et al., 1990). However, Inskeep and Bloom (1987) did not find the Fe soil test level as a factor. A North Dakota study by Franzen and Richardson (2000) found inconsistent results regarding DTPA-extractable Fe values. Some of the field locations within the study found differences in the DTPA-extractable Fe values between chlorotic and non-chlorotic areas. However, other field locations did not have differences. A Minnesota study by Hansen et al. (2003) found significant differences in DTPA-extractable Fe concentrations by field positions. Higher concentrations of Fe were observed in non-chlorotic field positions compared to chlorotic field positions.

Severity of IDC can be influenced by other factors. The micronutrient Mn can interfere with Fe uptake, which induces IDC (Roomizadeh and Karimina, 1996). Moraghan et al. (1986) observed that two soybean varieties grown on calcareous soils accumulated high Mn concentrations. The reason for high Mn concentrations is unknown. However, it is thought that Fe stressed plants may reduce or solubilize insoluble Mn (Brown et al., 1972; Moraghan et al., 1986). There was no interaction between Fe and Mn in a Minnesota study by Hansen et al. (2003), as Mn was lower in chlorotic areas. The presence of pests and diseases can also influence the severity of IDC (Charlson et al., 2004). The major pest of soybeans is the soybean cyst nematode (SCN) (*Heterodera glycmes).* It is thought that IDC may predispose the soybean to SCN infection in fields with a history of IDC and SCN. The SCN infection intensifies the severity of IDC. Further research of soil properties that influence IDC is necessary.

Cultural practices also influence the severity of IDC, such as seeding rate (Goos and Johnson, 2000). It was found that increasing seeding rates reduce IDC severity and increase yields. In fields where IDC is a problem, implement wheel tracks sometimes are green compared to IDC affected soybeans between the wheel tracks (Rehm, 2005). This indicates that soil properties, such as compaction, affect IDC.

Best management practices for IDC rotational soybean begins with the selection of cultivars less susceptible to IDC, as discussed. Currently, the private and public industries are developing soybean cultivars less susceptible to IDC by plant breeding. The plant breeding process includes genotype x environment interactions. The difficulty of developing a cultivar less susceptible to IDC is the environmental interaction. A cultivar less susceptible to IDC in one environment may be highly susceptible to IDC in other environments (Hansen et al., 2004). Thus, cultivars should be developed considering the specific environmental conditions present. Other control measures include foliar sprays (Goos and Johnson, 2000), soil-applied FeEDDHA (Goos and Johnson, 2000; Schenkeveld et al., 2008), cultivar mixtures (Fehr and Rodriguez, 1974) and companion crops (Naeve, 2006).

The "Chlorosis Paradox".

Soybean leaflets severely affected by IDC often contain higher Fe concentrations compared to healthy leaflets. This physiological situation is entitled "the chlorosis paradox" (Haussling et al., 1985; Römheld and Marschner, 2000). There are two hypotheses regarding the cause of IDC. The first hypothesis is that HCO_3^- inhibits the uptake, translocation, and utilization of Fe in plants (Chaney et al., 1972; Venkatraju and Marschner, 1981; Marschner, 1995; Römheld, 1986; Römheld, 2000). Bicarbonate

decreases soluble inorganic Fe by buffering the soil pH at a level where Fe availability is very low (Marschner, 1995). Strategy I responses are impaired, such as reduced efficiency of H^+-pump, decreasing release of phenolics, and decreased Fe (III) reduction at the plasma membrane (Marschner, 1995). Kolesch et al. (1984) and Dockendorf and Höfner (1990) indicated high HCO_3^- concentrations decrease the uptake and transport of Fe into the shoot system. The obtained Fe may be sequestered in root vacuoles, which inhibits Fe transport to the shoot. However, the extent of Fe accumulation of organic acids in vacuoles and contribution to reduced transport into the shoot is unknown (Marchner, 1995). The Fe transported into the shoot may be unevenly distributed within the leaflet tissues (Rutland and Bukavac, 1971; Rutland, 1971). This situation is further discussed in the second hypothesis. The high HCO_3^- concentrations reduce shoot growth before IDC (McCray and Matocha, 1992; Shi et al., 1993). Thus, high concentrations of Fe in leaflets may be due to the limitation of growth factors, such as leaf expansion, chloroplast development, and chlorophyll formation. The second hypothesis is the inactivation of Fe within the leaf apoplast by alkalinization due to a distant HCO_3^- effect (Mengel and Bubl, 1983; Römheld, 2000).

Biological Nitrogen Fixation.

Legumes and rhizobia bacteria benefit sustainable agriculture by BNF. Biological nitrogen fixation is the process where atmospheric N_2 is reduced to NH_4^+ by free-living soil microorganisms or within symbiotic associations of soil microorganisms and plants. The rhizobial species specific to soybeans is *Bradyrhizobium japonicum*. Nitrogen fertilizer is not recommended for soybean production as increased NO_3^- may increase IDC severity and suppress fixation (Kandel, 2010). Seed inoculation with *Bradyrhizobium japonicum* is

recommended especially on fields with no previous history of soybean (Berglund and Helms, 2003). Inoculating seed is an inexpensive insurance to assure an adequate population of rhizobia bacteria.

Bradyrhizobium japonicum is a free-living soil bacterium that can form a symbiotic association with soybean roots, inhabiting nodules and reducing atmospheric N_2 to a plant available form. The atmospheric N_2 reduced to NH_4^+ provides the soybean plant with an adequate level of N for biological functions with the following crop rotation receiving a nitrogen credit. Biological nitrogen fixation is an energy expensive process. The production of $2NH_3$ requires 16Mg-ATP molecules ($N_2 + 8H^+ + 8e^- + 16Mg\text{-}ATP \rightarrow 2NH_3 + H_2 + 16Mg\text{-}ADP + 16Pi$) (Marschner, 1995; Howard and Ress, 2006). However, the benefit of BNF outweighs the energy cost. The process of BNF requires an infected cell, unaffected cell, and xylem as atmospheric N_2 is reduced to NH_3, the NH_3 from the bacteroid is acidified to NH_4^+ by diffusing into the symbiosome space (White et al., 2007). Bacteroids and the surrounding peribacteroid membrane together are termed symbiosomes. Ammonium is then transported across the symbiosome membrane into the infected cell cytosol and into an adjacent uninfected cell for ureide synthesis (Smith and Atkins, 2002; White et al., 2007). The synthesized ureides are translocated into the plant shoot system via xylem. The presence of ureides indicates BNF, as ureides are not present without BNF in soybeans. Ureides, composed of allantoin and allantoic acids, are quantified by colormetric methods (Vogels and Van der Drift, 1970; Patterson et al., 1982). The ratio of water-extractable ureide-N in soybean tissues divided by the sum of ureide-N plus nitrate-N is an approximation of the current proportion of N coming from fixation verses the soil-N (Patterson and LaRue, 1983).

The process of BNF by rhizobia is initiated upon the exchange and recognition of chemical signals between the plant root and bacteria (Mylona et al., 1995). The chemical signals can be from the indigenous population or seed inoculated *Bradyrhizobium japonicum* having indigenous populations of *Bradyrhizobium japonicum* may suppress the seed inoculated *Bradyrhizobium japonicum* (Uma and Kalaiarasu, 2010). It is important that inoculant companies select *Bradyrhizobium japonicum* strains with the ability to compete with indigenous populations (Uma and Kalaiarasu, 2010). Upon positive recognition from both symbionts, the bacteria attaches to a root hair. Signaling molecules from the bacteria known as nodulation (*Nod)* factors induce curling of the root hair (Mylona et al., 1995). Cortical cells are mitotically reactivated by *Nod* factors with the location of cortical cells being species dependent (Mylona et al., 1995). *Bradyrhizobium japonicum* reactivates the outer cortical cells (Kijne, 1992). Pericycle cells, opposite to the reactivated cortical cells, also reactivate due to direct or indirect *Nod* factors (Mylona et al., 1995). A nodule is formed when the dividing cortical and pericycle cells merge and nodule vascular tissue develops. The nodule vascular tissue connects the nodule to the plant via xylem and phloem of the stele. For bacterium entry, an infection thread forms by the hydrolysis of the plant cell walls and the invagination of the plasma membrane of the curled root hair (Callaham and Torrey, 1981; Van Spronsen et al., 1994). The bacteria enter the developing nodule by a process resembling endocytosis from the infection thread (Bassett et al., 1977). Following entry, the bacteria differentiate into bacteroids, specialized cells that contain the rhizobia bacteria. An infected cell can contain as many as 50,000 rhizobia bacteria (Smith and Atkins, 2002). Biological nitrogen fixation is catalyzed by nitrogenase within the bacteroids with an interface with the peribacteroid

membrane. The peribacteroid membrane regulates the exchange of metabolites including NH_4^+, heme, and carbon sources (O'Gara and Shanmugam, 1976; Nadler and Avissar, 1977; De Bruijn et al., 1989; Werner, 1992). Heme is the prosthetic group of the oxygen transport protein leghemoglobin. Bacteroids and the surrounding peribacteroid membrane together are termed symbiosomes. Outside the symbiosomes, leghemoglobin buffers oxygen in the nodule, as nitrogenase is oxygen sensitive. Leghemoglobin has a high affinity for O_2. Functioning nodules will have a characteristic pink appearance from the leghemoglobin when dissected. Ineffective and developing nodules will appear green or white when dissected.

Arbuscular Mycorrhizas Associations.

The predominant mycorrhizas in agricultural crops and soils are AMF or vesicular-arbuscular mycorrhizal associations (VAM) (Trappe, 1987; Brundrett, 2004). It is important to note the terminology discrepancy between AMF and VAM. As of late, AMF has been the preferred term compared to VAM. Vesicles are not produced by all AMF, as indicated by the former, yet still used term, VAM (Brundrett, 2004).

Arbuscular mycorrhizal fungi are balanced mycorrhizal associations that assist in mineral nutrient uptake, especially P, by extending the rooting network (Vivekanandan and Fixen, 1991; Brundrett, 2004). Balanced mycorrhizal associations refer to the dynamic exchange process of AMF and the host plant as opposed to the term mutualistic association (Brundrett, 2004). When a plant host is colonized, it receives nutrients and in exchange provides AMF with carbon sources (Novero et al., 2001). Land management, such as crop rotation, is the most important component in maintaining AMF populations (Wood and Cummings, 1992; Gentili and Jumpponen, 2006). Arbuscular mycorrhizal fungi inoculants

have not been readily utilized in large-scale agriculture due to the extensive costs, slow turnover time, and colonization challenges related to root pathogens. High value nursery stocks commonly utilize AMF inoculants (Gentili and Jumpponen, 2006). Delayed planting, fallow systems, waterlogged soils, and some agricultural crops reduce AMF populations, as AMF are obligate symbionts (Brundrett, 2004). Sugarbeet (*Beta vugaris* L.), and canola (*Brassica napus* L.) are common crops of North Dakota that do not support AMF populations (Peterson et al., 2004). Planting crops, such as maize (*Zea mays*), wheat (*Triticum* spp.), and barley (*Hordeum vulgare*) that vigorously support AMF populations will maintain and increase the AMF populations.

Arbuscular mycorrhizal fungi colonization of a host plant is an asynchronous process that can be broadly described in stages: (1) AMF spore germination, (2) AMF penetration to host root, (3) colonization, (4) proliferation, and (5) senescence (Strack et al., 2003). The AMF spore germinates upon the positive exchange of chemical signals from a host plant, such as soybean. The hyphae that develop from the AMF spore penetrate the host plant root via appressorium resulting in colonization (Javaid, 2009). The AMF hyphae colonize the root cortex intercellularly and/or intracellulary (Javaid, 2009). The integrity of the root cortex cells is not affected as the architecture of the plant cell is modified upon colonization (Timonen and Peterson, 2002). The hyphae develop arbuscules, highly branched haustorium-like structures, within the cortex cell (Javaid, 2009). Arbuscules are accountable for nutrient exchange between the host plant and AMF (Javaid, 2009). The arbuscules have a short functioning lifespan, as senescence occurs after four to ten days of symbiosis (Sanders et al., 1977). Vesicles, intercellular storage organs, and extraradical spores may form depending on genus and species of AMF (Javaid, 2009).

Arbuscular mycorrhizal fungi obtain the mineral nutrient P in the form of $H_2PO_4^-$ and HPO_4^{2-}. Phosphorus, a macronutrient, is a key component of nucleic acids, phospholipids, and the adenosine triphosphate participating in every exchange reaction (Karandashov and Bucher, 2005). Phosphorus is very immobile in the soil (Wiederholt and Johnson, 2005). Therefore, the rhizosphere often develops a zone of depletion as plant roots readily utilize the available P originally in the rhizosphere (Smith and Read, 1997). However, it has been reported that AMF increased P uptake by 833% in grapevine (*Vitis vinifera*) (Schreiner, 2007). The increase of P is due to the exploration by AMF hyphae extending the rhizosphere (Giovannetti and Avio, 2002).

Light microscopy is the standard technique in determining the root colonization by AMF (Vierheilig et al., 2005). Arbuscular mycorrhizal fungi in roots are not easily visualized due to the natural pigments and cell contents of the plant root. The clearing process enables the internal plant root structures to be viewed by utilizing chemical agents to remove the cell contents and cell wall pigments (Gardner, 1975). Non-vital stains, such as aniline blue, further highlight AMF structures by binding to the fungal structures (Vierheilig et al., 2005).

Symbiont Interaction.

The symbionts are important factors in a sustainable agricultural system, as the symbionts in association with soybeans acquire additional nutrients (Keyser and Li, 1992). It has been observed that AMF enhance nodulation and efficiency of BNF in legumes (Patreze and Cordeiro, 2004; Kuster et al., 2007). The effect of AMF on improved nodulation and BNF by rhizobia may be due to the greater uptake of P and trace elements (Smith et al., 1979; Linderman, 1992). Competition between *Bradyrhizobium japonicum*

and AMF for colonization sites does not occur, perhaps due to chemical signaling (Barea and Azcon-Aguilar, 1983; Xie et al., 1995; Tobar et al., 1996). However, it was observed that one symbiont inhibited the development of the other symbiont in stressful environments (Bethlenfalvey et al., 1985).

Studies on the effect of iron deficiency on the development of BNF in soybean could not be located. However, effects of iron deficiency on BNF in blue lupine (*Lupinus angustifolius* L.) have been previously studied. Iron deficiency may limit symbiotic nitrogen fixation by effecting nodule initiation, development, and function (Tang et al., 1990; Tang et al., 1992a; Tang et al., 1992b). Blue lupine contained a higher nitrogen concentration when Fe supply was extremely limited compared to moderately Fe deficient plants (Tang et al., 1990). This indicated that plant growth was stunted by iron deficiency rather than by nitrogen deficiency. Microscopically, nodulation impairment of blue lupine was affected during the initiation stage by iron deficiency (Tang et al., 1990). Iron deficiency limited further divisions of root cortical cells by inhibiting *Bradyrhizobia* production from establishing nodule meristems (Tang et al., 1990). This impaired the release of *Bradyrhizobia* from the infection threads and proliferation of *Bradyrhizobia* within the cytoplasm (Tang et al., 1992b).

Auxin and cytokinin, phytohormones, produced by *Bradyrhizobia* are involved in nodulation formation (Libbenga and Torrey, 1973; Libbenga et al., 1973; Dart, 1977). Phytohormones are required for high metabolic activity or the stimulation of cortical cells to form nodules (Tang et al., 1990). The infection sites with low quantity of *Bradyrhizobia* are limited in the production of phytohormones as result of Fe deficiency (Tang et al., 1990). It is unclear if the lack of nodule initiation is from a low internal Fe supply, or

external Fe deficiency (Tang et al., 1990).

Iron deficiency is a common environmental stress observed in the Northern Great Plains. The relationship among IDC, BNF, and AMF are undefined (Novero et al., 2001). It has been observed that IDC may limit nodulation of *Bradyrhizobium japonicum* and AMF colonization (Porter et al., 1982; Franzen and Richardson, 2000). A reduction in nodulation and colonization, respectively, may decrease BNF and nutrient exploration and acquisition. It has been suggested that established and functioning AMF discontinues at the development of IDC (Porter et al., 1982). Furthermore, the relationship between IDC and AMF at North Dakota is unknown. Field research is necessary to observe the relationship between IDC and AMF in North Dakota.

Study Objectives.

The objectives of this thesis were to examine relationships between IDC and BNF by evaluating ureide concentrations utilizing an unpublished diacetyl monoxime analytical method (Goos method) and between IDC and AMF by a modified magnified line-intercept method (McGonigle et al., 1990). A comparative study of published ureide methods was compared to the unpublished Goos method.

PAPER 1. A COMPARATIVE STUDY OF UREIDE ANALYSES

Abstract

Ureides, the transport compounds of symbiotic nitrogen fixation in the soybean plant, are quantified by two key reactions (1) allantoin + base + heat → allantoic acid, and (2) allantoic acid + acid + heat → 2 urea + glyoxylate. The Vogels and Van der Drift (1970) method measures the glyoxylate structure present in allantoin and allantoic acids. The more time efficient Patterson et al. (1982) method measures ammonium-containing compounds after removal of most non-ureide compounds with an exchange resin. The concentration of ureides can also be quantified as urea with the Goos method (unpublished). This study was conducted to compare the Vogels and Van der Drift, Patterson et al., and Goos methods. 'Glacier' soybean plants, containing differing amounts of ureide-N, were analyzed by all three methods. The Vogels and Van der Drift and Goos methods specifically measured the chemical aspects of ureides. The Patterson et al. method had greater ureide concentrations due to the non-specific measurement of ammonium compounds. All methods had a linear absorbance by the determination of allantoin concentrations. The Goos and Patterson et al. methods agreed closely in ureide analyses while the Vogels and Van der Drift did not closely agree.

Introduction

Ureides, composed of allantoin and allantoic acids, are the transport compounds of symbiotic nitrogen fixation in the soybean plant (Patterson et. al., 1982). Thus, the ureide concentration in the above ground plant tissues indicates the relative rate of nitrogen fixation by the root nodules. The two key reactions in quantifying ureide concentrations are (1) allantoin + base + heat → allantoic acid, and (2) allantoic acid + acid + heat → 2

urea + glyoxylate. The Patterson et al. (1982) and Vogels and Van der Drift (1970) methods are commonly utilized for ureide analyses. For convenience, the Vogels and Van der Drift and Patterson et al. methods, respectively, will be referred to as the Vogels and Patterson methods. Both methods differ in the chemical quantification of ureides. The Vogels method detects the glyoxylate structure present in allantoin and allantoic acids, after treatment with base and acid as shown in the two reactions above. The Patterson method is non-specific in measuring ammonium-containing compounds. In comparison, the Patterson method is a quick non-specific colormetric method while the Vogels method requires use of hazardous chemicals, but specifically measures the glyoxylate structure. In the Patterson method, the sample extract is treated with H^+-resin, removing ammonium and other cationic ammonium-containing compounds, like amino acids, to make the determination specific for ureides.

Considering the chemistry of allantoin and allantoic acids, the concentration of ureides can be quantified as urea. The Goos method (unpublished) is a colormetric diacetyl monoxime method that measures the urea present in allantoin and allantoic acids. The chemistry of the Goos method is similar to human blood or soil extracts (Douglas and Bremner, 1970; Mulvaney and Bremner, 1979; Greenan et al., 1995; Ochei and Kolhatkar. 2000). The objective of this study is to compare the Vogels, Patterson, and Goos methods.

Material and Methods

'Glacier' soybean seeds were inoculated with *Bradyrhizobium japonicum* and greenhouse grown within on a mixture of 1 kg sand and 1 kg Renshaw soil (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, frigid Calcic Hapludolls) per pot. Four plants per pot were grown from seed for 3, 4, and 5 weeks. Plants were watered according

to weight based on the water-holding capacity of the soil-sand mixture and rotated daily. There were two treatments: unamended (control) and 200 mg N pot^{-1} as ammonium nitrate. The ureide concentrations of soybean tops were measured according the three methods being compared.

The plant samples were dried (60°C, 36 hr), ground, weighed (0.400 g) and placed in a screw-top culture tubes with 20 mL of deionized water per culture tube. The screw-top culture tubes were placed in a 90°C water bath for 30 minutes, and the suspensions filtered. Approximately half of the filtrate was used for the Vogels method. For the Patterson and Goos methods, approximately 2 g of H^+ resin were added to the remaining extract and culture tubes were agitated for 30 minutes. The plant extract was filtered and resin recovered. The concentration of ureide-N was estimated by the three methods and the concentration of ureide-N in the plant sample was calculated multiplying by the appropriate dilution factor.

Vogels Method.

The Vogels method measures the glyoxylate structure present in allantoin and allantoic acids. In a 15 mL screw-top culture tube, 0.5 mL of standard or extract, 0.5 mL water, and 1 mL 0.5 M NaOH were mixed. The 15 mL screw-top culture tubes were optically matched to also serve as a spectrophotometer tube. Two sets of standards were read per twelve samples. A standard set consisted of 0, 5, 10, 15, and 20 mg allantoin-N L^1. The tubes containing the standards or plant extracts were mixed and placed in a 90°C water bath for 30 minutes. The samples were removed, allowed to cool, 1 mL of 0.65 M HCl added and mixed. The samples were placed in a 90°C water bath for 30 minutes and allowed to cool to room temperature. One mL of phosphate buffer (1 M KH_2PO_4 buffer,

pH 6.5) was added to each tube. After mixing each sample, 1 mL phenyl hydrazine solution (0.10 g $C_6H_5NHNH_2$ and 30 mL of water) was added, mixed, and allowed to react for 5 minutes. Concentrated HCl at 0°C was added to each tube at 1.25 mL increments totaling 5 mL. One mL of ferricyanide solution (0.50 g potassium ferricyanide and 30 mL water) was added and mixed. The samples were cooled to room temperature in the dark for15 minutes prior to reading the color intensity. Color intensity was measured with a spectrophotometer at 535 nm.

Patterson Method.

The Patterson method is non-specific in measuring ureide ammonium compounds. In a 15 mL screw-top culture tube, 1mL of standard or sample, and 1mL 0.2 M phthalate buffer was added. The 15 mL screw-top culture tubes were optically matched to the standard spectrophotometer tube. Two sets of standards were read per twelve samples. A standard set consisted of 0, 5, 10, 15, and 20 mg allantoin-N L^{-1}. The samples were mixed following the addition of 0.05 mL diluted household chlorine bleach (10 mL bleach and 30 mL water). After 5 minutes of standing time, 2 mL of color developing reagent (15 mL 20% NaOH, 40 mL phenol solution (135 g phenol, 100 mL water, and 250 mL methanol) was added to each spectrophotometer tube. The samples were allowed to stand for 10 minutes followed by the addition of 5.5 mL water. Samples were read by the spectrophotometer at 625 nm transmittance.

Goos Method.

The Goos method is a diacetyl monoxime method that measures the urea present in allantoin and allantoic acids. The standard or sample extract was pipetted (0.5 mL) into 15 mL screw-top culture test tubes with 0.5 mL of 0.5 N NaOH. The 15 mL screw-top culture

tubes were optically matched to the standard spectrophotometer tube. Two sets of standards were read per twelve samples. A standard set consisted of 0, 5, 10, 15, and 20 mg allantoin-N L^{-1}. The sample-NaOH tube mixtures were placed in a 90°C water bath for 30 minutes. Following cooling to room temperature, 2.33 mL deionized water, and 5 mL color developing reagent were added. The color developing reagent was prepared daily. The color developing reagent was composed of 150 mL acid reagent (960 mL phosphoric acid and 40 mL sulfuric acid), 7.5 mL diacetyl monoxime (DAM) solution (3.75 g $C_4H_7NO_2$ and 100 mL water), and 4.5 mL thiosemicarbazide (TSC) solution (0.375 $NH_2CSNHNH_2$ and 100 mL water). The tubes were mixed and placed in a 90°C water bath for 55 minutes. The water bath was covered to block light, as the reaction is light sensitive. Samples were cooled in the dark and read at 525 nm transmittance by the spectrophotometer.

Results and Discussion

The allantoin concentration and absorbance of standards by the Vogels, Patterson, and Goos methods are shown in Figure 1. All methods gave a linear relationship between allantoin concentration and absorbance. The Vogels and Goos methods were the most similar. However, the Goos method had a tendency to measure a lower absorbance compared to the Vogels method. The Patterson method had greater absorbance values compared to the Patterson and Goos methods, as the Patterson is non-specific in measuring ureide-N ammonium compounds.

The Patterson and Goos methods are compared in Figure 2. The Patterson method measured slightly higher ureide concentrations than the Goos method, as the Patterson method measures non-specific ammonium compounds. The Goos method specifically

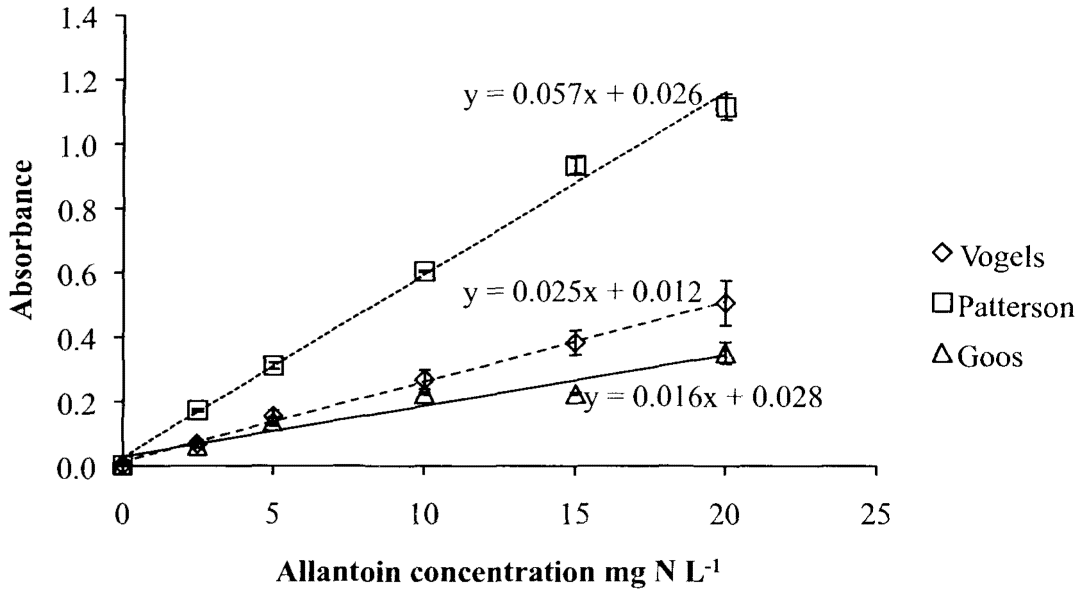

Figure 1. The allantoin concentration and absorbance of standards by three methods of ureide analyses with standard deviations of the mean.

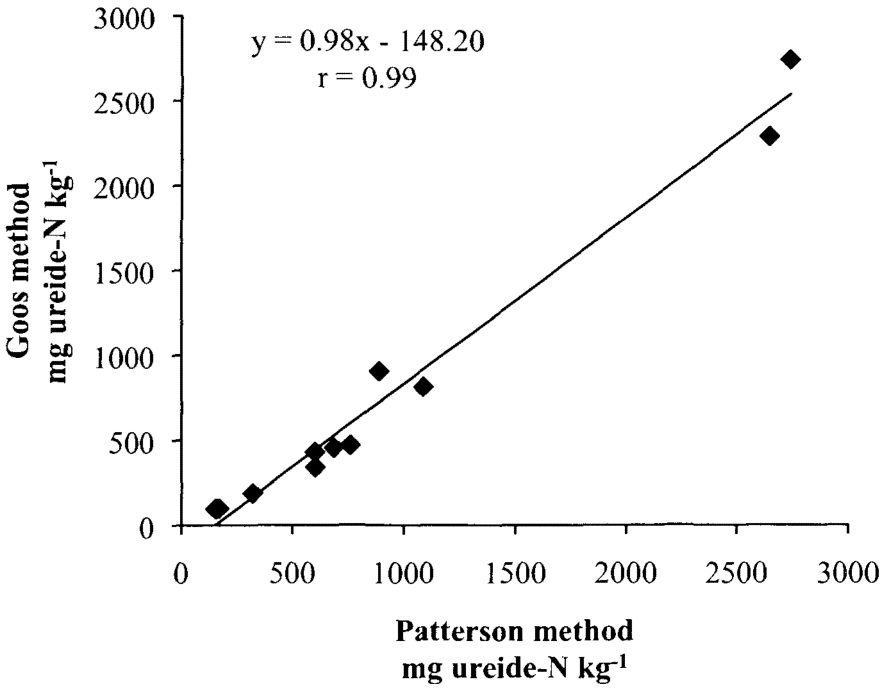

Figure 2. A comparison of ureide analysis: the Goos and Patterson methods.

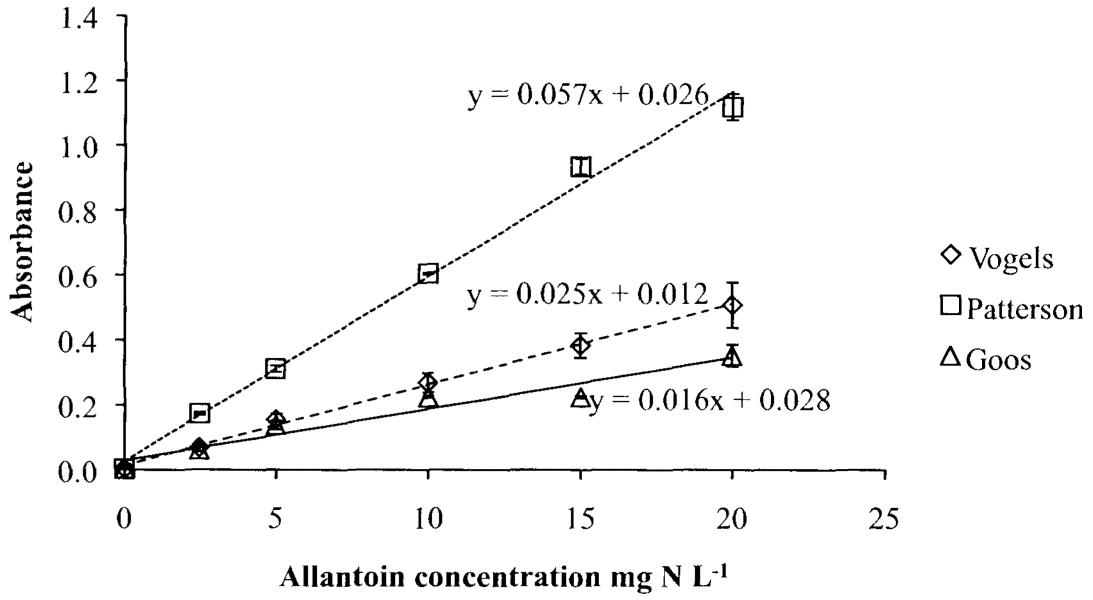

Figure 1. The allantoin concentration and absorbance of standards by three methods of ureide analyses with standard deviations of the mean.

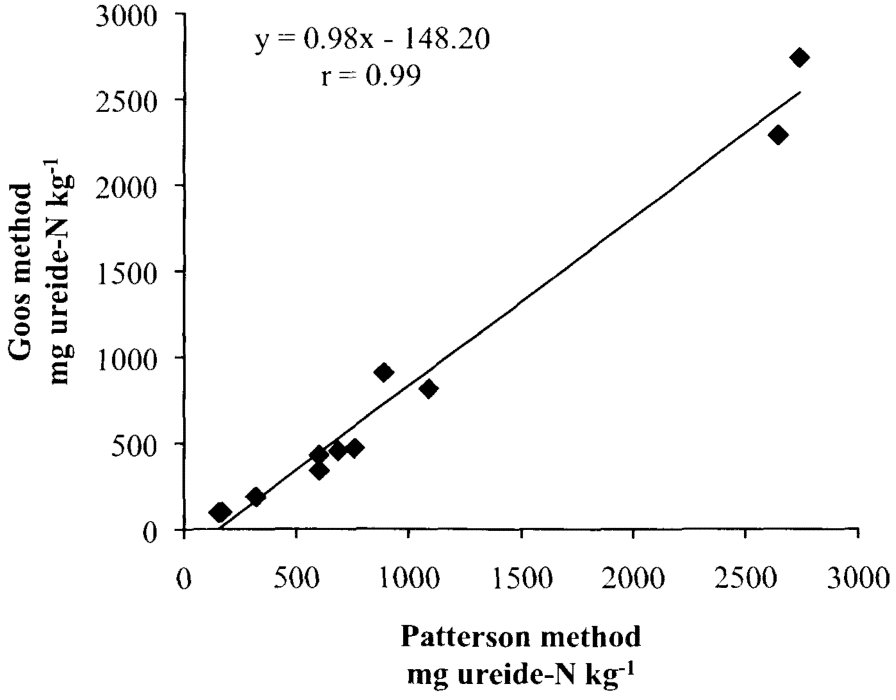

Figure 2. A comparison of ureide analysis: the Goos and Patterson methods.

measures urea. According to the correlation coefficient, the Patterson and Goos method gave nearly identical results. Thus, the Patterson and Goos methods are comparable methods in ureide analyses.

The concentration of ureide-N by the difference between the Goos and Patterson methods are shown in Figure 3. The Goos method usually measured lower ureide concentrations than the Patterson method, as most of the points fell beneath the zero line. The difference between methods may be due to the compound being quantified. The Goos method specifically quantifies urea while the Patterson method quantifies ammonium-containing compounds. The Patterson method may be measuring nitrogen-containing compounds not associated with ureides.

The Patterson and Vogels methods are compared in Figure 4. The Patterson method quantified higher ureide concentrations than the Vogels method, especially at ureide-N levels greater than 500 mg kg^{-1}. The Patterson method had approximately twice the ureide concentration than the Vogels method in the upper absorbance reading. The Patterson method may be measuring nitrogen-containing compounds not associated with ureides, or there were components in the plant extracts that interfered with the Vogels method.

The concentration of ureide-N by difference between the Vogels and Patterson methods is shown in Figure 5. The two methods gave about the same reading between 500-1000 mg ureide-N kg^{-1}, but the two methods diverged dramatically at higher or lower levels of plant ureide-N. The Patterson method may be measuring nitrogen-containing compounds not associated with ureides while the Vogels methods specifically measures the glyoxylate structure.

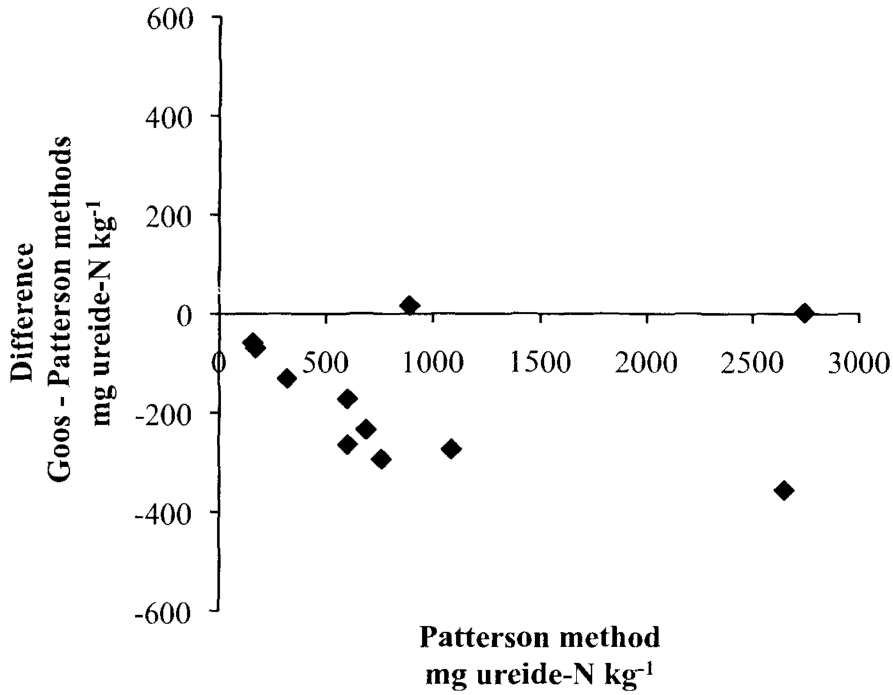

Figure 3. The difference between the Goos and Patterson methods.

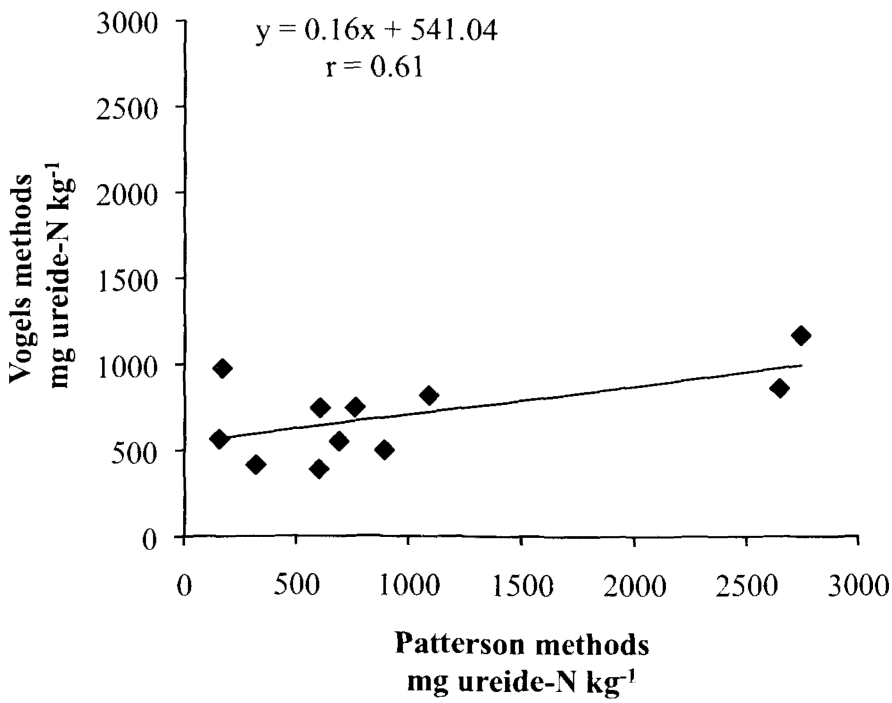

Figure 4. A comparison of ureide analysis: the Patterson and Vogels methods.

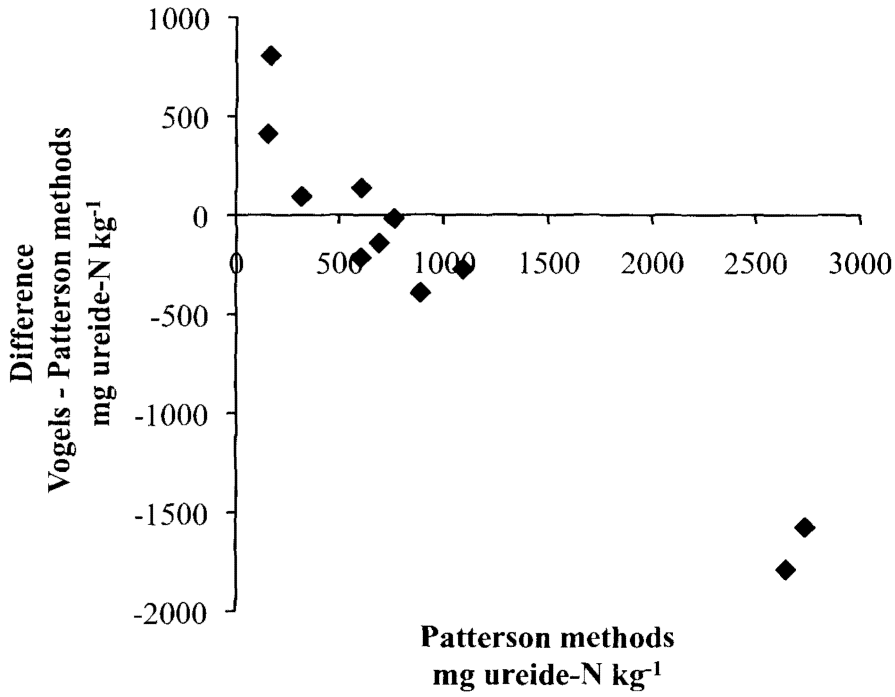

Figure 5. The difference between the Vogels and Patterson methods.

Summary and Conclusions

The method utilized did influence the concentration of ureides determined. All methods gave a linear relationship between allantoin concentration and absorbance. The Patterson method was more sensitive than the Vogels or Goos methods, as indicated by greater absorbance readings for a given amount of allantoin. The Goos and Patterson methods are comparable methods in ureide analyses, as the both methods gave nearly identical results though the Patterson method tended to quantify higher ureide concentrations. The Vogels and Patterson methods had a greater difference in ureide concentrations than the Goos and Patterson methods. The difference between the Vogels method and Patterson methods may be due the plant extract. The plant extract utilized for the Patterson methods required agitation with H^+-resin that removed non-ureide ammonium compounds while the Vogel method did not require H^+-resin. The Goos method also

utilized H^+-resin for removal of non-ureide ammonium compounds. Until further investigation, difference between the Vogels and Patterson methods may be due to a compound within the Vogels plant extract.

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PAPER 2. RELATIVE NITROGEN FIXATION RATE AND ARBUSCULAR MYCORRHIZAL FUNGI COLONIZATION OF IRON DEFICIENT SOYBEANS

Abstract

Soybeans are a symbiont of two beneficial associations: biological nitrogen fixation (BNF) with *Bradyrhizobium japonicum*, and arbuscular mycorrhizal fungi (AMF). Within the Northern Great Plains of the USA, iron deficiency chlorosis (IDC) of soybean is a yield-limiting factor. The effects of IDC on BNF and AMF are not well defined. This study was conducted to determine the effects of IDC on BNF and AMF. Three field studies were conducted in eastern North Dakota on sites with a history of producing IDC in soybean. The three cultivars, in the order of decreasing chlorosis susceptibility, were NuTech NT-0886, Roughrider Genetics RG 607, and Syngenta S01-C9 RR. The three treatments were control (unamended), grain sorghum (*Sorghum bicolor* L.) companion crop planted with the soybean seed, and FeEDDHA applied with the soybean seed. Chlorosis severity was the greatest and least for the NuTech and Syngenta cultivars, respectively. The FeEDDHA treatment decreased chlorosis severity. Ureide levels were abnormally high in plants severely stunted by IDC. The excess accumulation of ureides in IDC-stunted plants suggests that plant growth was reduced more than the rate of nitrogen fixation. The AMF population was at adequate levels at field studies, and not affected by cultivar or treatment, in general, which may be contributed to crop rotations that support AMF populations.

Introduction

Soybeans grown in the Northern Great Plains of the USA may exhibit iron deficiency chlorosis (IDC). Symptoms of IDC are yellowing leaves, interveinal chlorosis, stunting, and a reduced yield (Franzen and Richardson, 2000; Goos and Johnson, 2001). Severity of IDC is influenced by pH, soil temperature, HCO_3^- concentration in the soil solution, soil $CaCO_3$ content, and soil water content (Inskeep and Bloom, 1986; Moraghan and Mascagni, 1991). Goos and Johnson (2001) recommend planting IDC tolerant soybeans to alleviate chlorosis symptoms in IDC-prone soils. The effects of IDC on the two symbiotic relationships, biological nitrogen fixation (BNF) by *Bradyrhizobium japonicum* and arbuscular mycorrhizal fungi (AMF), are not well defined. The presence of IDC may limit early BNF nodule development, and AMF colonization (Porter et al., 1982; Tang et al., 1992; Franzen and Richardson, 2000). The objective of this study was to compare the relationship of cultivar selection, and seed treatments with BNF and AMF colonization in IDC-prone soils.

Material and Methods

Three field studies were conducted on locations in Cass County, North Dakota, near the towns of Ayr, Hunter, and, Leonard during the 2009 growing season, on sites with a history of producing IDC in soybean (see Appendix B Table 1B). The soil series Ayr, Hunter, and Leonard sites were Hamerly (Fine-loamy, mixed, superactive, frigid Aeric Calciaquolls), Glyndon (Coarse-silty, mixed, superactive, frigid Aeric Calciaquolls), and Hamerly, respectively (Omodt et al., 1966; Prochnow et al., 1985). Previous crop history was a soybean-maize (*Zea mays* L.) rotation for all locations. Field soil was collected from six locations at 0-15 cm and 0-61 cm depths by a bucket soil auger. The collected soil was

mixed, subsampled, air-dried and crushed to pass a 2 mm sieve for laboratory analyses. The experimental design was a factorial of three cultivars x three treatments. The treatments were arranged in a completely random design with four replications. The three cultivars, in the order of decreasing chlorosis susceptibility, were NuTech NT-0886, Roughrider Genetics RG 607, and Syngenta S01-C9 RR. For convenience, these three cultivars will be referred to as the susceptible, intermediate, and resistant cultivars, respectively. The three treatments were control (unamended), grain sorghum (*Sorghum bicolor* L.) companion crop planted with the soybean seed, and FeEDDHA (Soygreen, 6% iron chelate with 80% orthro-orthro isomer) applied with the soybean seed. A plot consisted of a single row, 4.9 m long. Row spacing was 76 cm. The soybean and grain sorghum seeding rates, and FeEDDHA rate were 229,710 seeds ha^{-1} for all soybean cultivars, 459,420 seeds ha^{-1} for grain sorghum, and 3.36 kg ha^{-1} for FeEDDHA. The FeEDDHA was placed with the seed impregnated on inert perlite. The FeEDDHA (94 g) was dissolved in 300 mL of water, mixed with 563 g of sieved (2-4 mm) perlite, and air-dried. The appropriate amount of FeEDDHA-impregnated perlite was weighed and planted with the soybean seed. The FeEDDHA was obtained from West Central, Inc., Fargo, North Dakota. Grain sorghum was selected as the companion crop due to the strong AMF relationship and rapid growth (Ellis et al., 1992). Mechanical and hand weeding maintained weed control.

Plants were rated and sampled at the V2 to V3 stages and two weeks later at approximately the V4 to V8 stages depending on IDC severity. Plants were rated for IDC with chlorosis score ±0.5 units (Goos and Johnson, 2000). The index was scaled from 1 to 5 with 1–no chlorosis, 2–slight chlorosis of the upper leaflets, 3–interveinal chlorosis of

upper leaflets with no stunting or necrosis, 4–interveinal chlorosis with stunted growth or some leaflet necrosis, and 5–growing point and upper leaflets necrotic or entire plant dead (Goos and Johnson, 2000). A harvest zone of 1 m long was measured from the middle of the plot row and marked for crop yield determination. Following chlorosis rating and harvest zone designation, 10 plants per plot were excavated with roots intact outside the harvest zone. The excavated plants were selected at random with 5 plants excavated from either side of the designated harvest area. The average relative chlorophyll content (Minolta SPAD meter) per plot was measured on the most fully developed vegetative leaflet stage during the V2-V3 and V4-V8 stages. Grain sorghum was chemically removed (0.84 glyphosate kg ha^{-1}) following the V2-V3 sampling, as the soybeans were Round-up Ready. Excavated plants were separated by the shoot (defined by the plant parts above the soil surface) and root systems (below the soil surface) and placed in separate bags for transportation. Above ground plant parts were divided into leaflets and axes (stems plus petioles). Plant material was stored at 5°C until processed for drying. Shoots and roots were, respectively, processed for drying within 4 and 10 hr from field removal. The leaflets were rinsed with water, dried (60°C, 36 hr), and weighed. The axes were dried (60°C, 36 hr) and weighed. Roots were rinsed in water and cut into 1 cm increments after nodules were counted (Giovannetti and Mosse, 1980). Seed yield was measured at maturity within the designated harvest area by cutting plants at the soil surface. Grain was threshed, cleaned, and weighed.

Ureides, composed of allantoin acid and allantoic acid, are the transport compounds of symbiotic nitrogen fixation in the soybean plant (Patterson et al., 1982). The concentration of ureides in the above soil plant tissue (axes) indicates the relative rate of

nitrogen fixation by the root nodules. Ureide concentrations were quantified according to a diacetyl monoxime method (R.J. Goos, unpublished). The Goos method measures ureides as urea after an alkaline hydrolysis step. Nitrate concentrations in the axes were measured according to the salicylic acid method (Cataldo et al., 1975). The nitrate concentrations are of interest as N inhibits legume nodules (Salvagiotti et al., 2008; Kandel, 2010). Agvise Laboratories located at Northwood, North Dakota analyzed the dried leaflets for nutrient element contents.

Arbuscular mycorrhizal fungi colonization was quantified according to a magnified line-intercept method (McGonigle et al., 1990). The roots were rehydrated with distilled water and 0.2 g of root was placed in biopsy cartridges (VWR Premium Biopsy Cassette). The biopsy cartridges and roots were placed in 10% KOH at 90°C water bath for 10 min and rinsed with distilled water. Roots were stained with aniline blue for 5 min (Grace and Stribley, 1991). The biopsy cartridges-roots were distained with distilled water (Vierheilig and Piché, 1998). Stained roots were placed on slides with 20 roots per slide (VWR 3x1 mm slides). Slides were made semi-permanent with the addition of Polyvinyl-Lacto-Glycerol (PVGL) (PVG 99-100% hydrolyzed) and 22 x 60 mm cover slip (Omar et al., 1978). One hundred roots were assessed to assure an adequate percent colonization (Biermann and Linderman, 1981). The intercept line was randomly selected at 200 x magnification (McGonigle et al., 1990). The data analysis for this paper was generated using SAS software, Version 9.2 of the SAS System for Windows, GLM command (SAS Institute, 2008).

Results and Discussions

Soil Chemical Characteristics.

The soil chemical characteristics at three North Dakota locations are shown in Table 1. The soils at all locations were alkaline (pH > 7) and non-saline (EC < 1). The risk for IDC on soybeans based on $CaCO_3$ content and soluble for Ayr is high while a moderate risk for Hunter and Leonard (Agvise, Laboratories, 2001). Available P was within the mid range for Ayr and Hunter while very low for Leonard (Kandel, 2010). Available K was in the very low range for Ayr and Leonard while Hunter was within the middle range (Kandel, 2010). The Ayr site had the greatest available K and sulfate-S compared to the other locations. Hunter had the greatest nitrate-N. Leonard had the greatest $CaCO_3$, and available Fe compared to Ayr and Hunter. Hunter was the lowest in available K.

Visual Chlorosis Ratings, Chlorophyll Content, and Plant Matter.

The effects of cultivar, companion crop, and FeEDDHA on soybean plants for the V2-V3 soybean stages at Ayr are shown in Table 2. The chlorosis scores indicated severe chlorosis for the susceptible cultivar, as the chlorosis score was 4.3 for the control. The severity of ICD decreased in the intermediate and resistant cultivars. The application of FeEDDHA reduced the chlorosis scores of all cultivars. The trends observed with chlorosis score were reflected in the relative chlorophyll readings. Cultivar effects ($p < 0.05$) were substantial, as cultivars more resistant to IDC had greater chlorophyll readings. The relative chlorophyll content increased with the FeEDDHA treatment for all three cultivars. The susceptible cultivar dramatically increased the relative chlorophyll content with the FeEDDHA treatment. Cultivar ($p < 0.05$) and FeEDDHA ($p < 0.05$) also

Table 1. Soil chemical characteristics at three North Dakota locations in 2009.

Measurement†	Units	Location‡		
		Ayr	Hunter	Leonard
0-15 cm depth				
pH		8.0	8.3	8.4
EC	d Sm^{-1}	0.9	0.4	0.3
$CaCO_3$	g kg^{-1}	58	37	155
Organic Matter	g kg^{-1}	44	30	52
Avail. P	mg kg^{-1}	9	11	3
Avail. K	mg kg^{-1}	400	45	195
Avail. Fe	mg kg^{-1}	2.7	2.1	4.2
0-61 cm depth				
Nitrate-N	kg ha^{-1}	87	112	47
Sulfate-S	kg ha^{-1}	1121	115	129

†pH, and EC determined on a 1:1 soil: water suspension, CaCO3 by pressure calcimetry, organic matter by weight loss on ignition, available P by the Olsen method, available K by ammonium acetate method, available Fe by the DTPA method, nitrate-N water extraction by the salicylic acid method, sulfate-S monocalcium phosphate extraction by turbidimetric determination.

‡The location is named according to the nearest city.

significantly affected the aboveground dry matter.

The effects of cultivar, companion crop, and FeEDDHA on soybean plants for the V4-V8 soybean stages at Ayr are shown in Table 3. The chlorosis scores for the susceptible cultivar was high indicating severe chlorosis. Chlorosis was severe for the susceptible cultivar and control, as the chlorosis score was 4.6. The FeEDDHA treatment reduced chlorosis severity for all cultivars. The relative chlorophyll content increased with the FeEDDHA treatment compared to the control treatment for all cultivars. However, the relative chlorophyll content for the companion crop treatment was greater than the FeEDDHA treatment for the susceptible and intermediate cultivars. The greatest dry matter production among all three cultivars was observed with the FeEDDHA treatment.

Table 2. Chlorosis score, relative chlorophyll content, and aboveground dry matter for the V2-V3 soybean stages at Ayr, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Chlorosis score[§]	Relative chlorophyll[¶]	Dry matter
				g plant^{-1}
Susceptible	Control	4.3	9.3	0.34
	C. crop	4.3	14.8	0.39
	FeEDDHA	2.5	30.1	0.77
Intermediate	Control	2.8	19.4	0.73
	C. crop	2.8	21.2	0.60
	FeEDDHA	2.5	26.6	0.94
Resistant	Control	2.0	25.3	0.68
	C. crop	1.3	24.7	0.70
	FeEDDHA	1.4	30.1	0.85
	Sig. of F[#]			
	Cultivar	*	*	*
	Treatment	*	*	*
	Cultivar x Treatment	*	*	NS
	LSD[††]			
	Cultivar	0.5	2.4	0.10
	Treatment	0.5	2.4	0.10
	Cultivar x Treatment	0.8	4.2	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.

[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.

[§] Chlorosis score, 1 = no chlorosis, 5 = most severe chlorosis.

[¶] Relative chlorophyll content using a Minolta SPAD meter.

[#] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.[#] NS = not significant.

[††] Least significant difference at the 0.05 probability level.

Table 3. Chlorosis score, relative chlorophyll content, and aboveground dry matter for the V4-V8 soybean stages at Ayr, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Chlorosis score[§]	Relative chlorophyll[¶]	Dry matter
				g plant^{-1}
Susceptible	Control	4.6	1.4	0.53
	C. crop	3.9	11.5	0.63
	FeEDDHA	3.1	9.4	1.62
Intermediate	Control	2.9	11.9	1.62
	C. crop	2.3	17.6	1.22
	FeEDDHA	2.0	16.5	2.48
Resistant	Control	1.6	24.4	1.62
	C. crop	1.5	20.9	1.59
	FeEDDHA	1.3	25.1	2.27
	Sig. of F[#]			
	Cultivar	*	*	*
	Treatment	*	*	*
	Cultivar x Treatment	NS	*	NS
	LSD[††]			
	Cultivar	0.3	2.9	0.3
	Treatment	0.3	2.9	0.3
	Cultivar x Treatment	-	5.0	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.

[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.

[§] Chlorosis score, 1 = no chlorosis, 5 = most severe chlorosis.

[¶] Relative chlorophyll content using a Minolta SPAD meter.

[#] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.

[††] Least significant difference at the 0.05 probability level.

The aboveground dry matter content was significantly affected by cultivar ($p < 0.05$) and treatment ($p < 0.05$).

The effects of cultivar, companion crop, and FeEDDHA on soybean plants for the V2-V3 soybean stages at Hunter are shown in Table 4. The susceptible cultivar chlorosis scores indicated severe chlorosis, especially for the control treatment. The chlorosis severity was reduced with cultivars resistant to IDC, as indicated by the chlorosis scores. The FeEDDHA treatment reduced the chlorosis scores for all cultivars, although not significantly different. The resistant and intermediate cultivars had greater relative chlorophyll contents compared to the susceptible cultivar. The FeEDDHA treatment significantly increased the relative chlorophyll content in the susceptible cultivar, as the relative chlorophyll content for the control and FeEDDHA, respectively, was 2.6 and 24.8. The FeEDDHA treatment also increased the relative chlorophyll contents for the cultivars resistant to IDC. Aboveground dry matter was significantly affected by cultivar ($p < 0.05$) and treatment ($p < 0.05$). The FeEDDHA treatment increased dry matter in all cultivars. The companion crop dry matter per plant was not significantly different from the control (means separations not displayed).

The effects of cultivar, companion crop, and FeEDDHA on soybean plants for the V4-V8 soybean stages at Hunter are shown in Table 5. The chlorosis scores were the greatest in the susceptible cultivar. The chlorosis scores decreased with cultivars more resistant to IDC and with the FeEDDHA treatment. The FeEDDHA treatment appeared to suppress the relative chlorophyll content for the susceptible and intermediate cultivars. The FeEDDHA treatment increased the aboveground dry matter production, but not the relative chlorophyll content for the susceptible and intermediate cultivar. The dry matter

Table 4. Chlorosis score, relative chlorophyll content, and aboveground dry matter for the V2-V3 soybean stages at Hunter, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Chlorosis score[§]	Relative chlorophyll[¶]	Dry matter
				g plant^{-1}
Susceptible	Control	4.1	2.6	0.21
	C. crop	3.8	5.2	0.22
	FeEDDHA	2.1	24.8	0.60
Intermediate	Control	3.0	13.7	0.41
	C. crop	3.3	10.5	0.38
	FeEDDHA	2.5	24.9	0.82
Resistant	Control	2.3	19.2	0.48
	C. crop	2.1	21.6	0.46
	FeEDDHA	1.9	29.8	0.74
	Sig. of F[#]			
	Cultivar	*	*	*
	Treatment	NS	*	*
	Cultivar x Treatment	NS	NS	NS
	LSD[††]			
	Cultivar	0.7	4.3	0.07
	Treatment	-	7.4	0.12
	Cultivar x Treatment	-	-	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.

[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.

[§] Chlorosis score, 1 = no chlorosis, 5 = most severe chlorosis.

[¶] Relative chlorophyll content using a Minolta SPAD meter.

[#] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.

[††] Least significant difference at the 0.05 probability level.

Table 5. Chlorosis score, relative chlorophyll content, and aboveground dry matter for the V4-V8 soybean stages at Hunter, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Chlorosis score[§]	Relative chlorophyll[¶]	Dry matter
				g plant^{-1}
Susceptible	Control	4.4	11.7	0.32
	C. crop	4.4	11.6	0.31
	FeEDDHA	3.6	6.5	1.26
Intermediate	Control	3.9	13.6	0.84
	C. crop	3.8	10.5	0.75
	FeEDDHA	3.3	11.0	1.90
Resistant	Control	2.8	23.0	1.30
	C. crop	2.9	20.2	1.21
	FeEDDHA	2.5	23.0	1.86
	Sig. of F[#]			
	Cultivar	*	*	*
	Treatment	*	NS	*
	Cultivar x Treatment	NS	NS	NS
	LSD[††]			
	Cultivar	0.4	3.7	0.25
	Treatment	0.4	-	0.25
	Cultivar x Treatment	-	-	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.

[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.

[§] Chlorosis score, 1 = no chlorosis, 5 = most severe chlorosis.

[¶] Relative chlorophyll content using a Minolta SPAD meter.

[#] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.

[††] Least significant difference at the 0.05 probability level.

per plant was the least in the susceptible cultivar, but significantly increased for the intermediate and resistant cultivars.

The effects of cultivar, companion crop, and FeEDDHA on soybean plants for the V2-V3 soybean stages at Leonard are shown in Table 6. The chlorosis scores for the susceptible cultivar were high, as the chlorosis score was 3.9 for the control. The chlorosis scores decreased with more resistant cultivars indicating a reduction in chlorosis severity. The companion crop treatment reduced the chlorosis scores for all cultivars perhaps due to the utilization of soil water, nitrate, or solubilize soil-Fe from phytosiderophores. The relative chlorophyll content increased with the FeEDDHA treatment for all three cultivars. The amount of dry matter increased as the cultivar resistance to IDC increased. The resistant cultivar control treatment produced almost double the amount of dry matter than the susceptible cultivar control treatment. The FeEDDHA treatment approximately doubled the dry matter produced in the susceptible cultivar compared to the control treatment. The resistant and intermediate cultivars dry matter also increased with the FeEDDHA treatment. The companion crop treatment effects on dry matter were statically similar to the control treatment (means separation not displayed).

The effects of cultivar, companion crop, and FeEDDHA on soybean plants for the V4-V8 soybean stages at Leonard are shown in Table 7. The susceptible cultivar had the more severe chlorosis, as the chlorosis score for the control was 3.9. The cultivars with more IDC resistant had lower chlorosis scores. The FeEDDHA treatment reduced the chlorosis scores in the susceptible and resistant cultivars. For the intermediate cultivar, the control had less chlorosis compared to the FeEDDHA treatment. The companion crop treatment reduced chlorosis score for the susceptible cultivar. The intermediate and

Table 6. Chlorosis score, relative chlorophyll content, and aboveground dry matter for the V2-V3 soybean stages at Leonard, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Chlorosis score[§]	Relative chlorophyll[¶]	Dry matter
				g plant^{-1}
Susceptible	Control	3.9	9.9	0.35
	C. crop	3.3	17.1	0.38
	FeEDDHA	2.8	16.7	0.61
Intermediate	Control	2.5	16.7	0.67
	C. crop	1.7	21.0	0.66
	FeEDDHA	2.4	21.5	0.93
Resistant	Control	1.6	23.6	0.72
	C. crop	1.4	18.6	0.63
	FeEDDHA	1.3	25.4	0.74
	Sig. of F[#]			
	Cultivar	*	*	*
	Treatment	*	NS	*
	Cultivar x Treatment	*	NS	NS
	LSD[††]			
	Cultivar	0.3	3.3	0.07
	Treatment	0.5	-	0.13
	Cultivar x Treatment	-	-	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.

[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.

[§] Chlorosis score, 1 = no chlorosis, 5 = most severe chlorosis.

[¶] Relative chlorophyll content using a Minolta SPAD meter.

[#] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.

[††] Least significant difference at the 0.05 probability level.

Table 7. Chlorosis score, relative chlorophyll content, and aboveground dry matter for the V4-V8 soybean stages at Leonard, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Chlorosis score[§]	Relative chlorophyll[¶]	Dry matter
				g plant^{-1}
Susceptible	Control	3.9	9.0	0.66
	C. crop	3.4	9.6	0.78
	FeEDDHA	3.1	11.8	1.43
Intermediate	Control	1.9	16.8	1.62
	C. crop	2.4	19.5	1.65
	FeEDDHA	2.1	22.0	2.54
Resistant	Control	1.4	26.5	1.92
	C. crop	1.5	23.8	1.61
	FeEDDHA	1.1	27.0	2.24
	Sig. of F[#]			
	Cultivar	*	*	*
	Treatment	NS	NS	*
	Cultivar x Treatment	NS	NS	NS
	LSD[††]			
	Cultivar	0.3	2.7	0.29
	Treatment	-	-	0.29
	Cultivar x Treatment	-	-	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.

[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.

[§] Chlorosis score, 1 = no chlorosis, 5 = most severe chlorosis.

[¶] Relative chlorophyll content using a Minolta SPAD meter.

[#] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.

[††] Least significant difference at the 0.05 probability level.

resistant cultivars chlorosis scores were slightly greater with the companion crop treatment. The relative chlorophyll content and aboveground dry matter increased with the FeEDDHA treatment for all three cultivars. Thus, the trends observed with relative chlorophyll content were reflected in the aboveground dry matter. Regardless of chlorosis scores, the intermediate cultivar followed the trends in relative chlorophyll content and aboveground dry matter. The companion crop treatment increased the relative chlorophyll content and dry matter for the susceptible and intermediate cultivars. The resistant cultivar companion crop treatment slightly decreased the relative chlorophyll content and dry matter.

Ureide and Nitrate Concentrations.

The ureide and nitrate concentrations for the V2-V3 and V4-V8 soybean stages at Ayr are shown in Table 8. Ureide concentrations decreased as the cultivar resistance to IDC increased, as the ureide concentrations for the first and V4-V8 soybean stages were abnormally high in the susceptible cultivar. Young, healthy soybean plants in North Dakota typically contain 500-1000 mg kg^{-1} ureide-N in the plant axes (Goos et al., 2002). The companion crop treatment reduced ureide concentrations for all cultivars at the V2-V3 soybean stages, and for the susceptible and intermediate cultivar for the V4-V8 soybean stages. The FeEDDHA treatment reduced ureide concentrations for the V2-V3 soybean stages susceptible and intermediate cultivars and all cultivars for the V4-V8 soybean stages. Uriede concentrations increased as growth increased, being the greatest for the susceptible cultivar, decreasing with the intermediate cultivar, and the lowest for the resistant cultivar.

Nitrate concentrations decreased as the cultivar resistance to IDC increased. The nitrate concentrations decreased from the V2-V3 and V4-V8 soybean stages for the

Table 8. Ureide and nitrate concentrations of soybean axes at Ayr, North Dakota, 2009.

Cultivar†	Treatment‡	V2-V3 soybean stages		V4-V8 soybean stages	
		Ureide	Nitrate	Ureide	Nitrate
		mg N kg^{-1}			
Susceptible	Control	1988	3444	4339	1175
	C. crop	768	2594	830	1193
	FeEDDHA	530	1820	695	1338
Intermediate	Control	497	1933	638	920
	C. crop	294	903	445	866
	FeEDDHA	359	1205	517	1007
Resistant	Control	249	97	420	852
	C. crop	220	55	470	629
	FeEDDHA	254	112	385	736
	Sig. of F§				
	Cultivar	*	*	*	*
	Treatment	*	*	*	NS
	Cultivar x Treatment	*	*	*	NS
	LSD¶				
	Cultivar	0.3	2.7	0.29	
	Treatment	-	-	0.29	
	Cultivar x Treatment	-	-	-	93

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

susceptible and intermediate cultivars. The resistant cultivar nitrate concentrations increased between samplings. The FeEDDHA treatment decreased the nitrate concentrations for the V2-V3 soybean stages susceptible and intermediate cultivars and the V4-V8 soybean stages susceptible and resistant cultivars. The companion crop treatment reduced nitrate concentrations in the V2-V3 soybean stages for all cultivars.

The ureide and nitrate concentrations for the V2-V3 and V4-V8 soybean stages at Hunter are shown in Table 9. The ureide concentrations were abnormally high in the susceptible cultivar in all treatments. For the V2-V3 soybean stages, the companion crop treatment increased the ureide concentrations for the susceptible and resistant cultivars. The FeEDDHA application decreased ureide concentrations in the susceptible cultivar. The resistant cultivar had the lowest ureide concentration, presumably because growth was not as limited by IDC. The ureide concentrations for the V4-V8 soybean stages were the greatest for the susceptible cultivar and lower for the other two cultivars.

Nitrate concentrations decreased as the cultivar resistance to IDC decreased. The companion crop decreased nitrate concentrations in the V2-V3 soybean stages cultivars, but increased all V4-V8 soybean stages cultivars. Nitrates for the V2-V3 soybean stages increased as FeEDDHA was added to the susceptible cultivar, probably due to increased root growth. Nitrate values were over 1500 mg N kg^{-1} for all cultivars for the V4-V8 soybean stages indicating an abundance of nitrate-N in the soil (Table 1).

The ureide and nitrate concentrations at Leonard are shown in Table 10. Ureide concentrations were abnormally high in the susceptible cultivar, especially within for the control treatment. At the V2-V3 soybean stages, the companion crop decreased the ureide concentrations for all cultivars. In addition, the FeEDDHA application decreased ureide

Table 9. Ureide and nitrate concentrations of soybean axes at Hunter, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	V2-V3 soybean stages		V4-V8 soybean stages	
		Ureide	Nitrate	Ureide	Nitrate
		mg N kg^{-1}			
Susceptible	Control	2134	500	2974	3230
	C. crop	2593	344	1239	3483
	FeEDDHA	947	2790	3322	3855
Intermediate	Control	868	2390	583	3509
	C. crop	402	2132	641	3689
	FeEDDHA	688	2613	1525	3544
Resistant	Control	321	2179	282	1597
	C. crop	360	2115	500	1652
	FeEDDHA	348	1692	583	3093
	Sig. of F[§]				
	Cultivar	*	*	*	*
	Treatment	*	*	*	NS
	Cult x Trt	*	*	*	NS
	LSD[¶]				
	Cultivar	0.3	2.7	0.29	
	Treatment	-	-	0.29	
	Cultivar x Treatment	-	-	-	99

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
[§] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
[¶] Least significant difference at the 0.05 probability level.

Table 10. Ureide and nitrate concentrations of soybean axes at Leonard, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	V2-V3 soybean stages		V4-V8 soybean stages	
		Ureide	Nitrate	Ureide	Nitrate
		mg N kg^{-1}			
Susceptible	Control	3275	949	2706	2511
	C. crop	834	772	2675	2198
	FeEDDHA	427	707	3062	2425
Intermediate	Control	485	293	647	1970
	C. crop	167	279	687	847
	FeEDDHA	538	326	493	399
Resistant	Control	334	228	410	597
	C. crop	217	141	746	597
	FeEDDHA	133	312	298	611
	Sig. of F[§]				
	Cultivar	*	*	*	*
	Treatment	*	+	NS	*
	Cult x Trt	*	*	*	*
	LSD[¶]				
	Cultivar	0.3	2.7	0.29	
	Treatment	-	-	0.29	
	Cultivar x Treatment	-	-	-	99

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
[§] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
[¶] Least significant difference at the 0.05 probability level.

concentrations for the susceptible and resistant cultivars. The lowest ureide concentration was in the resistant cultivar, as plant growth was not limited. For the V4-V8 soybean stages, ureide concentrations were the greatest for the susceptible cultivar and lower for the other two cultivars.

Nitrate concentrations decreased as the cultivar resistance to IDC decreased. The companion crop decreased nitrate concentrations in the cultivars at the V2-V3 soybean stages. Nitrate concentrations also decreased with the FeEDDHA treatment in all cultivars. The FeEDDHA application decreased nitrate concentrations in all cultivars. For the V4-V8 soybean stages, the susceptible cultivar nitrate values were over 1500 mg N kg^{-1} indicating an abundance of nitrate-N in the soil (Table 1).

Nodules.

The nodule number and mass of the V2-V3 soybean stages at Ayr are shown in Table 11. The number of nodules per plant and nodule fresh weight mg per plant was not significantly different. Although, the companion crop decreased the number of nodules per plant for the susceptible and intermediate cultivars though the decrease was not significant. The FeEDDHA treatment significantly increased the nodule fresh weight mg per nodule. The nodule fresh weight mg per nodule for the control and companion crop was similar.

The nodule number and mass for the V4-V8 soybean stages at Ayr are shown in Table 12. The number of nodules per plant and nodule fresh weight mg per plant was not significantly different. The nodule fresh weight mg per nodule increased as cultivar resistant increased. The FeEDDHA treatment increased nodule fresh weight mg per nodule. The intermediate cultivar FeEDDHA treatment had the greatest nodule fresh weight mg per nodule while the susceptible cultivar control treatment and susceptible

Table 11. Nodule number and mass for the V2-V3 soybean stages at Ayr, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Nodules	----- Nodule fresh weight -----	
		no. plant^{-1}	mg plant^{-1}	mg nodule^{-1}
Susceptible	Control	10	23	2
	C. crop	9	19	2
	FeEDDHA	25	128	5
Intermediate	Control	32	115	3
	C. crop	17	60	4
	FeEDDHA	21	99	4
Resistant	Control	18	60	3
	C. crop	34	107	3
	FeEDDHA	25	97	4
	Sig. of F[§]			
	Cultivar	NS	NS	NS
	Treatment	NS	NS	*
	Cultivar x Treatment	NS	NS	NS
	LSD[¶]			
	Cultivar	-	-	-
	Treatment	-	-	0.8
	Cultivar x Treatment	-	-	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
[§] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
[¶] Least significant difference at the 0.05 probability level.

Table 12. Nodule number and mass for the V4-V8 soybean stages at Ayr, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Nodules	----- Nodule fresh weight -----	
		no. plant^{-1}	mg plant^{-1}	mg nodules^{-1}
Susceptible	Control	16	48	2
	C. crop	8	25	2
	FeEDDHA	22	173	8
Intermediate	Control	35	213	5
	C. crop	18	101	5
	FeEDDHA	26	212	8
Resistant	Control	25	173	6
	C. crop	32	231	7
	FeEDDHA	21	160	7
	Sig. of F[§]			
	Cultivar	NS	*	*
	Treatment	NS	NS	*
	Cultivar x Treatment	NS	NS	*
	LSD[¶]			
	Cultivar	-	101	1
	Treatment	-	-	1
	Cultivar x Treatment	-	-	2

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
[§] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
[¶] Least significant difference at the 0.05 probability level.

cultivar companion crop treatment had the least nodule fresh weight mg per nodule.

The nodule number and mass for the V2-V3 soybean stages at Hunter are shown in Table 13. The intermediate cultivar had the greatest nodule number per plant while the resistant cultivar had the least nodule number per plant. The FeEDDHA treatment increased nodule number per plant for the susceptive and resistant cultivars though not significantly different. The FeEDDHA treatment significantly increased the nodule fresh weight per plant and nodule fresh weight per nodule for all cultivars. The nodule fresh weight mg per nodule was approximately doubled with the FeEDDHA application. The companion crop nodule fresh weight mg per plant and nodule fresh weight mg per nodule was statically similar to the control treatment.

The nodule number and mass of the V4-V8 soybean stages at Hunter are shown in Table 14. The control treatment had less number of nodules per plant compared to the companion crop and FeEDDHA treatment for all cultivars. The nodule fresh weight per plant was the greatest in the control treatment for all cultivars. However, the nodule fresh weight per nodule was increased with the FeEDDHA treatment. This indicated that the FeEDDHA treatment assisted in nodule fresh weight per nodule more than nodule fresh weight per plant.

The nodule number and mass of the V2-V3 soybean stages at Leonard are shown in Table 15. The intermediate cultivar significantly increased the nodule number per plant while the susceptible cultivar significantly decreased the nodule number per plant. The nodule fresh weight mg per plant and nodule fresh weight mg per nodule significantly decreased with the susceptible cultivar. The FeEDDHA treatment significantly increased the nodule fresh weight mg per plant and nodule fresh weight mg per nodule. The

Table 13. Nodule number and mass for the V2-V3 soybean stages at Hunter, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Nodules	----- Nodule fresh weight -----	
		no. plant^{-1}	mg plant^{-1}	mg nodule^{-1}
Susceptible	Control	10	16	2
	C. crop	12	21	2
	FeEDDHA	16	73	4
Intermediate	Control	21	33	2
	C. crop	12	20	2
	FeEDDHA	21	90	4
Resistant	Control	8	19	3
	C. crop	10	18	2
	FeEDDHA	11	48	4
	Sig. of F[§]			
	Cultivar	*	NS	NS
	Treatment	NS	*	*
	Cultivar x Treatment	NS	NS	NS
	LSD[¶]			
	Cultivar	3.6	-	-
	Treatment	-	16	0.7
	Cultivar x Treatment	-	-	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
[§] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
[¶] Least significant difference at the 0.05 probability level.

Table 14. Nodule number and mass for the V4-V8 soybean stages at Hunter, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Nodules	----- Nodule fresh weight -----	
		no. plant^{-1}	mg plant^{-1}	mg nodule^{-1}
Susceptible	Control	11	22	2
	C. crop	12	23	2
	FeEDDHA	19	117	6
Intermediate	Control	14	35	3
	C. crop	16	36	2
	FeEDDHA	25	157	6
Resistant	Control	9	35	4
	C. crop	13	36	3
	FeEDDHA	14	86	7
	Sig. of F[§]			
	Cultivar	*	*	*
	Treatment	*	*	*
	Cultivar x Treatment	NS	*	NS
	LSD[¶]			
	Cultivar	3.5	17	1
	Treatment	3.5	17	1
	Cultivar x Treatment	-	30	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
[§] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
[¶] Least significant difference at the 0.05 probability level.

Table 15. Nodule number and mass for the V2-V3 soybean stages at Leonard, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Nodules	----- Nodule fresh weight -----	
		no. plant^{-1}	mg plant^{-1}	mg nod^{-1}
Susceptible	Control	19	57	3
	C. crop	15	61	4
	FeEDDHA	19	95	5
Intermediate	Control	34	126	4
	C. crop	22	118	5
	FeEDDHA	28	159	6
Resistant	Control	22	113	5
	C. crop	23	105	5
	FeEDDHA	23	121	5
	Sig. of F[§]			
	Cultivar	*	*	*
	Treatment	NS	*	*
	Cultivar x Treatment	NS	NS	NS
	LSD[¶]			
	Cultivar	4.0	22	0.8
	Treatment	-	22	0.8
	Cultivar x Treatment	-	-	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
[§] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
[¶] Least significant difference at the 0.05 probability level.

companion crop treatment was statically similar to the control treatment. The intermediate cultivar FeEDDHA treatment had the greatest nodule number per plants, nodule fresh weight mg per plant, and nodule fresh weight mg per nod.

The nodule number and mass of the V4-V8 soybean stages at Leonard are shown in Table 16. The intermediate cultivar had the most number of nodules per plant and nodule fresh weight mg per plant while the susceptible cultivar had the least number of nodules per plant and nodule fresh weight mg per plant. The susceptible cultivar control treatment had the least nodule fresh mg per nodule. The FeEDDHA treatment significantly increased the nodule fresh weight per nodule.

AMF Colonization and Yield.

The AMF colonization and soybean yield at Ayr are shown in Table 17. There was no significance in AMF colonization in all cultivars and treatments. It was interesting that the resistant cultivar FeEDDHA treatment had slightly greater colonization while the intermediate cultivar FeEDDHA treatment had the least colonization. The FeEDDHA treatment increased yield in all cultivars, especially in the susceptible cultivar, though not significantly different. The susceptible cultivar failed to yield with the control and companion crop treatments. Thus, selecting an IDC resistant cultivar is more critical than a FeEDDHA treatment, but the FeEDDHA treatment increased yield. In conjunction with an IDC resistant cultivar, the FeEDDHA treatment increased yield for the intermediate and resistant cultivars, respectively, approximately 50% and 15%. The FeEDDHA treatment with the intermediate cultivar had the greatest yield. However, the resistant cultivar had greater yields in the control and companion crop treatments.

The AMF colonization and soybean yield at Hunter is shown are Table 18. The

Table 16. Nodule number and mass for the V4-V8 soybean stages at Leonard, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	Nodules	----- Nodule fresh weight -----	
		no. plant^{-1}	mg plant^{-1}	mg nodule^{-1}
Susceptible	Control	18	108	6
	C. crop	17	145	8
	FeEDDHA	15	152	10
Intermediate	Control	31	228	8
	C. crop	26	258	10
	FeEDDHA	24	251	11
Resistant	Control	20	226	11
	C. crop	22	200	9
	FeEDDHA	17	192	11
	Sig. of F[§]			
	Cultivar	*	*	*
	Treatment	NS	NS	*
	Cultivar x Treatment	NS	NS	*
	LSD[¶]			
	Cultivar	4	50.5	0.8
	Treatment	-	-	0.8
	Cultivar x Treatment	-	-	2.6

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.

[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.

[§] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.

[¶] Least significant difference at the 0.05 probability level.

Table 17. Arbuscular mycorrhizal fungi colonization and soybean yield at Ayr, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	AMF	Yield
		%	kg ha^{-1}
Susceptible	Control	45	0
	C. crop	45	0
	FeEDDHA	45	1368
Intermediate	Control	45	1768
	C. crop	44	1817
	FeEDDHA	41	2694
Resistant	Control	47	2211
	C. crop	43	2274
	FeEDDHA	50	2533
	Sig. of F[§]		
	Cultivar	NS	*
	Treatment	NS	*
	Cultivar x Treatment	NS	NS
	LSD[¶]		
	Cultivar	-	745
	Treatment	-	745
	Cultivar x Treatment	-	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
[§] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
[¶] Least significant difference at the 0.05 probability level.

Table 18. Arbuscular mycorrhizal fungi colonization and soybean yield at Hunter, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	AMF	Yield
		%	kg ha^{-1}
Susceptible	Control	36	0
	C. crop	47	43
	FeEDDHA	50	784
Intermediate	Control	39	1010
	C. crop	41	190
	FeEDDHA	42	1988
Resistant	Control	47	2205
	C. crop	46	2474
	FeEDDHA	43	2871
	Sig. of F[§]		
	Cultivar	NS	*
	Treatment	NS	*
	Cultivar x Treatment	NS	NS
	LSD[¶]		
	Cultivar	-	461
	Treatment	-	461
	Cultivar x Treatment	-	-

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
[§] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
[¶] Least significant difference at the 0.05 probability level.

AMF population was not influenced by cultivar or treatment. Although not significant AMF population was not influenced by cultivar or treatment. Although not significant difference in AMF colonization was observed between treatments, the highest and lowest AMF colonization occurred in the susceptible cultivar. The control had the lowest AMF colonization while the FeEDDHA treatment had the highest colonization AMF though no significant difference. The yield was influenced by cultivar and treatment. The susceptible cultivar had a significantly poor yield while the resistant cultivar had a significantly greater yield. The FeEDDHA treatment significantly increased yield for all cultivars. Thus, selecting an IDC resistant cultivar is more critical than a FeEDDHA treatment, as resistant cultivar had a greater yield in all treatments. However, a resistant cultivar with FeEDDHA treatment optimizes the yield.

The AMF colonization and soybean yield at Leonard in shown are Table 19. The AMF colonization was influenced by treatment at $p \leq 0.05$. The FeEDDHA treatment significantly increased colonization in the susceptible and intermediate cultivars. The companion crop treatment had the least AMF colonization, which was unexpected as grain sorghum has a strong colonization rate. The intermediate cultivar had the greatest yield. The FeEDDHA treatment increased yield for the susceptible and resistant cultivars. The intermediate cultivar had a greater yield with the control treatment than the FeEDDHA treatment. The control treatment yield for all cultivars was greater than the companion crop yield.

Nutrient Analyses of Soybean Leaflets: N, P, and K.

The N, P, and K soybean leaflet analyses at Ayr for both sampling is shown in Table 20. At the V2-V3 soybean stages, the susceptible cultivar had the greatest N, P, and

Table 19. Arbuscular mycorrhizal fungi colonization and soybean yield in Leonard, North Dakota, 2009.

Cultivar†	Treatment‡	AMF	Yield
		%	kg ha^{-1}
Susceptible	Control	38	896
	C. crop	37	512
	FeEDDHA	39	1549
Intermediate	Control	39	2204
	C. crop	30	1865
	FeEDDHA	42	2037
Resistant	Control	44	1885
	C. crop	32	1801
	FeEDDHA	39	1949
	Sig. of F§		
	Cultivar	NS	*
	Treatment	*	*
	Cultivar x Treatment	NS	*
	LSD¶		
	Cultivar	-	458
	Treatment	8.2	458
	Cultivar x Treatment	-	794

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

Table 20. Soybean leaflet nutrient analyses for N, P, and K at Ayr, North Dakota, 2009.

Cultivar†	Treatment‡	V2-V3 soybean stages			V4-V8 soybean stages		
		N	P	K	N	P	K
		g kg^{-1}					
Susceptible	Control	53.9	4.5	22.4	54.8	4.3	23.4
	C. crop	49.6	3.6	21.2	48.7	3.0	19.3
	FeEDDHA	46.1	3.2	21.1	56.1	3.6	19.0
Intermediate	Control	45.7	3.5	23.6	53.0	3.9	22.8
	C. crop	43.5	3.0	22.2	44.9	2.7	19.4
	FeEDDHA	42.5	3.0	21.6	51.7	3.3	20.8
Resistant	Control	39.9	2.9	26.5	45.7	2.9	23.2
	C. crop	37.3	2.7	27.4	43.4	3.0	23.8
	FeEDDHA	39.9	2.8	25.9	46.8	3.0	23.9
	Sig. of F§						
	Cultivar	*	*	*	*	*	*
	Treatment	*	*	NS	*	*	*
	Cultivar x Treatment	NS	*	NS	NS	*	NS
	LSD¶						
	Cultivar	3.04	0.3	1.6	2.34	0.3	1.85
	Treatment	3.04	0.3	-	2.34	0.3	1.85
	Cultivar x Treatment	-	0.4	-	-	0.5	-

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

K concentrations. The V2-V3 soybean stages N, P, and K concentrations were the greatest in the control treatment for the susceptible and intermediate cultivars. The resistant cultivar control treatment was the greatest in P V2-V3 soybean stages. The V2-V3 soybean stages N and P control and FeEDDHA treatments were the greatest for the resistant cultivar. Potassium was the greatest in the companion crop resistant cultivar in the V2-V3 soybean stages. Similar trends were observed in the V4-V8 soybean stages. The N, P, and K concentrations were greater in the susceptible cultivar. The N, P, and K concentrations were the greatest in the control treatments for the intermediate cultivar. The susceptible and resistant cultivars FeEDDHA treatment had the greatest concentration N content. The resistant cultivar K concentration was the greatest in the FeEDDHA treatment. The P concentration was the greatest in the companion crop and FeEDDHA treatments.

The effects of N, P, and K soybean leaflet analyses at Hunter for both samplings are shown in Table 21. The N, P, and K concentrations V2-V3 soybean stages were greater in the susceptible cultivar compared to the resistant cultivars. The V2-V3 soybean stages FeEDDHA treatment for all cultivars suppressed the concentration of N, P, and K. The V2-V3 soybean stages control treatment had the greatest N, P, and K concentrations in the susceptible and resistant cultivars. The V2-V3 soybean stages control treatment had greater concentration in N, P, and K compared to the other treatments. For the V4-V8 soybean stages, the N, P, and K concentrations were greater in the susceptible cultivar, as observed in the V2-V3 soybean stages. Unlike the V2-V3 soybean stages, the FeEDDHA treatment increased N, P, and K concentrations in the resistant cultivar. The FeEDDHA treatment also had increased concentrations of N and P in the susceptible cultivar. The

Table 21. Soybean leaflet nutrient analyses for N, P, and K at Hunter, North Dakota, 2009.

Cultivar†	Treatment‡	V2-V3 soybean stages			V4-V8 soybean stages		
		N	P	K	N	P	K
		g kg^{-1}					
Susceptible	Control	58.9	5.7	16.4	50.5	4.9	15.5
	C. crop	57.2	5.0	15.5	46.4	4.0	12.4
	FeEDDHA	55.4	5.0	13.1	54.1	5.1	13.7
Intermediate	Control	55.4	4.8	17.1	50.1	4.5	15.6
	C. crop	55.7	4.3	15.6	47.1	4.1	13.8
	FeEDDHA	52.0	4.6	13.2	49.2	4.9	14.2
Resistant	Control	49.2	4.3	15.0	44.0	3.8	11.9
	C. crop	47.5	3.4	15.0	42.5	3.5	12.0
	FeEDDHA	46.7	3.7	12.7	44.7	4.0	12.2
	Sig. of F§						
	Cultivar	*	*	NS	*	*	*
	Treatment	*	*	*	*	*	NS
	Cultivar x Treatment	NS	NS	NS	NS	NS	NS
	LSD¶						
	Cultivar	2.32	0.3	-	2.19	0.3	1.54
	Treatment	2.32	0.3	1.24	2.19	0.3	1.54
	Cultivar x Treatment	-	-	-	-	-	-

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

control treatment had greater concentration in K for the susceptible and intermediate cultivars.

The effect of N, P, and K soybean leaflet analyses at Leonard for both samplings are shown in Table 22. The V2-V3 soybean stages N, P, and K concentrations were greater in the susceptible cultivar. The N and P concentrations at the V2-V3 soybean stages were the least in the companion crop treatment. Cultivar effected K concentration more than treatment, as the resistant cultivar had the lowest K content. For the V4-V8 soybean stages, there was no significance in the treatment effect on N, P, and K. However, the susceptible cultivar had greater N, P, and K concentrations compared to the other cultivars.

Nutrient Analyses of Soybean Leaflets: S, Fe, and Mn.

The effects S, Fe, and Mn soybean leaflet analyses at Ayr for both samplings are shown in Table 23. The S, Fe, and Mn concentrations were greater in the susceptible cultivar for both samplings, although, the Fe and Mn concentrations were not significant. The S concentration for both samplings was the greatest with the control treatment. The Fe concentration showed not significant differences at the V2-V3 soybean stages. For the V4-V8 soybean stages, the control treatment had the greatest Fe concentration while the FeEDDHA treatment had the least Fe content. For the V2-V3 soybean stages, Mn concentration was not significantly affected by cultivar, but was significantly affected by treatment. The control treatment had the greatest for Mn content. The FeEDDHA application had the lowest Mn content for all cultivar. It has been documented that FeEDDHA increases Fe concentrations, but decreases Mn concentrations (Moraghan, 1985; Ghasemi-Fasaei et al., 2003). The antagonistic effect of Fe on Mn can be attributed to the dilution effect and plant growth (Moraghan, 1985). A similar trend was observed in

Table 22. Soybean leaflet nutrient analyses for N, P, and K at Leonard, North Dakota, 2009.

Cultivar†	Treatment‡	V2-V3 soybean stages			V4-V8 soybean stages		
		N	P	K	N	P	K
		g kg^{-1}					
Susceptible	Control	44.1	3.5	20.4	48.1	3.4	19.1
	C. crop	38.3	2.4	17.7	48.6	3.5	20.6
	FeEDDHA	43.6	2.8	18.4	52.7	3.8	21.0
Intermediate	Control	39.0	2.8	20.8	49.1	3.6	21.9
	C. crop	32.7	2.2	20.6	44.3	3.1	21.5
	FeEDDHA	38.9	2.4	20.3	44.8	3.2	22.1
Resistant	Control	33.7	2.1	21.5	42.3	2.9	23.3
	C. crop	32.0	1.8	20.1	41.8	2.6	21.0
	FeEDDHA	33.0	2.2	22.9	42.2	2.6	22.7
	Sig. of F§						
	Cultivar	*	*	*	*	*	*
	Treatment	*	*	NS	NS	NS	NS
	Cultivar x Treatment	NS	NS	NS	*	NS	NS
	LSD¶						
	Cultivar	2.32	0.3	1.24	2.19	0.3	1.54
	Treatment	2.32	0.3	-	-	-	-
	Cultivar x Treatment	-	-	-	3.79	-	-

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.

‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.

§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.

¶ Least significant difference at the 0.05 probability level.

Table 23. Soybean leaflet nutrient analyses of S, Fe, and Mn at Ayr, North Dakota, 2009.

Cultivar†	Treatment‡	V2-V3 soybean stages			V4-V8 soybean stages		
		S	Fe	Mn	S	Fe	Mn
		g kg^{-1}	---- mg kg^{-1} ----		g kg^{-1}	---- mg kg^{-1} ----	
Susceptible	Control	5.5	217	293	5.5	192	384
	C. crop	5.5	216	299	6.1	150	364
	FeEDDHA	3.8	160	112	4.6	94	200
Intermediate	Control	4.0	160	273	4.3	100	329
	C. crop	4.5	193	272	5.8	110	312
	FeEDDHA	4.5	148	146	5.4	89	226
Resistant	Control	4.2	169	317	4.0	96	383
	C. crop	2.5	167	269	2.7	89	326
	FeEDDHA	3.5	166	143	4.1	93	313
	Sig. of F§						
	Cultivar	*	NS	NS	*	*	NS
	Treatment	NS	NS	*	NS	*	*
	Cultivar x Treatment	NS	NS	NS	NS	*	NS
	LSD¶						
	Cultivar	1.1	-	-	1.2	17	41.0
	Treatment	-	-	35.7	-	17	41.0
	Cultivar x Treatment	-	-	-	-	30	-

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

Mn at the V4-V8 soybean stages, as Mn concentration was the greatest in the control treatment. The resistant cultivar had the most Mn concentration followed by the susceptible cultivar. In contrast, the resistant cultivar accumulated the least Fe concentration ($p < 0.05$).

The effects of S, Fe, and Mn soybean leaflet analyses at Hunter for both samplings are shown in Table 24. The S, and Fe concentrations were greater in the susceptible cultivar for both samplings. In contrast, the Mn concentrations were greater in the resistant cultivar for both samplings. The S, Fe, and Mn concentrations were the lowest in the FeEDDHA treated plants for both samplings.

The S, Fe, and Mn soybean leaflet analyses at Leonard for both samplings are shown in Table 25. The S, and Fe concentrations were greater in the susceptible cultivar for both samplings although only S was significantly higher. The susceptible cultivar had the lowest Mn concentration for the V2-V3 soybean stages, but the greatest Mn concentration for the V4-V8 soybean stages and not significant. The control treatment had the greatest S concentration for both samplings. Treatment was not significant for Fe concentration for the V2-V3 soybean stages. In contrast, FeEDDHA treatment for Fe concentration ($p < 0.05$) had the least accumulation for the V4-V8 soybean stages. Cultivar was not significant for Mn concentration for both samplings. However, treatment was significant for the first and V4-V8 soybean stages, respectively, 95% probability level. The companion crop treatment had the greatest Mn concentration for the V2-V3 soybean stages but not significant while the control treatment had the greatest Mn concentration for the V4-V8 soybean stages.

Table 24. Soybean leaflet nutrient analyses of S, Fe, and Mn at Hunter, North Dakota, 2009.

Cultivar†	Treatment‡	V2-V3 soybean stages			V4-V8 soybean stages		
		S	Fe	Mn	S	Fe	Mn
		g kg^{-1}	---- mg kg^{-1} ----		g kg^{-1}	---- mg kg^{-1} ----	
Susceptible	Control	3.4	187	177	3.1	155	253
	C. crop	3.3	153	165	2.9	156	228
	FeEDDHA	2.8	113	74	2.9	75	143
Intermediate	Control	3.0	123	198	2.8	114	252
	C. crop	2.9	127	180	2.7	122	251
	FeEDDHA	2.6	98	95	2.8	82	178
Resistant	Control	2.7	114	232	2.5	88	285
	C. crop	2.6	112	254	2.4	89	288
	FeEDDHA	2.3	100	111	2.5	85	249
	Sig. of F§						
	Cultivar	*	*	*	*	*	*
	Treatment	*	*	*	NS	*	*
	Cultivar x Treatment	NS	NS	NS	NS	*	NS
	LSD¶						
	Cultivar	0.2	19	18.1	0.2	11	24.3
	Treatment	0.2	19	18.1	-	11	24.3
	Cultivar x Treatment	-	-	-	-	19	-

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

Table 25. Soybean leaflet nutrient analyses for S, Fe, and Mn at Leonard, North Dakota, 2009.

Cultivar†	Treatment‡	----- V2-V3 soybean stages -----			--- V4-V8 soybean stages ---		
		S	Fe	Mn	S	Fe	Mn
		g kg^{-1}	---- mg kg^{-1} ----		g kg^{-1}	---- mg kg^{-1} ----	
Susceptible	Control	2.8	78	178	2.8	102	270
	C. crop	2.3	75	181	2.9	108	278
	FeEDDHA	2.6	66	110	3.0	91	249
Intermediate	Control	2.3	64	159	2.9	107	308
	C. crop	2.1	76	175	2.6	106	267
	FeEDDHA	2.2	66	110	2.7	89	252
Resistant	Control	2.0	65	177	2.5	96	318
	C. crop	1.9	70	181	2.4	86	268
	FeEDDHA	2.0	66	131	2.3	88	287
	Sig. of F§						
	Cultivar	*	NS	NS	*	*	NS
	Treatment	*	NS	*	NS	*	*
	Cultivar x Treatment	NS	NS	NS	NS	NS	NS
	LSD¶						
	Cultivar	0.2	-	-	0.2	13	-
	Treatment	0.2	0	19	-	13	28.1
	Cultivar x Treatment	-	-	-	-	-	-

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

Summary and Conclusions

Cultivar had a strong influence on the severity of IDC, because the susceptible cultivar had significantly greater IDC severity compared to the intermediate and resistant cultivars. The companion crop, grain sorghum, was generally ineffective in alleviating chlorosis. The FeEDDHA application significantly reduced IDC in all cultivars though cultivar selection was more effective than the FeEDDHA application. Ureide concentrations were abnormally high in plants severely affected by IDC. The plants severely affected by IDC were significantly stunted in growth, as indicated by reduced dry matter per plant. Thus, the excess accumulation of ureides in IDC-stunted plants suggests that plant growth was reduced more then the rate of nitrogen fixation. The AMF colonization, in general, was not affected by cultivar or treatment indicating an adequate AMF population. Cultivar, also, had a strong influence on yield. The susceptible cultivar had a significantly reduced yield compared to the intermediate and resistant cultivars. Yield was increased by the FeEDDHA application though cultivar selection is a major factor. The selection of a cultivar less susceptible to IDC is more critical than a FeEDDHA treatment, as the resistant and intermediate cultivars had a greater yield in all treatments. However, selecting a cultivar less susceptible to IDC with a FeEDDHA treatment optimized yield.

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GENERAL CONCLUSIONS

The method utilized for ureide analyses did influence the ureide concentrations. The Patterson and Goos methods were comparable methods for ureide analyses. The Patterson method was more sensitive than the Vogels or Goos methods. The Goos and Patterson methods are comparable methods in ureide analyses. The Vogels and Patterson methods had a greater difference in ureide concentrations than the Goos and Patterson methods. The difference between the Vogels and Patterson methods may be due to the preparation of the plant extract. Further investigation is necessary to determine the affect of H^+-resin on all methods.

An effective regression curve between two methods was not determined due to the small sample size. The regression curve developed was not effective due to the data points clustering in two zones and the small sample population. Increasing the sample population will assist in determining the type of regression curve. Another consideration in developing an effective regression curve is the cultivar selected for the ureide analyses, as it is probable that cultivars have varying degrees of BNF.

Within the field study, cultivar had a strong influence on the severity of IDC. The companion crop was generally ineffective in alleviating chlorosis. The FeEDDHA application substantially reduced IDC, but was a less effective control measure than cultivar selection. Soybean with severe IDC had an abnormally high ureide concentrations indicating that plant growth was reduced by IDC more than BNF. The AMF colonization, in general, was not affected by cultivar or treatment. Yield was increased by the FeEDDHA application though cultivar selection was a more effective control measure.

Grain sorghum, the companion crop, was ineffective in alleviating chlorosis.

Gramineae species utilize Strategy II to obtain Fe by releasing phytosiderophores to chelate Fe (III) in the rhizosphere (Römheld and Marschner, 1986; Hell and Stephan, 2003). The companion crop was chemically removed following the V2-V3 soybean stages to prevent plant competition between species in addition to IDC of soybean. Thus, the companion crop for the V4-V8 soybean stages was possibly decomposing root hairs, and root exudates, such as phytosiderophores, remaining in the rhizosphere. It would be interesting to have left the companion crop to yield though it is hypothesized that plant competition would have affected the results more than the IDC.

The magnified line-intercept method was utilized to determine the colonization of AMF. This method and similar microscopic methods are considered standards in determining AMF colonization. However, the standard microscopic methods lack analytical consistency outside of DNA based methods (Rosier et al., 2008). Glomalin is a protein produced by AMF that can serves a biomarker for AMF. The concentration of glomalin was not examined in this study. Research has indicated that roots colonized by AMF have greater concentration of glomalin compared to roots not colonized by AMF (Rosier et al., 2008).

Iron deficiency chlorosis is a nutrient deficiency that affects soybean qualities. Soybean qualities were not investigated within the study. However, soybean qualities are an important aspect for potential soybean buyers. Soybean qualities include protein, oil, and fiber contents. Future research should consider the effect of cultivar, location, and IDC severity upon soybean qualities.

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APPENDIX A

Table 1A. Relative chlorophyll and plant matter of soybeans.

Time[†]	Treatment[‡]	Relative chlorophyll[§]	Fresh matter	Dry matter
Weeks			--------- g per plant^{-1} ---------	
3	Yes	36.40	15.71	3.10
	No	17.30	12.11	2.69
4	Yes	38.80	23.30	4.91
	No	26.81	17.27	3.60
5	Yes	29.75	33.01	7.69
	No	31.16	21.49	4.67
	Sig. of F[¶]			
	Time	*	*	*
	Treatment	*	*	*
	Time x Treatment	*	*	*
	LSD[††]			
	Time	1.50	1.47	0.34
	Treatment	1.22	1.20	0.28
	Time x Treatment	2.18	2.14	0.49

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.

[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.

[§] Relative chlorophyll content using a Minolta SPAD meter.

[¶] Significance of F
NS = not significant;
* = significant at the 0.05 probability level.

[††] Least significant difference at the 0.05 probability level.

APPENDIX B

Table 1B. Location, classification and crop history of North Dakota field sites.

Location†	Legal Description	Soil	2008 Crop
Ayr	Sec. 31, T142N, R54W	Hamerly	Maize
Hunter	Sec. 30, T143N, R52W	Glyndon	Maize
Leonard	Sec 21, T137N, R52W	Hamerly	Soybean

†The location is named according to the nearest city.

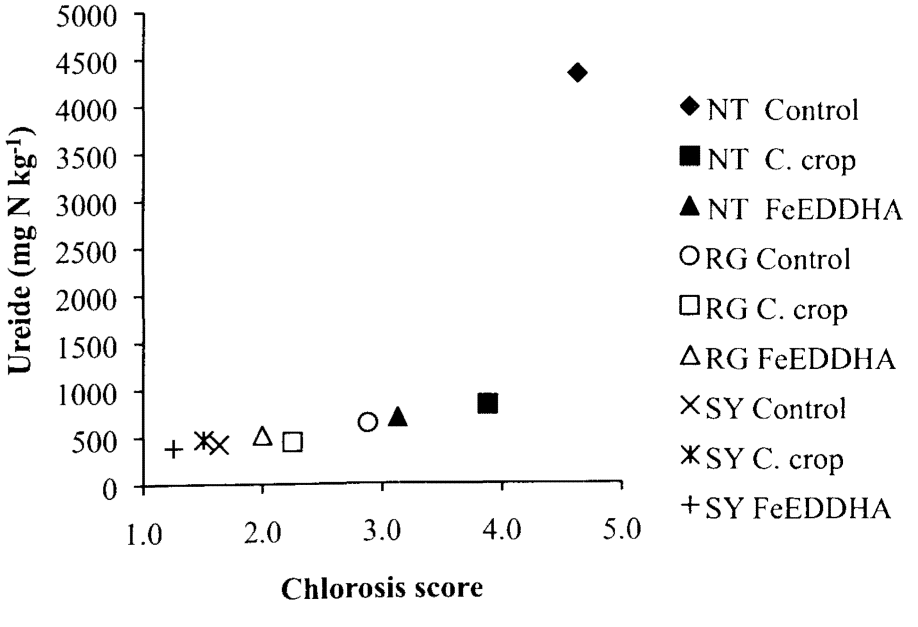

Figure 1B. The chlorosis scores and ureide concentrations for the V4-V8 soybean stages at Ayr, North Dakota, 2009.

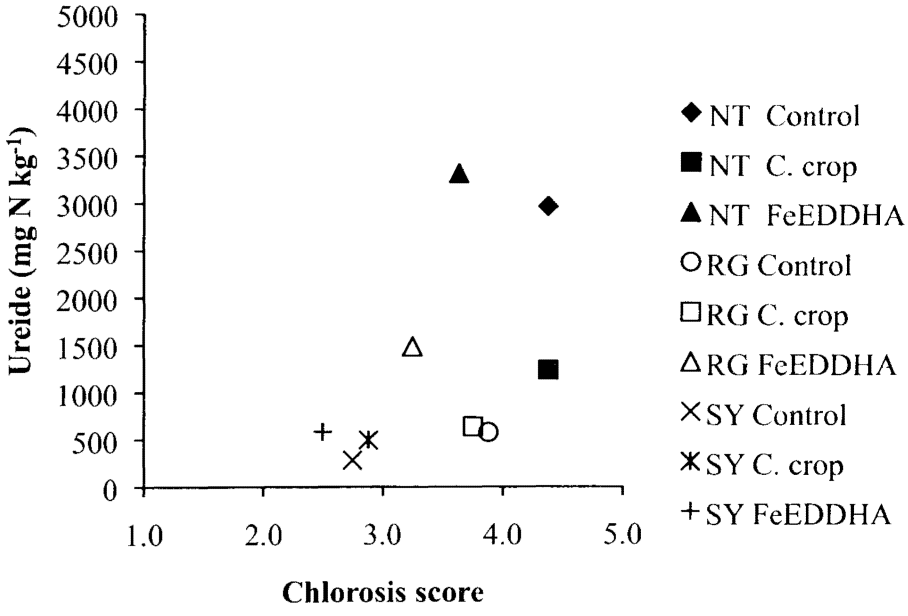

Figure 2B. The chlorosis scores and ureide concentrations for the V4-V8 soybean stages at Hunter, North Dakota, 2009.

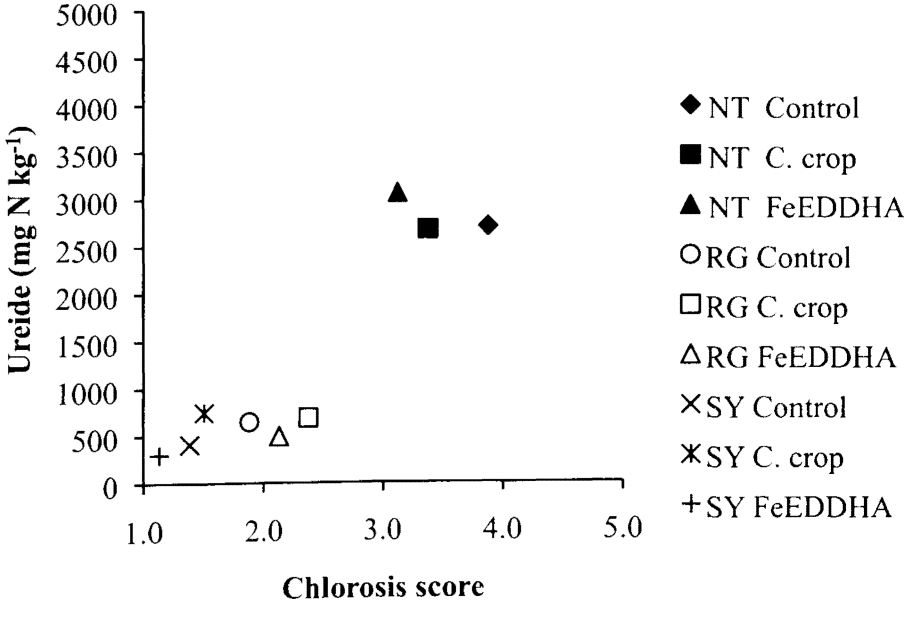

Figure 3B. The chlorosis scores and ureide concentrations for the V4-V8 soybean stages at Leonard, North Dakota, 2009.

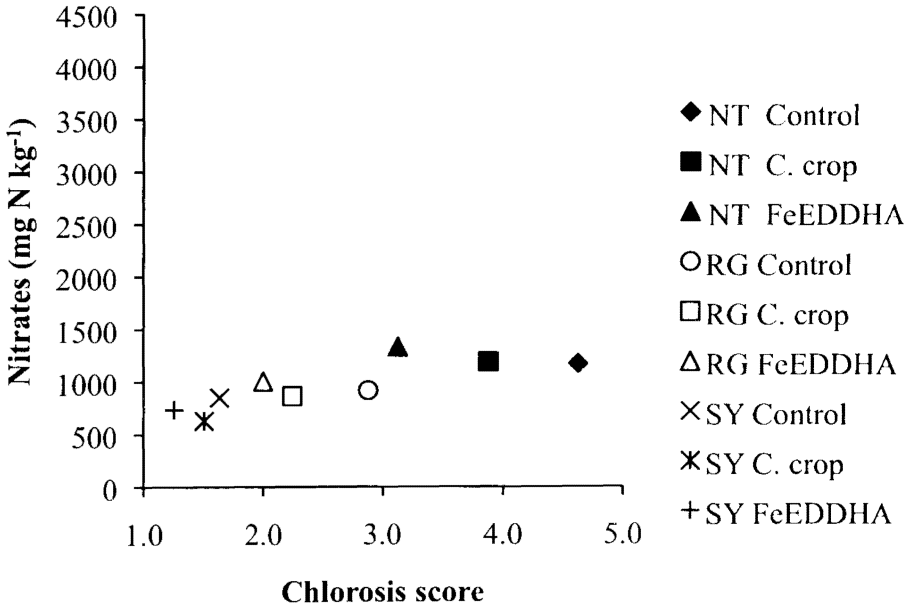

Figure 4B. The chlorosis scores and nitrate concentration for the V4-V8 soybean stages at Ayr, North Dakota, 2009.

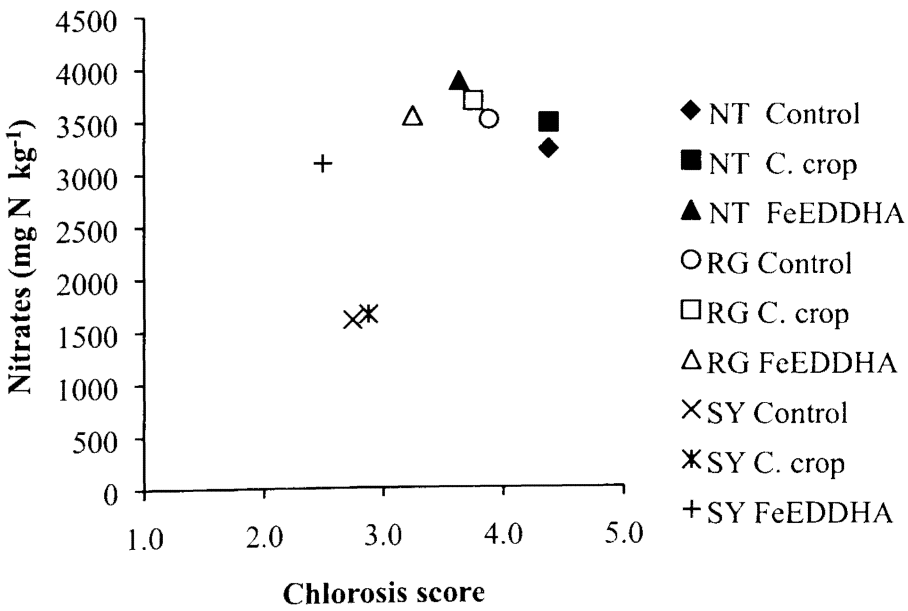

Figure 5B. The chlorosis scores and nitrate concentrations for the V4-V8 soybean stages at Hunter, North Dakota, 2009.

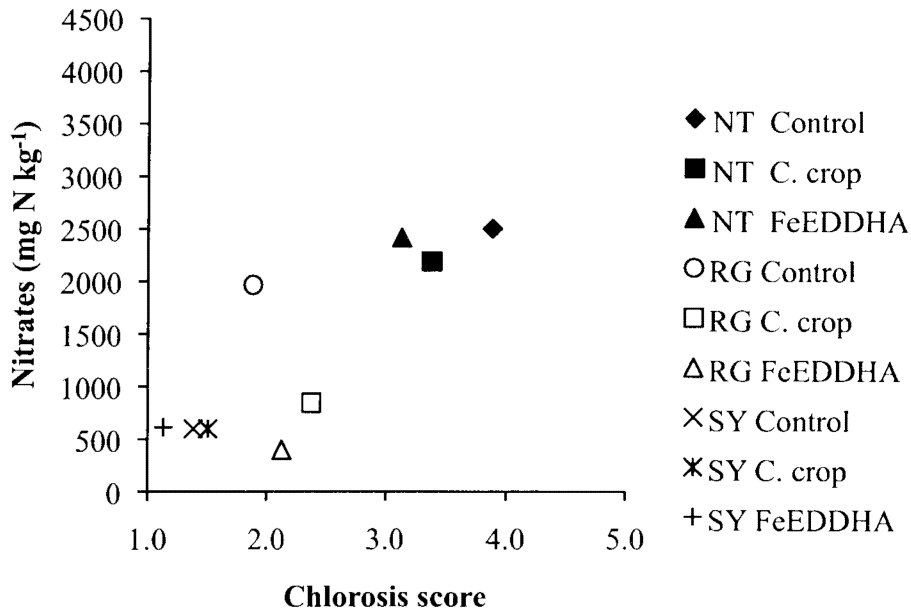

Figure 6B. The chlorosis scores and nitrate concentrations for the V4-V8 soybean stages at Leonard, North Dakota, 2009.

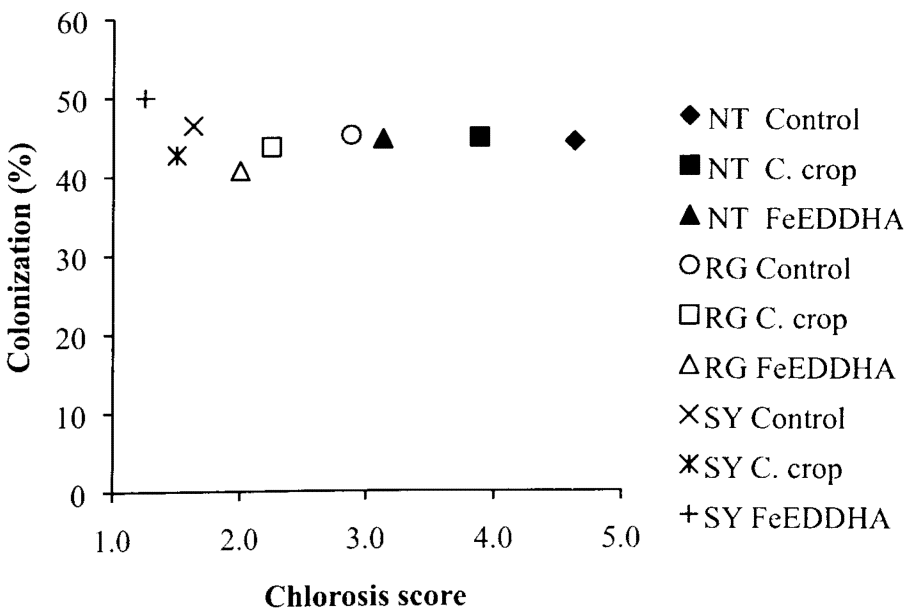

Figure 7B. The chlorosis scores and arbuscular mycorrhizal fungi colonization for the V4-V8 soybean stages at Ayr, North Dakota, 2009.

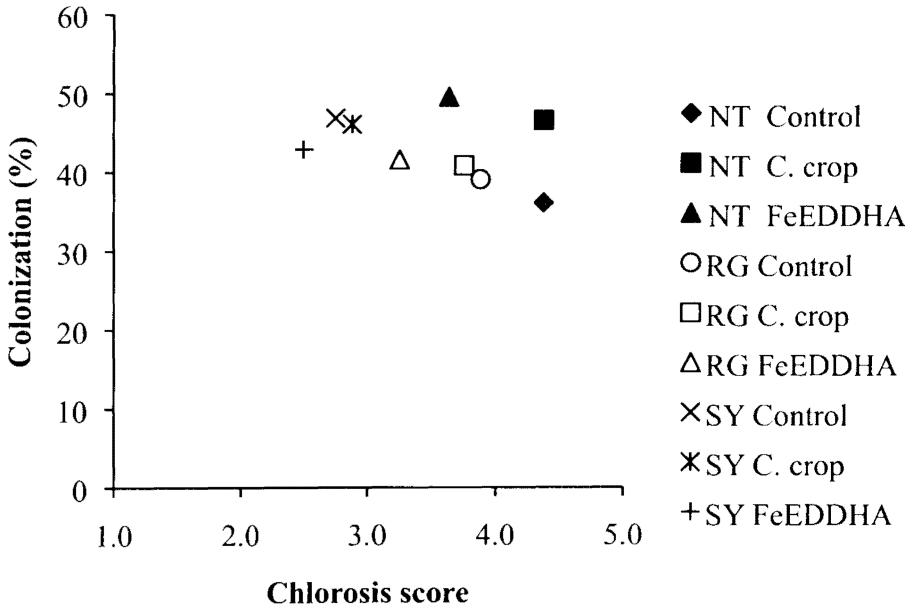

Figure 8B. The chlorosis scores and arbuscular mycorrhizal fungi colonization for the V4-V8 soybean stages at Hunter, North Dakota, 2009.

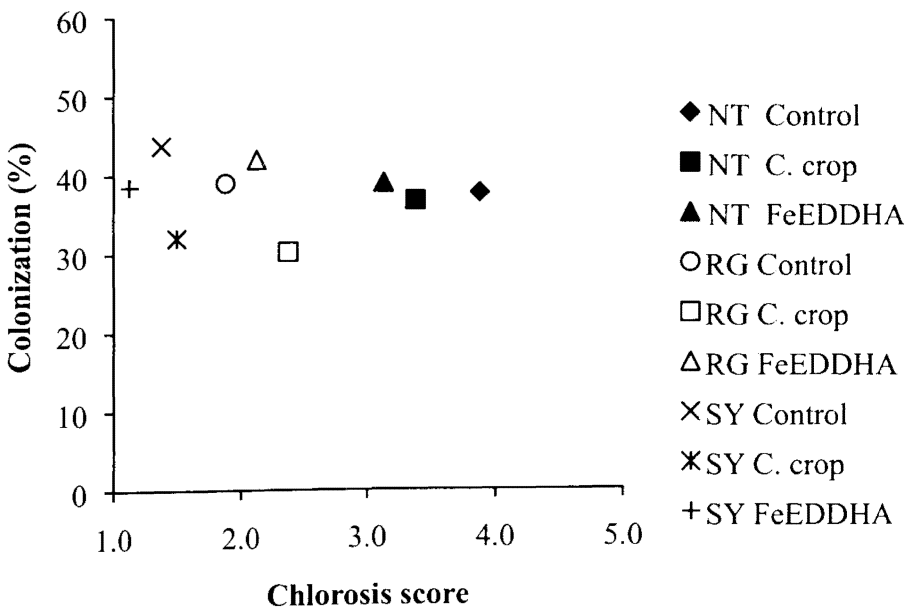

Figure 9B. The chlorosis scores and arbuscular mycorrhizal fungi colonization for the V4-V8 soybean stages at Leonard, North Dakota, 2009.

Table 2B. Soybean leaflet nutrient analyses for Cu, B, and Zn at Ayr, North Dakota, 2009.

Cultivar[†]	Treatment[‡]	V2-V3 soybean stages			V4-V8 soybean stages		
		Cu	B	Zn	Cu	B	Zn
		mg kg^{-1}					
Susceptible	Control	17	54	34	17	64	38
	C. crop	14	52	29	11	59	26
	FeEDDHA	16	42	27	12	50	30
Intermediate	Control	14	48	27	11	57	31
	C. crop	13	55	23	8	66	21
	FeEDDHA	14	45	24	10	51	26
Resistant	Control	14	53	23	11	64	24
	C. crop	15	49	25	11	57	23
	FeEDDHA	21	46	28	10	59	24
	Sig. of F						
	Cultivar	NS	NS	*	*	NS	*
	Treatment	NS	*	NS	*	*	*
	Cultivar x Treatment	NS	NS	NS	*	NS	*
	LSD[¶]						
	Cultivar	-	-	4.1	2	-	3.1
	Treatment	-	4.6	-	2	6.4	3.1
	Cultivar x Treatment	-	-	-	8	-	6.0

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
[‡] Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
[§] Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
[¶] Least significant difference at the 0.05 probability level.

Table 3B. Soybean leaflet nutrient analyses for Cu, B, and Zn at Hunter, North Dakota, 2009.

Cultivar†	Treatment‡	V2-V3 soybean stages			V4-V8 soybean stages		
		Cu	B	Zn	Cu	B	Zn
		mg kg^{-1}					
Susceptible	Control	9	54	28	12	57	27
	C. crop	9	64	26	11	65	22
	FeEDDHA	8	45	20	7	52	23
Intermediate	Control	11	50	25	7	53	21
	C. crop	7	53	20	6	58	19
	FeEDDHA	9	45	18	7	54	21
Resistant	Control	9	56	21	5	59	17
	C. crop	7	61	17	5	63	17
	FeEDDHA	7	50	17	6	60	18
	Sig. of F§						
	Cultivar	NS	*	*	*	*	*
	Treatment	NS	*	*	*	*	NS
	Cultivar x Treatment	NS	*	NS	*	NS	NS
	LSD¶						
	Cultivar	-	2.3	2.4	1	5.0	2.1
	Treatment	-	2.3	2.4	2	5.0	-
	Cultivar x Treatment	-	4.0	-	2	-	-

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

Table 4B. Plant nutrient analyses for Cu, B, and Zn in Leonard, North Dakota, 2009.

Cultivar†	Treatment‡	V2-V3 soybean stages			V4-V8 soybean stages		
		Cu	B	Zn	Cu	B	Zn
		mg kg^{-1}					
Susceptible	Control	12	65	25	14	76	27
	C. crop	9	61	19	15	78	27
	FeEDDHA	12	61	24	15	74	30
Intermediate	Control	11	59	21	13	82	27
	C. crop	9	63	18	11	79	26
	FeEDDHA	10	57	18	13	77	26
Resistant	Control	9	55	17	12	76	25
	C. crop	8	58	15	10	74	24
	FeEDDHA	9	54	18	11	69	22
	Sig. of F§						
	Cultivar	*	*	*	*	NS	*
	Treatment	*	NS	*	NS	NS	NS
	Cultivar x Treatment	NS	NS	NS	NS	NS	NS
	LSD¶						
	Cultivar	1	3	2	1	-	2
	Treatment	1	3	2	-	-	-
	Cultivar x Treatment	2	-	-	-	-	-

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

Table 5B. Soybean leaflet nutrient analyses for Ca, Mg, and Na at Ayr, North Dakota, 2009.

Cultivar†	Treatment‡	V2-V3 soybean stages			V4-V8 soybean stages		
		Ca	Mg	Na	Ca	Mg	Na
		g kg^{-1}					
Susceptible	Control	15.6	7.5	0.1	19.7	8.8	0.1
	C. crop	17.3	7.6	0.1	18.3	8.1	0.1
	FeEDDHA	15.1	7.0	0.1	16.5	7.9	0.1
Intermediate	Control	17.1	7.6	0.1	17.3	7.9	0.1
	C. crop	17.4	8.1	0.1	17.7	8.7	0.1
	FeEDDHA	16.0	6.9	0.1	17.7	7.8	0.1
Resistant	Control	16.6	8.3	0.1	17.0	8.4	0.1
	C. crop	16.1	7.2	0.1	16.8	7.5	0.1
	FeEDDHA	14.3	6.4	0.1	16.5	7.7	0.1
	Sig. of F§						
	Cultivar	*	NS	NS	*	NS	NS
	Treatment	*	*	NS	NS	NS	NS
	Cultivar x Treatment	NS	NS	NS	NS	NS	NS
	LSD¶						
	Cultivar	1.09	-	-	0.92	-	-
	Treatment	1.09	0.6	-	-	-	-
	Cultivar x Treatment	-	-	-	-	-	-

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

Table 6B. Soybean leaflet nutrient analyses for Ca, Mg, and Na at Hunter, North Dakota, 2009.

Cultivar†	Treatment	V2-V3 soybean stages			V4-V8 soybean stages		
		Ca	Mg	Na	Ca	Mg	Na
		g kg^{-1}					
Susceptible	Control	16.1	14.5	0.1	16.3	13.9	0.1
	C. crop	15.5	15.4	0.1	16.5	15.5	0.1
	FeEDDHA	13.5	13.9	0.1	15.5	14.7	0.1
Intermediate	Control	15.1	13.6	0.1	16.1	13.7	0.1
	C. crop	14.5	14.2	0.1	16.1	15.0	0.1
	FeEDDHA	13.6	13.8	0.1	16.2	15.6	0.1
Resistant	Control	14.7	15.1	0.1	15.3	15.6	0.1
	C. crop	16.3	14.3	0.1	16.8	14.8	0.1
	FeEDDHA	13.1	13.1	0.1	15.0	14.5	0.1
	Sig. of F§						
	Cultivar	NS	NS	NS	NS	NS	NS
	Treatment	*	NS	NS	NS	NS	NS
	Cultivar x Treatment	NS	NS	NS	NS	NS	NS
	LSD¶						
	Cultivar	-	-	-	-	-	-
	Treatment	0.98	-	-	-	-	-
	Cultivar x Treatment	-	-	-	-	-	-

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

Table 7B. Soybean leaflet nutrient analyses for Ca, Mg, and Na at Leonard, North Dakota, 2009.

Cultivar†	Treatment‡	V2-V3 soybean stages			V4-V8 soybean stages		
		Ca	Mg	Na	Ca	Mg	Na
		g kg^{-1}					
Susceptible	Control	1.2	0.9	0.0	1.5	1.1	0.0
	C. crop	1.2	0.9	0.0	1.6	1.2	0.0
	FeEDDHA	1.1	0.9	0.0	1.4	1.1	0.0
Intermediate	Control	1.2	0.9	0.0	1.6	1.1	0.0
	C. crop	1.2	0.9	0.0	1.6	1.1	0.0
	FeEDDHA	1.1	0.8	0.0	1.5	1.1	0.0
Resistant	Control	1.2	0.8	0.0	1.5	1.1	0.0
	C. crop	1.1	0.8	0.0	1.4	1.0	0.0
	FeEDDHA	1.1	0.8	0.0	1.4	1.0	0.0
	Sig. of F§						
	Cultivar	NS	*	NS	NS	*	NS
	Treatment	*	NS	NS	NS	NS	NS
	Cultivar x Treatment	NS	NS	NS	NS	NS	NS
	LSD¶						
	Cultivar	-	0.6	-	-	0.7	-
	Treatment	0.8	-	-	-	-	-
	Cultivar x Treatment	-	-	-	-	-	-

† Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.
‡ Control, no treatment;
C. crop, sorghum companion crop;
FeEDDHA, 3.3 kg ha-1 of FeEDDHA.
§ Significance of F
NS = not significant.
* = significant at the 0.05 probability level.
¶ Least significant difference at the 0.05 probability level.

Table 8B. Companion crop dry matter weight during the V2-V3 soybean stages matter per plant at three locations, 2009.

Cultivar[†]	Treatment[‡]	Location		
		Ayr	Hunter	Leonard
		g plant^{-1}		
Susceptible	C. crop	0.33	0.33	0.31
Intermediate	C. crop	0.33	0.28	0.27
Resistant	C. crop	0.31	0.25	0.40

[†] Susceptible = NT, NuTech NT-0886;
Intermediate = RG, Roughrider Genetics RG 607;
Resistant = SY, Syngenta S01-C9 RR.

[‡] C. crop, sorghum companion crop;