NITROGEN MANAGEMENT IN DRY BEAN AND SOYBEAN

A Thesis
Submitted to the Graduate Faculty
Of
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

April 2015

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Nitrogen Management in Dry Bean and Soybean

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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

MASTER OF SCIENCE

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ABSTRACT

Legume crops, dry bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* (L.) Merr.), can form symbiotic relationships with nitrogen-fixing bacteria. Nitrogen (N) fertilizer may be necessary for optimal yields. Three experiments were conducted on dry bean and on soybean in North Dakota. Objectives of the research were to evaluate yield and growth differences between different N management strategies. Inoculant applied to dry bean increased nodulation in one environment. Nodule formation was highest in the Lariat pinto bean and lowest in Vista navy bean. Application of N increased yield at Park River in 2014. Applying N to dry bean may not be necessary if soil N reserves are adequate. N application to soybean aggravated iron deficiency chlorosis (IDC), but increased yield. When fertilizer cost was accounted for there were no differences between treatments. Application of N to leguminous crops is not recommended, but it may increase yield under certain conditions.

ACKNOWLEDGMENTS

I am extremely grateful for the assistance of my graduate advisor, Dr. Hans Kandel, for allowing me to continue my professional education. I will be leaving graduate school a better man because of the guidance and encouragement, along with the opportunities I have experienced over the past two years. I would also like to thank my committee members, Dr. Joel Ransom, Dr. Burton Johnson, and Dr. David Franzen for their assistance and advice in experiment implementation and for taking the time to read this thesis and offer critical comments to make it the best it can be.

I am also extraordinarily thankful to have worked with Chad Deplazes and Grant Mehring, the support and assistance I received from them along with the help of Paula Petersen, Garret Lungren, Erin Endres, Lindsey Forward, Diego Ronchi, Courage Mudzongo, Ethan Sweep, and Shelby Hanson in data collection and plot maintenance allowed for the research to be conducted accurately and efficiently. Grant Mehring also assisted greatly in statistical analysis and interpretation of the experiments. Great thanks to my fellow graduate students for assisting in the completion of my research tasks and helping to make the most of my graduate education: Kellie Podliska, Jacob Muir, Lucas Holmes, Matt Chaput, and Matthew Taylor. The memories I have made over the past two years are unforgettable. To my parents, family, friends, and fraternity brothers: Thank you for always being there and for the love and support you provided for me to pursue graduate education. Thank you to everyone else that has helped me in anyway.

Special thanks to the North Dakota (ND) Soybean Council and the Pembina and Walsh County Crop Improvement Associations for funding this research. Also a large thank you to Dave Hankey from Hankey farms for providing land to conduct dry bean research.

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INTRODUCTION

Nitrogen (N) is an essential nutrient for plant growth (Wetzel and Likens, 1991) and fertilizers containing N are applied to fields worldwide to improve crop yields. Legume crops form a symbiotic relationship with root-colonizing rhizobacteria, which transform atmospheric N_2 gas in the soil into plant useable ammonium, this is known as N fixation.

Nitrogen fixation in legume crops alone may not provide enough N for a legume's needs throughout a growing season. However, N fertilizer application may reduce N fixation in root nodules (Edje et al., 1975). A balance between N fixation and application of N fertilizer may be required for optimal yields. For example, adding N at different times during the development of the plant, or by applying a slow-release fertilizer, the plant may benefit from the rhizobacteria early in the season, while the extra N supplied may be utilized by the crop later in the season to improve yield. If researchers can find a way to increase crop yield and more efficiently use fertilizer, farmers can manage their resources more economically, money can be saved, and optimal N use may be increased.

For the research reported in this thesis two species of legume crops, dry beans and soybean, were utilized with several N application rates and timing in ND. Two experiments were conducted on dry beans and one experiment on soybean during multiple years. After a general literature review, the thesis is split into separate chapters for the dry bean and soybean experiments.

Literature Review

Dinitrogen Fixation in Legumes

One of the most yield-limiting nutrients for crops is N; however, N can exist in the soil system in many forms and can transform from one form to another. There are several possible supply sources of N for plant growth. A large supply of N is in the atmosphere as 78% of air is N_2 gas. Most plants cannot directly utilize this resource due to the triple bond between the two N atoms (Brady and Weil, 2010), but some soil bacteria and fungi can fix atmospheric N. In addition, atmospheric fixation by lightning can deposit N into soil through precipitation. The enzyme, *nitrogenase*, catalyzes the reduction of N_2 to ammonia in biological fixation. Organisms that can fix N have a relatively high requirement for molybdenum (Mo), iron (Fe), phosphorus (P), and sulfur (S) as these nutrients are either a part of the *nitrogenase* molecule or are needed for its synthesis and use (Brady and Weil, 2010).

$$\mathrm{N}_2 + 8H^+ + 6e^- \xrightarrow{(Nitrogenase)} 2NH_3 + H_2$$

Leguminous plants, such as soybean and dry bean, are able to utilize atmospheric N through N fixation with nodule-forming rhizobacteria. According to Ohyama et al. (2009), legume roots excrete species specific isoflavonoid compounds to be recognized by compatible rhizobia. Nodule or NOD genes are expressed by specific isoflavanoid signals to make NOD factor. The NOD factor is a lipochitine oligosaccharide that induces nodule formation. Structures of NOD factors are different among rhizobia species, and host plants only recognize certain NOD factors. After NOD factor expression, the rhizobia move to the host's roots and proliferate near the root surface. As the rhizobia become attached to the root hair by an adhesive substance the root hair entraps the rhizobia through curling. The bacteria create an intracellular tunnel known as an infection thread. Rhizobia can enter the root through this infection thread and are released into the proliferating nodule meristem cells where the bacterial filament takes over the plant cell nucleus. Plant cell division and rhizobium proliferation stimulate the development of the nodule structure. Nodule vascular bundles connect to the root vascular bundles so that the nodules and roots can exchange compounds through the phloem and xylem. Inside the formed nodules bacteroid, a symbiotic state of rhizobia, start to fix soil atmospheric N_2 into a form that plants can utilize (Ohyama et al., 2009).

Different rhizobacteria react with different legume species and there are variations in the amount of N fixed by these bacteria. Dry beans have been shown to fix 22-90 kg ha^{-1} of N with a typical amount of 44 kg ha^{-1}, while soybeans have been shown to fix 44-290 kg ha^{-1} of N during a growing season with a typical amount of 112 kg ha^{-1}. There are many factors that can influence the effectiveness of N fixation by rhizobacteria. Factors such as reduced light intensity, moisture stress in the form of flooding or drought, and low temperature will reduce the rate of plant photosynthesis in turn reducing atmospheric N_2 fixation (Havlin et al., 2005).

Nitrogen supplied only by biological fixation may be insufficient for the vigorous vegetative growth necessary for optimal yields, while overly vigorous vegetative growth can be detrimental to yield. High levels of available N in the soil tend to depress biological N fixation. Plants only make the energy investment, required for symbiotic N fixation, when there are short supplies of mineral N (Brady and Weil, 2010). Nitrogen sources other than biologically fixed N are soil organic matter, crop residues, animal manures, and commercial fertilizers (O'Leary et al., 2002). Managing N by utilizing biological N fixation and N fertilizer applications, while avoiding over fertilization but supporting high yields can be difficult. Scientists are exploring tools such as optical sensors to determine the need for in-season N application and N use efficiency (NUE).

Optical Sensors

According to Cassman et al. (2002), NUE of a cropping system is the proportion of all N removed in harvested crop biomass, contained in recycled crop residues, and incorporated into soil organic matter and inorganic N pools compared with all the N inputs. Applied N not taken up by the crop or immobilized in soil organic N pools is vulnerable to losses from volatilization, denitrification, and leaching. The overall NUE of a cropping system can be increased by achieving greater uptake efficiency from applied N inputs by reducing the amount of N lost from soil organic and inorganic N pools (Cassman et al., 2002).

In corn (*Zea mays* L.) and wheat (*Triticum aestivum* L. emed. Thell.) algorithms have been empirically produced that use data from optical sensors to manage in-season N applications. By relating sensor readings to yield and using a response index to variable apply N at 1 m^2 spatial resolution, NUE was improved by >15% compared with traditional application practices using uniform N rates (Raun et al., 2002).

The chlorophyll carotenoid pigments in plant leaves absorb visible light from 400 to 700 nm for use in photosynthesis and reflect green light. Bullock and Anderson (2008) found the Minolta SPAD 502 (SPAD) reading to be useful as a diagnostic aid rather than a tool for N management in corn. The SPAD could provide a measure of the relative greenness of living leaves at a specific point in time. SPAD correlation to corresponding N concentration improved over time through the growing season. Chlorophyll readings, such as SPAD, can be useful in detecting N deficiencies in growing crops when compared to a non N limiting standard in the field. Leaf chlorophyll content is related to photosynthetic activity, stress condition and nutritional status of a plant, and by quantifying this; it can be used as an N management tool.

Fritschi and Ray (2007) assessed genotypic variation in soybean chlorophyll content with the use of SPAD meter readings to see if these data could be used as a rapid screening method to predict genotypic variation in leaf tissue N content. They found that chlorophyll content was related to SPAD readings and leaf N content; however the relationships found were not sufficiently consistent for chlorophyll to be useful as a predictive tool for leaf N content in soybean. If SPAD readings were correlated to yield in legume crops it could be a highly useful management tool.

Late Season Applications

Urea (46-0-0) is a granular fertilizer that is commonly used because of its high N analysis compared to other dry or liquid forms of fertilizer N, is easy and safe to handle and store, and has a relatively low price. Urea is readily soluble in water and is used in the formulation of urea ammonium nitrate (UAN) and compound fertilizers. However, it is subject to volatilization, if surface applied, and it can produce severe seedling damage if seed placed rates are too high (Grant, 2004). A number of products have been developed to delay the N transformation process so that N is available later into the growing season. The product Environmentally Smart Nitrogen (ESN) (Agrium) is a polymer coated urea that delays urease activity through physical separation of urea from the soil (Franzen, 2010). The polymer coating breaks down later in the season, slowly releasing N. Marketed as Agrotain (Koch Agronomic Services, LLC, Wichita, KS), the chemical NBPT (N-(n-butyl) thiophopshoric acid triamide) is used to decrease urea volatilization through locking onto the urease enzyme binding sites (Manunza et al., 1999). Dicyandiamide (DCD), marketed as Super-U (Koch Agronomic Services, LLC, Wichita, KS) and other fertilizer materials, can be used as a nitrification inhibitor. While Agrotain is a urease inhibitior, the product Super-U is a urease and a nitrification inhibitor. Malzer et al. (1989) found that yield differences from fertilizer treated with DCD and fertilizer alone were inconsistent and limited to those soils and conditions where nitrate was lost through leaching or denitrification.

As the dry bean plant develops, it takes up N from the soil if there is sufficient moisture. If N is applied early in plant development there may not be enough in the soil to meet the full extent of the plant's needs during pod fill. To make up for this it may be beneficial to apply a product that makes the N available later in the growing season such as a N stabilizer applied to urea or a slow release encapsulated urea. An alternative to these products would be to apply urea later in the season or to split the supplemental N with an early application along with a later application. In Canada, a field trial was established to determine the effect of late N application on yield of irrigated dry bean. Treatments included 25 kg N ha^{-1} as ammonium nitrate applied at seeding, early flower, mid-late flower, and at early pod fill. Seed yield was significantly increased with late N application at early pod fill compared to the control treatment (Canada-Saskatchewan Irrigation Diversification Centre, 2012).

Research at South Dakota State University (2013) reported the soybean plant at early pod set has accumulated approximately 30% of its total seasonal N requirement. When the plant adds pods and fills seeds it may possibly require more N than the N fixation can provide. Shibles (1998) indicated that N fixing capacity in rhizobacteria begins to decline rapidly after growth stage R5 (Fehr et al., 1971 and Ritchie et al., 1994), which is about the same time as peak N demand for protein synthesis. It could be possible that by supplying N to the plant later in the season one could increase yield potential.

Takahashi et al. (1991) found that with deep placement of polymer-coated urea (ESN) soybean growth was improved and it did not significantly depress the N fixation activity during the maturation stage. Furrowed N application increased seed yield, however top dressing of the coated urea inhibited the nodule activity after the R3 stage and the seed yield did not increase.

Research at the University of Illinois by Nafziger (2015) found that N fertilizer in some cases increased yield. With the cost of fertilizer and application the yield would need to increase 188 to 250 kg ha^{-1} in order to break even with 2015 prices. Nafziger (2015) applied urea, urea with urease inhibitor, and polymer-coated urea (ESN) with rates from 112 to 336 kg ha^{-1} and applications between first flower (R1) and full pod stages. Significant yield increases were found in two of the 33 trials, and a significant yield decrease in one trial.

Schmitt et al. (2001) conducted research on in-season applications to soybean at 12 sites in Minnesota. The study included timing of application, placement method, and N source (urea vs. poly-coated urea). Seed yield did not respond to the fertilizer N treatments at any of the 12 sites; however, a combined analysis indicated a significant yield increase from using polymer-coated urea or applying the urea in August.

NITROGEN MANAGEMENT PRACTICES OF DRY BEAN

Abstracts

Nitrogen Fertilization and Inoculation Effects on Dry Bean

Dry bean has the ability to form symbiotic relationships with N-fixing bacteria. For optimal yield, multiple methods of N management can be utilized. Objectives of this research were to evaluate yield and growth differences between 3 varieties in different market classes of dry bean with different N management combinations including application of urea fertilizer and 2 rhizobacterial inoculants. An increase in yield was found in pinto bean in 2014 at Park River, ND, with addition of 56 kg ha^{-1} of N. There was an increase in root nodules with inoculant in Park River, 2013. Lariat pinto bean had the most nodules followed by Eclipse black bean across environments. Application of 56 kg ha^{-1} of N did result in more vigorous plants, but decreased kernel weight. Inoculation and application of fertilizer on dry bean may not be necessary if rhizobacteria and N levels in the soil are sufficient.

Efficiency of Fertilizer Management on Lariat Dry Bean

Dry bean can form a symbiotic relationship with N-fixing bacteria. Various methods of N application can be used. Objectives of this research were to evaluate yield and growth differences among rates and timing of application and different N products. An increase in yield was found in 2014 at Park River, ND, with addition of 56 kg ha^{-1} (ESN) and 56 kg ha^{-1} (Super-U) over the control; however, the yield of these treatments was not higher than the application of 56 kg ha^{-1} (urea) and 84 kg ha^{-1} (urea). The yields with split application, fertilizer at emergence, and slow release products were not significantly different from the control with no addition of fertilizer. This may be due to rhizobacteria and adequate N levels in the soil. Application of N decreased kernel weight at Prosper, ND 2014.

Literature Review

Dry Bean as a Crop

Dry bean has been grown for thousands of years, and the most common types such as pinto originated in Africa, Asia, and the Middle East. Archeological evidence suggests that the first beans were brought to the Americas by nomads crossing the Bering Strait into Alaska (California Dry Bean Advisory Board, 2011).

The dry bean industry recognizes multiple market classes. Dry bean production in general is adaptable to a large range of growing regions. North Dakota is the number one dry bean producer in the United States with 32% of U.S. production (NASS, 1998). Due to its adaptability to ND conditions, pinto is the market class with the greatest number of hectares in the state. There is a substantial amount of navy, black, and several other classes of dry bean grown in the state as well.

Dry bean in ND has become an important crop in many farmers' rotations, and is adaptable to various growing situations. The symbiotic relationship of dry bean with nitrogen fixing bacteria may help improve crop performance on marginal soils (Goodwin, 2003). Some of the most important factors affecting yield are cultivar selection, tillage, soil fertility, planting practices, cropping systems, and post-planting management (Heatherly and Elmore, 2004).

Importance of Fertilizer

An important factor when growing crops is N nutrition. Nitrogen is one of the most important nutrients for plant growth and is involved in cellular respiration and chlorophyll synthesis. Nitrogen can help provide high yields; however, excessive N can cause delayed maturity and promote excessive leaf growth which could lead to lodging and increased risk of disease (Franzen, 2006). In order to maximize yield it is important to find an efficient N management system (Davis and Brick, 2009).

Since inoculation is inexpensive compared to supplemental N fertilizer applications, finding a way to consistently increase N fixation may benefit farmers. Most dry bean growers choose from four main N fertilization strategies: no inoculation or supplemental N, inoculation with a N-fixing bacteria at seeding, inoculation and supplemental N, or supplemental N only (Franzen, 2006). Each farmer generally knows which strategy will work best for each field from experience and is dependent on factors such as soil type and crop history. The strategy of no inoculation or supplemental N for example is possible if soil N is high, or if the field is known to already have sufficient levels of rhizobacteria present in the soil. Graham (1978) found that applications of as little as 15 kg N ha^{-1} may reduce rates of N fixation of dry bean by as much as 40%.

In dry bean nodulation and N fixation are typically low and variable. According to Edje et al. (1975), the reason for poor and variable N fixation of dry bean is not well understood, but has been attributed to many factors including short growing season, seasonal variation, and differences in growth habit between cultivars. Edje et al. (1975) observed the effect of six fertilizer levels (0, 40, 80, 120, 160, and 200 kg N ha^{-1}) on dry bean growth and yield. Grain yield increased significantly ($P<0.05$) with 40 kg N ha^{-1} from the control, but with all higher N treatments only a slight yield increase occurred and the treatments more than 40 kg N ha^{-1} were not significantly different from each other, however 200 kg N ha^{-1} was significantly higher yielding than 40 kg N ha^{-1}. Leaf area index, total dry matter, and general plant vigor increased at the higher N levels.

Eckert et al. (2001) conducted research on pinto bean cultivars and effects of row spacing and N. They reported that increasing the N level did not have a direct effect on the seed yield, yield potential, or seed weight of the cultivars tested in the study.

Inoculation

According to Kellman et al. (2005) most soils sown to dry bean contain indigenous rhizobia. Their results found that it should be possible to increase nodulation and yield of dry beans by combining suitable cultivars with an appropriate strain of rhizobia. Fageria et al. (2014) reported that inoculation with rhizobial strains improved grain yield.

Nitrogen-limited conditions are considered necessary for symbiotic relationships between legumes and rhizobia, but the effects of N rich conditions on this symbiotic status remain poorly understood. Research conducted by Nanjareddy et al. (2014) examined rhizobial symbiosis with dry bean under different N conditions. They found that high levels of N impaired nodule maturation and nodule numbers while low N conditions positively regulated nodule number, biomass, and extended the duration of N-fixing activity, this is consistent with observations made in the study by Edje et al (1975). This study will focus on dry bean inoculation and N fertility management.

Research Objectives

The objectives of this dry bean research were to: 1) compare multiple types of inoculant and fertilizer management, including variation in products applied and timing of application, in relation to yield; 2) compare nodule formation among treatments and its association with yield; 3) record observations on plant growth, utilizing visual scores, plant measurements, and optical sensors in order to better understand dry bean growth in relation to yield.

Materials and Methods

Experiment 1 Nitrogen Fertilization and Inoculation Effects on Dry Bean and Experiment 2 Efficiency of Fertilizer Management on Lariat Dry Bean

Two experiments were conducted on dry bean. In experiment 1 three varieties in different market classes of dry bean were used: black, 'Eclipse' (Osorno et al., 2009); navy, 'Vista' (Gen-Tec Seeds Ltd., South Woodslee, ON, released 1989); and pinto, 'Lariat' (Osorno et al., 2010). There were three inoculant treatments: control (no inoculant), *Rhizobium leguminsarum* bv. *phaseoli* 2 x 10^9 viable cells g^{-1} and *Bacillus subtilis* 2 x 10^8 viable spores g^{-1} (Hi-Stick, Becker Underwood Inc., Ames, Iowa), and *Rhizobium legumnosarum* bv. *phaseoli* 2 x 10^8 viable cells g^{-1} (RhizoStick, Becker Underwood Inc., Ames, Iowa). These inoculants were applied to seed prior to planting and both were peat based dry inoculants. In order to avoid inoculant treatment contamination all treatments without inoculant were planted first, then all plots containing RhizoStick were planted. After the cones were sanitized, the cones were coated with HiStick and the remaining plots which contained HiStick were planted.

There were two fertilizer treatments: no fertilizer (control) and 56 kg ha^{-1} of N (in the form of urea) at emergence.

The experimental design was a factorial arrangement in a randomized complete block with four replications.

A ragdoll germination test was conducted using a moist paper towel at room temperature to find a germination percentage and proper planting rates were determined from these results. Seeds were counted and packaged into envelopes to obtain a population of 222 400 plants ha^{-1} for Eclipse and Vista and 173 000 plants ha^{-1} for Lariat.

In experiment 2 there was no inoculant applied. Most fertilizer treatments were applied at emergence. Nitrogen was applied as granular urea (46-0-0). Along with urea, three products were used to allow for extended length of availability for N fertilizer. The products were N-(n-butyl) thiophosphoric triamide (Agrotain, Koch Agronomic Services LLC, Wichita, KS), a urease inhibitor applied to urea which was replaced in the 2014 experiment with Dicyandiamide (DCD), (Super-U, Koch Agronomic Services LLC, Wichita, KS) a nitrification inhibitor. The other product was polymer coated urea, ESN (Agrium, Calgary, AB), slow release fertilizer (44-0-0). There were seven fertilizer treatments: 1) no fertilizer (control); 2) 56 kg ha^{-1} of N (applied as urea) at emergence; 3) 28 kg ha^{-1} of N (urea) at emergence and 28 kg ha^{-1} of N (urea) at R2/R3 (LeBaron, 1974); 4) 84 kg ha^{-1} of N (urea); 5) 28 kg ha^{-1} of N (ESN) at emergence and 28 kg ha^{-1} of N (ESN) at R2/R3; 6) 56 kg ha^{-1} of N (ESN) at emergence; and 7) 56 kg ha^{-1} of N (Urea with Agrotain or Super-U in 2013 and 2014, respectively) at emergence. For treatments with a later application time the same application method was followed at the R2-R3 stage of growth. The experimental design was a randomized complete block with four replications.

In experiments 1 and 2 the cultivars were planted in four-row plots, 7.6 m long with a 45.7 cm row spacing. Plots were planted with a John Deere 71 flex four-row planter with a cone seed distribution system (John Deere, Moline, IL) pulled behind a Case 385 tractor (Case IH, Racine, WI). Seeds were planted at a depth of 2.5 to 3.8 cm.

Previous crop was wheat and fields with a history of no recent dry bean production were chosen. Research was conducted at Prosper, ND (47.000683, -97.111029) in 2010 and 2012. In 2013 and 2014 experiments were planned at Prosper and near Park River, ND (48.411681, -97.671029). Prosper is located in Cass County and the plot was located on Kindred-Bearden silty clay loam soil (USDA, 2013). Park River is located in Walsh County on Fairdale silt loam

(USDA, 2013). Soil tests were collected in the spring of 2013 and 2014 growing seasons (Table 1). Tests were conducted at the NDSU Soil Testing Lab (Fargo, ND).

Table 1. Soil test results for NO_3 at Prosper and Park River, ND, in 2013 and 2014.

		2013		2014	
Location	Soil Type†	0-30.5 cm	30.5-61cm	0-30.5 cm	30.5-61cm
		---------------------------kg ha^{-1}	---------------------		
Prosper	Kindred-Bearden silty clay loam	16	111	67	67
Park River	Fairdale silt loam	38	16	55	41

†USDA soil survey data.

Fertilizer treatments for experiment 1 were applied at emergence along with most treatments for experiment 2. Granular urea was the N source used. Furrows were hoed about 5 cm deep along each row, and the treatments were hand spread into the furrows and immediately covered. In order to achieve the correct amount of fertilizer, measuring cups were made for each treatment. The cups were made by measuring the amount of fertilizer needed per row for the different treatments, placing measured amount in a plastic container and then cutting the container to be level with the measured amount. This allowed for consistent and quick fertilizer application.

Dimethenamid-P: (S)-2chloro-N-[(1-methyl-2-methoxy) ethyl]-N (2, 4-dimethyl-thien-3-yl)-acetamide (Outlook, BASF, Research Triangle Park, NC) was applied for weed control immediately after planting using a backpack sprayer with 8001 VS nozzles at 275 KPa at a rate of 941 g ha^{-1}. Hand weeding was conducted throughout the season for extended weed control. On July 2nd of 2014 Prosper and Park River were sprayed with bentazon: (3-(1-methylethyl)-1H-2, 1, 3-benzothiadiazin-4 (3H)-one 2, 2-dioxide)) (Basagran, BASF, Research Triangle Park, NC) at 0.3 L ha^{-1} of a.i. and imazamox: 2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1HOimidazol-2-yl)5-(methoxymethyl)-3-pyridinexarboxylic acid) (Raptor, BASF, Research Triangle Park, NC) at 8.4 g ha^{-1} of a.i. Fungicide was applied in 2013 and 2014 for preventative measures. Application of boscalid: (3-pyridinecarboxamide, 2-chloro-N-(4-chloro(1,1-biphenyl)-

2-yl)) (Endura, BASF, Research Triangle Park, NC) was sprayed at a rate of 539 g ha^{-1} of a.i. during early reproductive growth stages.

Stand counts were recorded within the two middle rows for each plot with a 1.5 m measuring stick. A vigor score, scale of 1-9 with 9 being the most vigorous, were measured throughout the growing season along with a visual greenness score, with a scale of 1-5 with 5 being greener. SPAD meter readings, which are on a scale from 0 to 99.9 (although readings over 50.0 are considered less accurate) with higher readings being greener, were taken with a handheld SPAD meter (Spectrum Technologies, Aurora, IL). SPAD readings were taken from the upper-most fully developed leaf and readings were averaged over 6 plants from the inside rows.

A root excavation was done to count nodules per root two weeks after the R2-R3 stage of growth (Table 2). Roots were extracted using the Penn State shovelomics method (Penn State, 2012). Five plants per plot were extracted with shovels; the roots were cleaned in a bucket of water to remove soil before nodules were counted. Plants for nodule counts were taken from the end of the rows before alleys were cut. After counting nodules, the plants were brought back to the lab. The plants were then dried and weighed for total biomass, aboveground biomass, and root biomass.

Table 2. Dates of application and observations.

Measurement/Application	Prosper, ND		Park River, ND	
	--2013--	--2014--	--2013--	--2014--
Dry bean planted	28 May	29 May	29 May	24 May
Applied fertilizer	17 June	18 June	19 June	18 June
Vigor score 1	8 Aug.	9 July	6 Aug.	9 July
Green score 1	14 Aug.	9 July	6 Aug.	9 July
SPAD reading 1	N/A	9 July	N/A	9 July
Second fertilizer application	17 July	17 July	18 July	16 July
Root excavation/nodule count	31 July	7 Aug.	1 Aug.	1 Aug.
SPAD reading 2	19 Aug.	12 Aug.	15 Aug.	12 Aug.
Height measurements	12 Sept.	11 Sept.	4 Sept.	2 Sept.
Harvest	2 Oct.	26 Sept.	3 Oct.	23 Sept.

Alleys were cut at about R5 growth stage in between the replicates, perpendicular to the plot length, resulting in a harvested area of about 6 m x 1.8 m. A mechanical tiller was used to cut alleys and control weeds around the borders of the plot.

Before harvest, average plot plant height and average plot vine length were recorded (Table 2). Plant height was measured from the ground to the upper node of three random standing plants (height of the canopy) within the two center rows. Vine length was the actual extended length of the vines of the observed plants. These measurements were used to calculate the degree of lodging (standability percentage) by dividing the standing height by the vine length. Higher percentages indicate good standability while a lower percentage indicates more lodging. Plot lengths were measured in order to calculate the actual harvested area. If there were large gaps in stand in the middle of a plot not caused by treatment the harvested area was corrected. For example if out of 4 rows, 1 row had a 2 meter gap in stand then 0.5 meters would be reduced from plot length.

During harvest one row per plot was harvested by hand and threshed with a stationary Hege 125B combine (Hege Company, Waldenberg, Germany) in order to simulate “no” harvest loss. The remaining three rows were then mechanically harvested with a Hege 125B combine (Hege Company, Waldenberg, Germany) to represent the direct-harvesting method.

Yield loss was calculated for direct-harvesting by placing a measuring frame, 0.1 m^2 in size, three times within the plot, after the combine had passed. Beans were counted within the frame and used to estimate total yield loss ha^{-1}.

Once all samples were harvested and dried, they were cleaned with an air blast seed cleaner (Allan Machine Company, Nevada, IA) to remove dirt and plant material. The sample was weighed and analyzed for yield, test weight, and 1000 kernel weight. Moisture and test weight were measured using a GAC 2100 moisture tester (DICKEY-John Corp., Minneapolis, MN) and observations were corrected to 13.5% moisture content.

Statistical analyses for experiments 1 and 2 were conducted using standard procedures for a randomized complete block design. Data was analyzed in experiment 1 as a factorial using analysis of variance with SAS (SAS Institute Inc., Cary, NC). PROC MIXED procedure and Type 3 ANOVA tests were used to analyze treatment data. Each environment was first analyzed separately. Data for each individual year are reported in the following data tables.

Previous research data from 2010 and 2012 at Prosper were used for experiment 1 in the combined analysis with Prosper and Park River 2013 and identifying the location by year as an environment and random effect. The experiment was planted in 2011 however flooding damage caused the experiment to be abandoned. Fixed effects in the analysis of experiment 1 were variety, inoculant, and application of N with all other factors considered random effects. In the following data tables the combined data is based on the combined analysis. One variety (Lariat, a pinto bean) in experiment 1 was continued in 2014 at Prosper and Park River. The statistical analysis is similar as the described above, except the factorial had only inoculant (3 levels) and N (2 levels) applied to Lariat without the factor of variety.

Experiment 2 had the fixed effect of fertilizer application with all other factors considered random effects. Experiment 2 had 4 environments with Park River and Prosper in 2013 and 2014. The statistical analysis was done similarly as described in experiment 1. All means were separated using a paired t-test at the 5% level of significance.

Note: Mention of trade names, proprietary products, or vendors does not constitute a guarantee or warranty for the product by North Dakota State University (NDSU) and does not imply its approval to the exclusion of other products or vendors that may be suitable.

Results and Discussion

Weather for Experiment 1 Nitrogen Fertilization and Inoculation Effects on Dry Bean and Experiment 2 Efficiency of Fertilizer Management on Lariat Dry Bean

Air temperatures were close to historical averages each year (Tables 3, 4, and 5). While temperatures were near normal, there was noticeable variation in rainfall. At Prosper there was high rainfall (103.4 mm) recorded in July of 2010, and above average rainfall recorded in May (105.2 mm) and June (192.5mm) of 2013. Park River showed above average rainfall in August of 2014 (88.4 mm). These differences in rainfall may have impacted how the dry bean plants responded to each treatment and in times of heavy rainfall, root nodule activity may have been reduced.

Table 3. Monthly minimum, maximum, and mean temperatures and mean rainfall for Prosper, ND, in 2010 and 2012.

Month	Minimum air temp.			Maximum air temp.			Mean air temp.			Mean rainfall		
	2010	2012	Historical[†]	2010	2012	Historical	2010	2012	Historical	2010	2012	Historical
	°C									mm		
May	8	8	6	20	23	21	14	15	13	70	46	78
June	13	13	12	25	27	25	19	20	19	81	67	100
July	15	17	14	28	32	28	21	24	21	103	16	88
August	14	11	13	28	29	28	21	20	20	89	23	67
September	7	5	8	19	24	22	13	15	15	135	15	66
October	2	0	1	16	11	14	9	6	7	36	45	62

†Historical data represents a 30-yr average from 1981-2010 (NDAWN, 2015).

Table 4. Monthly minimum, maximum, and mean temperatures and mean rainfall for Forest River, ND, in 2013 and 2014.

Month	Minimum air temp.			Maximum air temp.			Mean air temp.			Mean rainfall		
	2013	2014	Historical[†]	2013	2014	Historical	2013	2014	Historical	2013	2014	Historical
	°C									mm		
May	6	7	6	18	19	21	12	13	14	253	63	70
June	13	13	12	25	23	25	19	18	19	85	134	88
July	15	13	15	27	26	28	21	19	21	71	41	86
August	13	13	13	27	26	27	20	20	20	23	88	63
September	10	8	8	23	22	22	16	15	15	50	31	47
October	1	2	1	11	14	13	6	8	7	57	9	47

†Historical data represents a 30-yr average from 1981-2010 (NDAWN, 2015). NDAWN station, about 11 miles from Park River, ND, experiment site).

Table 5. Monthly minimum, maximum, and mean temperatures and mean rainfall for Prosper, ND, in 2013 and 2014.

Month	Minimum air temp.			Maximum air temp.			Mean air temp.			Mean rainfall		
	2013	2014	Historical†	2013	2014	Historical	2013	2014	Historical	2013	2014	Historical
	°C									mm		
May	8	7	6	21	20	21	14	14	13	105	52	78
June	14	14	12	26	25	25	20	20	19	193	107	100
July	15	14	14	28	27	28	21	20	21	20	33	88
August	14	14	13	28	27	28	21	21	20	51	61	67
September	11	8	8	25	23	22	18	15	15	93	47	66
October	2	1	1	12	15	14	7	8	7	84	9	62

†Historical data represents a 30-yr average from 1981-2010 (NDAWN, 2015).

NITROGEN FERTILIZATION AND INOCULATION EFFECTS ON DRY BEAN

Results and Discussion

Inoculant

At Park River in 2013, application of rhizobacteria inoculant prior to planting resulted in an increased number of nodules amongst the three varieties of dry bean compared with the control; however, no difference in nodule count was found between the two inoculants. Addition of inoculant showed an increase in yield for the HiStick inoculant compared to the control but it was not different in yield with RhizoStick inoculant (Table 6). The slightly higher yield from HiStick may be related to the *Bacillus subtilis* in the product, which has natural anti-fungal properties or could be related to the slightly higher cell count g^{-1} of *Rhizobium leguminsarum* bv. *phaseoli* compared to RhizoStick. There were nodules on the control (no inoculant) (Table 6) this indicates natural rhizobacteria in the soil. There were no differences in nodule count at Prosper in either year. This may have been caused by natural rhizobacteria in the soil, or—most likely—the Prosper experiment site had inoculated dry bean grown in previous years.

Table 6. Direct harvest yield and nodule count for inoculants across N levels and varieties of dry bean at Park River, ND, in 2013.

Inoculant	Yield	Nodules
	--kg ha $^{-1}$--	--Nodule Count--
None	2317b†	10.7b
HiStick	2599a	16.2a
RhizoStick	2364ab	15.8a
LSD (0.05)	238	4.2

†Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).

Variety

When the data from Prosper 2010, 2012, and 2013 along with Park River 2014 were combined, differences were found among the varieties of dry bean for number of nodules, height, yield, and kernel weight (Table 7). Lariat had more nodules than both Eclipse and Vista. Lariat both yielded higher and was taller than Vista; however, Eclipse did not differ in yield or height from either variety. Lariat had a higher kernel weight, typical for pinto bean, than the Eclipse and Vista. Eclipse had a higher kernel weight and number of nodules than Vista (Table 7).

Table 7. Direct harvest yield and 1000 kernel weight averages of varieties across inoculants and N treatments averaged over 4 environments; Prosper, ND, in 2010, 2012, 2013, and Park River, ND, in 2013.

Variety	Nodules	Height	Yield	KWT
	--Nodule Count--	--cm--	--kg ha $^{-1}$--	--g--
Eclipse	13b†	46.7ab	2679ab	180.5b
Lariat	19a	48.6a	2820a	300.6a
Vista	9c	45.0b	2578b	169.3c
LSD (0.05)	3	2.2	169	9.8

†Within columns, means followed by the same letter are not significantly different at (P≤0.05).

Across varieties and inoculant there were no yield differences with and without application on N. However, when data was combined across locations, differences were found in vigor and kernel weight with application of N (Table 8). Application of 56 kg ha^{-1} of N across all varieties of dry bean showed an increase in vigor score and decrease in kernel weight. The N applied may have put more of the plants' energy toward vegetative growth or possibly more pods, causing a lower kernel weight as less energy would be going towards pod fill.

Table 8. Vigor score for N treatments across varieties and inoculants over 4 environments; Prosper, ND, in 2010, 2012, 2013, and Park River, ND, in 2013.

N applied	Vigor	KWT
--kg ha $^{-1}$--	--1-9†--	--g--
0	6.2b‡	221.8a
56	6.6a	211.8b
LSD (0.05)	0.3	8.0

†Visual score (1-9) with 1 as poor plant vigor and 9 indicating best plant vigor.
‡Within columns, means followed by the same letter are not significantly different at (P≤0.05).

The following section is describing Lariat with inoculant and N application across Prosper and Park River in 2013 and 2014.

SPAD Readings

Application of N resulted in differences in the green score and SPAD readings (Table 9). The SPAD readings were objective measurements, while green score was recorded visually.

Observations at Prosper indicated an increase in green score in both 2013 and 2014 with addition of fertilizer; however, the SPAD readings showed no difference between N treatments. Park River in 2014 also showed an increase in green score with application of N, but showed a lower SPAD score. Lack in consistency between green score and SPAD readings may be caused by the method of SPAD reading, as readings were taken from the uppermost developed leaf and may not have been representative of the whole plant. Park River in 2013 showed a decrease in green score and SPAD reading with application of N.

Table 9. Visual green score and SPAD readings of Lariat pinto bean across inoculants at Prosper and Park River, ND, in 2013 and 2014.

	Prosper				Park River			
N applied	Green†		SPAD‡		Green		SPAD	
--kg ha $^{-1}$--	2013	2014	2013	2014	2013	2014	2013	2014
0	4.2b§	3.6b	44.5	40.8	4.0a	2.9b	41.6a	39.1a
56	4.6a	4.1a	43.8	40.0	3.3b	4.1a	39.7b	37.0b
LSD (0.05)	0.4	0.4	ns	ns	0.5	0.5	1.7	1.8

†Visual score (1-5) with 1 as lighter green and 5 as darker green.
‡SPAD readings (0-99.9) with higher readings representing darker green.
§Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
ns = not significant.

Differences in green score and SPAD between inoculants were recorded only in 2014 at Park River (Table 10). The visual green score with HiStick had a higher score (greener) than no inoculant, but RhizoStick was not different from no inoculant or HiStick application. The SPAD readings presented different results as no inoculant and RhizoStick had higher readings than HiStick. These differences in results may have been influenced by fertilizer application, although no interaction was observed. While the control had no inoculant added, there was still nodulation as observed earlier in Table 6, which may have caused some increase in the greenness of the plant.

Table 10. Visual green score and SPAD readings of Lariat pinto bean across N applications at Prosper and Park River, ND, in 2013 and 2014.

	Prosper				Park River			
Inoculant	Green†		SPAD‡		Green		SPAD	
	2013	2014	2013	2014	2013	2014	2013	2014
None	4.6	3.9	44.3	41.0	3.6	3.1b§	39.4	40.2a
HiStick	4.3	3.8	44.5	40.0	3.5	4.0a	40.8	35.6b
RhizoStick	4.3	3.9	43.6	41.0	3.8	3.4ab	41.8	38.4a
LSD (0.05)	ns	ns	ns	ns	ns	0.6	ns	2.2

†Visual score (1-5) with 1 as lighter green and 5 as darker green.
‡SPAD readings (0-99.9) with higher readings representing darker green.
§Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
ns = not significant.

Nitrogen

Yield

At the Park River location a difference in yield was found between the N application treatments on Lariat. The application of 56 kg ha^{-1} of N increased yield by 7% in 2014 at Park River, but no difference was found in other years or location (Table 11). High rainfall in June of 2014 at Park River may have contributed to the response to N fertilizer with the wet conditions possibly delaying root nodule activity allowing for the plant to rely on fertilizer N. These findings are consistent with the findings from Eckert et al. (2001) that N application may not increase grain yield in dry bean, but conflict with the results of Edje et al. (1975), who reported a positive grain yield response to application of N. Therefore application of N to dry bean may increase yield in certain conditions, however it did not increase yield in all of the environments of our trial. Conservative N management would appear to be the appropriate approach when dealing with dry bean with various soil and weather conditions.

Table 11. Direct harvest yield for N treatment of Lariat pinto bean across inoculants at Prosper and Park River, ND, in 2013 and 2014.

	Prosper		Park River	
N applied	2013	2014	2013	2014
--kg ha $^{-1}$--	--kg ha $^{-1}$--			
0	3711	3416	2415	3036b†
56	3403	3291	2479	3275a
LSD (0.05)	ns	ns	ns	176

†Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
ns = not significant.

Conclusion

At Park River in 2014 there was an increase in grain yield of Lariat pinto bean with application of 56 kg ha^{-1} of N, but this increase was not found in other years or at Prosper. Application of N did not generally result in differences in yield, which conflicted with the results of Edje et al. (1975). Addition of fertilizer did not cause differences in number of nodules. Adding N did not increase yield, however soil available N and rhizobacteria already in the soil may have been confounding factors. Differences between different varieties of dry bean were found in number of nodules, yield, height, and kernel weight when averaged over four environments (Table 7), but no interactions occurred among variety and inoculant or fertilizer. Inoculation increased the number of nodules at Park River in 2013, but this increase was not found in other years or location and nodules were present on the control.

SPAD reading indicated differences in color of dry bean, but the method used in this study was not consistently representative of the whole plant and did not always match the visual observation. The method involving observation of the uppermost fully developed leaf may need to be improved upon, and more research into using a SPAD meter as a management tool in dry bean could be useful.

EFFICIENCY OF FERTILIZER MANAGEMENT ON LARIAT DRY BEAN

Results and Discussion

Vegetative Growth

In 2013 and 2014 differences were found in biomass growth of Lariat, pinto dry bean at Park River with addition of different fertilizer applications (Table 12). In 2013 above ground vegetation and total biomass showed differences. Three treatments; split application of 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2(urea), 56 kg ha^{-1}(Agrotain), and 84 kg ha^{-1}(urea) had more aboveground vegetation and total biomass than the control and all other treatments except 56 kg ha^{-1} (ESN) (Table 12). It was thought that the slow release function of ESN may feed the plant later into the season, meaning less vegetative growth and more N uptake during pod fill; however Table 12 shows that there was no difference in vegetative growth between the urea, ESN and control.

Table 12. Average weight of above ground vegetation and roots for N treatments of Lariat pinto bean at Park River, ND.

N applied	2013			2014			Combined		
	Vegetation	Root	Total	Vegetation	Root	Total	Vegetation	Root	Total
--kg ha^{-1}--	------------------------------			g			------------------------------		
0	85.5b§	10.0	95.5b	117.2	6.4bc	123.6	101.3	8.2	109.5
28 (urea)†	122.8a	11.5	134.2a	121.0	6.0c	127.0	121.9	8.8	130.6
28 (ESN)†	81.5b	8.1	89.6b	141.2	6.7bc	147.9	111.4	7.4	118.8
56 (urea)	83.9b	8.6	92.5b	157.1	8.5a	165.5	120.5	8.5	129.0
56 (ESN)	111.8ab	10.0	121.9ab	107.1	5.9c	113.1	109.5	8.0	117.5
56 (other) ‡	120.9a	11.3	132.2a	111.5	6.7bc	118.1	-	-	-
84 (urea)	121.6a	10.4	131.9a	134.1	8.1ab	142.2	127.8	9.2	137.0
LSD (0.05)	30.2	ns	31.3	ns	1.7	ns	ns	ns	ns

†Split application of 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2.
‡Urea plus Agrotain was applied in 2013 and Super-U was applied in 2014.
§Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
ns = not significant.

No differences in height were found at Prosper among N treatments. There were differences found in height for 2014 and the combined analysis at Park River. In 2014 all treatments except the split application of 28 + 28 kg ha^{-1} (ESN) were taller than the control. The shorter plant height with the application of ESN compared to other treatments may be explained by the slow release mechanism of the polymer encapsulated granule and the N in the second application may have been too late for plant height development. In the combined analysis only treatments 56 kg ha^{-1} and 84 kg ha^{-1} (urea) were significantly higher than the control (Table 13).

Table 13. Average height for N treatments of Lariat pinto bean at Park River, ND.

N applied	2013	2014	Combined
--kg ha^{-1}--	---------------------------------------cm---------------------------------------		
0	53.0	48.8b§	50.9c
28 (urea)†	50.8	56.3a	53.5bc
28 (ESN)†	51.5	50.0b	50.8c
56 (urea)	59.5	58.3a	58.9a
56 (ESN)	50.3	55.5a	52.9bc
56 (other) ‡	57.5	58.0a	-
84 (urea)	55.8	56.3a	56.0ab
LSD (0.05)	ns	5.4	5.0

†Split application of 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2.
‡Urea plus Agrotain was applied in 2013 and the product Super-U was applied in 2014.
§Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
ns = not significant.

There were differences in vigor among N treatments in 2014 at Prosper and Park River (Tables 14 and 15). The only treatments different in vigor from the control at Prosper were 56 kg ha^{-1} (urea), 56 kg ha^{-1} (Super-U), and 84 kg ha^{-1} (urea). In 2014, at Park River all treatments except the split application of 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2 (ESN) were more vigorous than no N application (Table 15).

Table 14. Average early vigor, green scores, and SPAD readings for N treatments of Lariat pinto bean at Prosper, ND.

N applied	Vigor			Green			SPAD		
	2013	2014	Combined	2013	2014	Combined	2013	2014	Combined
--kg ha^{-1}--	-----------------1-9†-------------			-------------1-5‡--------------			----------0-99.9§----------		
0	6.6	4.0c¶	5.3	4.3	3.8	4.0	44.1	40.5	42.3
28 (urea)††	5.4	4.8bc	5.1	4.8	4.0	4.4	42.9	39.9	41.4
28 (ESN)††	5.5	4.5bc	5.0	5.0	3.8	4.4	43.3	40.7	42.0
56 (urea)	4.9	5.8a	5.3	4.9	4.0	4.4	44.6	42.5	43.5
56 (ESN)	5.1	4.0c	4.6	4.3	3.8	4.0	43.9	43.1	43.5
56 (other)‡‡	6.8	5.3ab	-	4.1	4.3	-	43.9	41.5	-
84 (urea)	5.6	5.3ab	5.4	4.6	4.3	4.4	44.3	40.9	42.6
LSD (0.05)	ns	0.8	ns	ns	ns	ns	ns	ns	ns

†Visual score (1-9) with 1 as poor plant vigor and 9 indicating best plant vigor.
‡Visual score (1-5) with 1 as lighter green and 5 as darker green.
§SPAD readings (0-99.9) with higher readings representing darker green.
¶Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
††Split application of 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2.
‡‡Urea plus Agrotain was applied in 2013 and Super-U was applied in 2014.
ns = not significant.

The combined analysis at Prosper did not result in differences among N applications in vigor, green score, or SPAD readings (Table 14) and at Park River for green score and Spad readings (Table 15).

Table 15. Average early vigor, green scores, and SPAD readings of Lariat pinto bean at Park River, ND.

N applied	Vigor			Green			SPAD		
	2013	2014	Combined	2013	2014	Combined	2013	2014	Combined
--kg ha^{-1}--	-----------------1-9†-------------			-------------1-5‡--------------			----------0-99.9§----------		
0	6.6	4.9c¶	5.8b	3.6a	2.5b	3.1	39.9	41.2	40.5
28 (urea)††	7.8	6.4ab	7.1a	3.8a	4.0a	3.9	41.9	36.9	39.4
28 (ESN)††	7.5	5.6bc	6.6a	3.6a	3.5a	3.6	42.3	40.8	41.6
56 (urea)	7.8	6.4ab	7.1a	3.6a	3.8a	3.7	38.9	39.2	39.1
56 (ESN)	7.1	5.9b	6.5ab	3.4a	3.5a	3.4	43.9	36.5	40.2
56 (other)‡‡	8.3	7.0a	-	2.3b	4.3a	-	41.7	36.9	-
84 (urea)	7.5	6.9a	7.2a	2.6b	4.3a	3.4	38.7	38.3	38.5
LSD (0.05)	ns	0.8	0.7	0.7	0.9	ns	ns	ns	ns

†Visual score (1-9) with 1 as poor plant vigor and 9 indicating best plant vigor.
‡Visual score (1-5) with 1 as lighter green and 5 as darker green.
§SPAD readings (0-99.9) with higher readings representing darker green.
¶Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
††Split application of 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2.
‡‡Urea plus Agrotain was applied in 2013 and Super-U was applied in 2014.
ns = not significant.

Yield

At Park River N application treatments resulted in differences in direct harvest grain yield in 2014. Applications of 56 kg ha^{-1} (ESN) and 56 kg ha^{-1} (Super-U) were significantly higher than the control; however, the yield of these treatments were not higher than application of 56 kg ha^{-1} (urea) and 84 kg ha^{-1}(urea) (Table 16). No differences in yield were observed for single row hand harvest and no differences in direct harvest grain loss. Grain yield results at Prosper did not indicate any N treatment differences in single row hand harvest, direct harvest and direct harvest loss (Table 17). Lack of differences in yield among treatments may be caused by the presence of rhizobacteria in the soil. Although there were no differences in nodule count among treatments, there were nodules present in the absence of rhizobacterial inoculation. Kernel weight differences among treatments were found in Prosper in 2014, showing a decrease in kernel weight with N application in all treatments except 56 kg ha^{-1} of Super-U. As there were no yield differences, but a decrease in kernel weight with application of N, it appears that N application increased number of pods, allowing for less energy going to filling each seed compared to a lower number of pods possibly in the control. This decrease in kernel weight with application of N is consistent with the other experiment in this thesis "Nitrogen Fertilization and Inoculation Effects on Dry Bean".

Table 16. Results for 1000 kernel weight, hand harvest, direct harvest, and harvest loss of N treatments for Lariat pinto bean at Park River, ND.

	KWT			Hand Harvest			Direct Harvest			Loss		
N applied	2013	2014	Combined	2013	2014	Combined	2013	2014	Combined	2013	2014	Combined
--kg ha^{-1}--	----------g----------			----------kg ha^{-1}----------								
0	277	311	294	2383	3884	3133	2224	3030b[§]	2627	510	276	393
28 (urea)[†]	263	317	290	3085	3955	3520	2341	2997b	2669	345	229	287
28 (ESN)[†]	274	315	294	2668	4014	3341	2785	3063b	2924	353	312	332
56 (urea)	282	305	293	2903	3955	3429	2643	3210ab	2926	345	212	278
56 (ESN)	267	326	296	2669	3763	3216	2129	3369a	2924	505	213	359
56 (other)[‡]	277	335	-	2402	3885	-	2478	3345a	-	445	235	-
84 (urea)	275	301	288	2809	3826	3318	2781	3213b	2997	544	171	358
LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	239	ns	ns	ns	ns

†Split application of 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2.
‡Urea plus Agrotain was applied in 2013 and Super-U was applied in 2014.
§Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
ns = not significant.

Table 17. Results for 1000 kernel weight, hand harvest, direct harvest, and harvest loss of N treatments for Lariat pinto bean at Prosper, ND.

	KWT			Hand Harvest			Direct Harvest			Loss		
N applied	2013	2014	Combined	2013	2014	Combined	2013	2014	Combined	2013	2014	Combined
--kg ha^{-1}--	----------g----------			----------kg ha^{-1}----------								
0	323	385a	354	3884	3511	3697	3739	3279	3509	503	820	661
28 (urea)[†]	338	356bc	347	3420	3772	3596	2808	3385	3096	650	577	613
28 (ESN)[†]	307	360bc	333	2926	3327	3126	3011	3366	3188	509	628	569
56 (urea)	296	348bc	322	3256	4119	3687	3424	3148	3286	811	420	615
56 (ESN)	307	346c	323	3172	3789	3480	2623	3335	2979	550	524	537
56 (other)[‡]	347	366ab	-	3931	3846	-	3347	3253	-	403	713	-
84 (urea)	346	356bc	351	3340	3823	3581	2885	3515	3200	645	511	578
LSD (0.05)	ns	19.3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

†Split application of 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2.
‡Urea plus Agrotain was applied in 2013 and Super-U was applied in 2014.
§Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
ns = not significant.

Conclusions

The experiment was repeated at 2 locations over 2 years and consistency among the effects of treatments could not be found. Rhizobacteria present in the soil nodulated the dry bean plants. The control (no additional N applied) yielded no differently than treatments with application of supplemental N. Besides rhizobacteria fixing N, there was residual N available as well as N mineralization, which was most likely the main confounding factor why there was not a yield benefit with the application of supplemental N. In Park River 2014 application of 56 kg ha^{-1} (ESN) and 56 kg ha^{-1} (Super-U) yielded significantly higher than the control; however, the yield of these treatments were not higher than the application of 56 kg ha^{-1} (urea) and 84 kg ha^{-1}(urea). While the application of ESN and Super-U resulted in a higher yield than the control, application of these products can be costly. This increase could not be repeated in other years or location and may be the result of high residual soil N. Available N and presence of rhizobacteria in the soil lowered the need for application of N fertilizer. More research should be done into the effect of supplemental N application on kernel weight as a kernel weight decrease was found with application of N compared to the control in Prosper 2014 (Table 17). The results that indicated a decrease in kernel weight conflict with the results found in Eckert et al. (2011).

SOYBEAN RESPONSE TO NITROGEN INPUTS UNDER TILE DRAINED CONDITIONS

Abstract

Soybean Response to Nitrogen Inputs under Tile Drained Conditions

Best management practices are needed to achieve optimal crop yields. Soybean has the ability to form a symbiotic relationship with N-fixing bacteria; however, it may be possible to increase yield through addition of synthetic N fertilizer. Objectives of this research were to evaluate yield and growth differences between six N management strategies applied to four soybean cultivars grown on tile vs. non-tiled conditions. We also evaluated the effect of tile and N application on the expression of IDC. Tile decreased IDC severity but yields were similar. Addition of N resulted in higher IDC severity. In 2013 there were no significant yield differences among N treatments and in 2014 yields were significantly increased from the control in four of five N treatments. Across environments N application increased yield. Financial returns with fertilizer were not different from no N application.

Literature Review

Soybean as a Crop

Soybean is a major food crop across the world and is an important source of amino acids, protein, and oil. Due to improved genetics and pest management options, soybean yields have increased steadily over the past thirty years giving great importance to the crop's production in the U.S. Midwest (Schmitt et al., 2001).

Tile Drainage

Subsurface drainage removes excess water from the soil profile through a network of perforated tubes. These tubes, commonly called tiles, are usually installed 0.6 to 1.2 m below the soil surface. Drainage prior to the growing season is important so that crops can be planted at the optimum time. Tile drained soil has less surface runoff, erosion, and soil attached phosphorous lost from the land compared to soil without subsurface drainage improvements. However, nitrate loss can occur from tile drained land as nitrate is very soluble and moves easily through the soil and into the tile lines (U.S. Environmental Protection Agency, 2012).

Water is essential for plant growth; however, when there is excess moisture in the soil it can limit air exchange with the atmosphere. This limit in air exchange does not stop the plant roots from respiring, hence causing a buildup in carbon dioxide in the soil. The amount of carbon dioxide in the soil is proportional to the amount of bicarbonate in the soil and as carbon dioxide increases so does bicarbonate. As the bicarbonate increases so does the pH of the soil (Franzen, 2012). University of Minnesota research has shown that more bicarbonate in the soil was positively correlated with higher iron-deficiency chlorosis (IDC) (Kaiser et al., 2011). Interveinal yellowing of the leaves with the leaf veins staying a dark green are IDC symptoms and it is caused by the plant not being able to take up enough iron (Fe), even if there is sufficient Fe in the soil (Kaiser et al., 2011). The form of Fe that plants can take up becomes less soluble at higher soil pH. Soil nitrates have been shown to increase the severity of the chlorosis. During the uptake of nitrates into the plant it must exchange with a bicarbonate ion. For the nitrate to be usable to the plant it must be converted to ammonium which increases the pH in the leaf sap, which in turn reduces the solubility of the necessary Fe (Kaiser et al., 2011). Tile drainage can help alleviate the effects of IDC by reducing excess moisture.

Nelson et al. (2012), observed multiple soybean cultivars on claypan soils with drainage or drainage plus subirrigation. They reported increased yields from 15 to 46% compared to the non-drained control. This research also showed a decrease in oil concentration on two cultivars by 0.3 percent with drainage, although there was no effect on three other cultivars. They concluded that it was important to match high yielding cultivars with appropriate drainage water management systems.

Importance of Fertilizer

Presently N application is not recommended for soybean according to the NDSU extension service due to a possibility of increasing the severity of IDC caused by high nitrates in the soil and under the present yield levels the economics of N application are not justified (Franzen, 2013). However, with better genetics and improved management resulting in high yield levels, highest recorded yield of 10 800 kg ha^{-1} by Kip Cullers in Missouri (Missouri Soybean Association, 2010), the plant may not be able to biologically fix and take up soil available N to maximize production.

Research Objectives

The objectives of this soybean research were to: 1) evaluate plant growth and grain yield with six different N treatments applied to soybean cultivars grown under tiled versus non-tiled conditions; 2) evaluate the effect of tile and application of N on the expression of IDC; 3) record observations on plant growth with visual scores, plant measurements, and with optical sensors to better understand soybean growth in relation to yield.

Materials and Methods

This experiment was conducted at the NW22 NDSU experiment station outside of Fargo, ND (46.931855, -96.859287), which consists of Fargo-Ryan silty clay (USDA, 2013). Soil samples were taken in the fall before planting in 2013 and during the growing season in 2014 (Table 18). The experimental field had tiled and non-tiled ground with 7.6 m tile spacing (Figure 1). There were four replications and the experiment was set up as a factorial (four cultivars and six N treatments) within a randomized complete block design with a split plot arrangement for evaluating tiled and non-tiled drained conditions. Water table levels were read throughout the growing season by recording measurements from the wells distributed throughout the station.

Table 18. Soil test results for NO_3 at Fargo, ND, in 2013 and 2014.

		2013		2014	
Location	Soil Type†	0-30.5 cm	30.5-61cm	0-30.5 cm	30.5-61cm
		--------------------------kg ha^{-1}-------------------------			
Fargo	Fargo-Ryan silty clay	49	27	59	40

†USDA soil survey data.

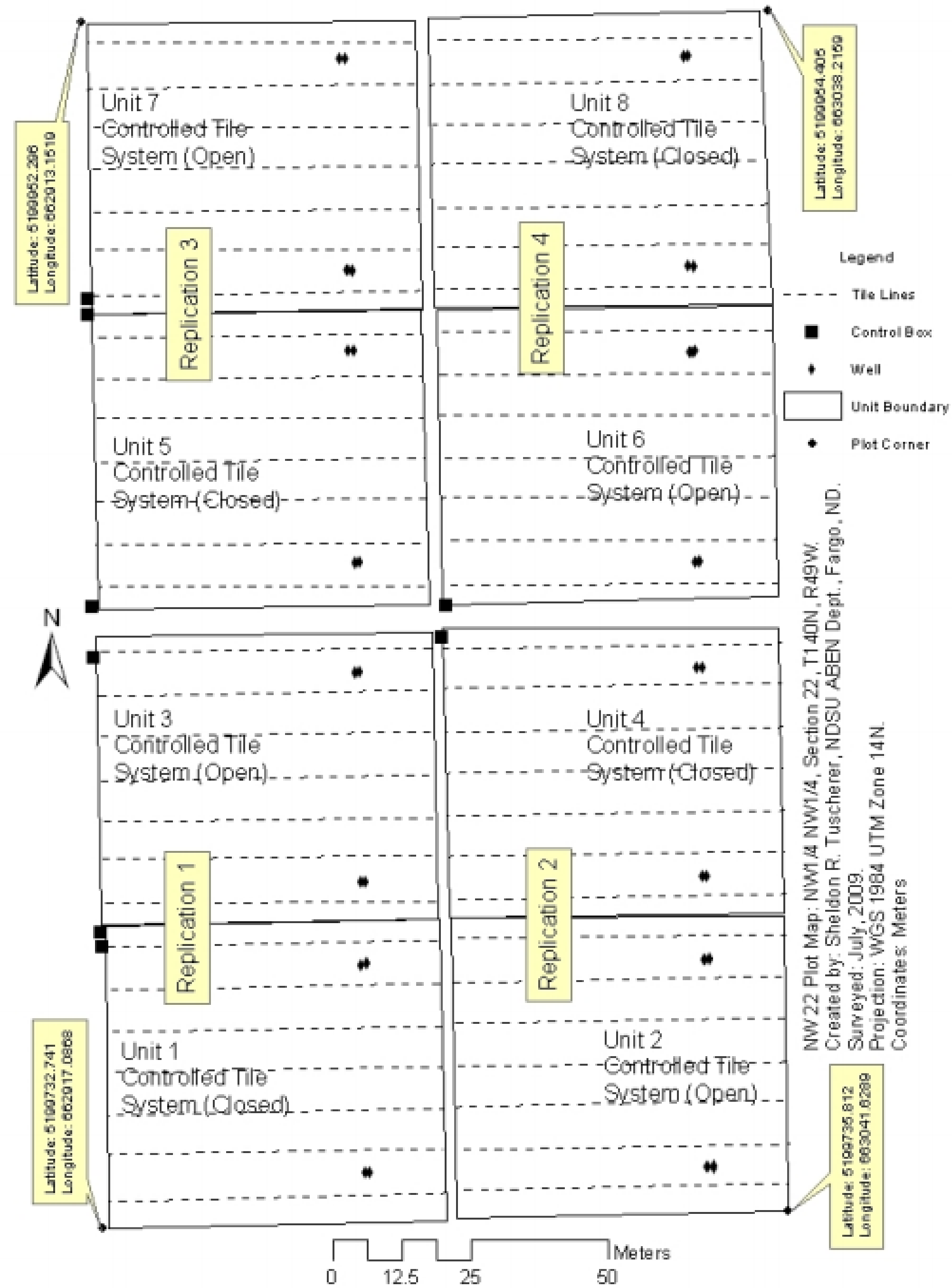

Figure 1. Experimental area near Fargo, ND. The area is divided into eight units: four simulating undrained and four drained (Hoppe, 2013).

Four soybean cultivars were used each year: 90Y41 (Pioneer), 04403 (Mustang), PFS 12R06 (Peterson Seed), 6088 (NuTech)/0906R2(Channel). The cultivar 0906R2 was used in the second season to replace 6088 because no seed was available in 2014; 0906R2 was selected as the best replacement due to similar maturity and IDC score. The selected cultivars have different maturity and IDC ratings along with different growth types.

Table 19. Characteristics of soybean cultivars included in field experiments.

Company	Cultivar	Maturity†	IDC
DuPont Pioneer	90Y41	0.4	2.0
Mustang	04403	0.4	2.0
Peterson Seed	12R06	0.6	2.5
NuTech§	6088	0.8	2.7
Channel§	0906R2	0.8	2.7‡

†Maturities and IDC are based on averaged performance score over multiple locations as reported in the 2012 ND Soybean Variety Trial Results and Selection Guide (Kandel et al., 2012) The scale is 1 to 5 with 5 being the most chlorotic.
‡IDC for cultivar 0906R2 is based on averaged performance score over multiple locations as reported in the 2013 ND Soybean Variety Trial Results and Selection Guide (Kandel et al., 2013).
§After being grown in 2013 NuTech's 6088 was replaced with Channel's 0906R2 in 2014 as 6088 seed was not available in the spring of 2014.

There were two main treatments of tile and non-tiled plots. There were six N fertilizer treatments applied to the plots. Granular urea (46-0-0) as well as a polymeric coated form of urea (44-0-0), ESN (Agrium, Calgary, AB), were applied. The N treatments were as follows: 1) no fertilizer (control), 2) 56 kg ha^{-1} N (urea) at emergence, 3) 84 kg ha^{-1} N (urea) at emergence, 4) 56 kg ha^{-1} N (urea) at the R2-3 stage, 5) 28 kg ha^{-1} N (urea) at emergence and 28 kg ha^{-1} at R2-3, and 6) 56 kg ha^{-1} N (ESN) at emergence.

Soybean seed received from seed companies without a fungicide/insecticide seed treatment were treated with the fungicide Apron Maxx RTA (Syngenta Crop Protection, Inc., Greensboro, NC) (a.i. mefenoxam and fludioxonil) at a rate of 3 ml kg^{-1} seed (a.i. mefenoxam 11.3 g L^{-1} and a.i. fludioxinil 7.55 g L^{-1}) (Table 20). Seeds were treated in a Hege 11 liquid seed treater (Hans-Ulrich, Hege, Waldenberg, Germany).

Table 20. Soybean cultivars and fungicide/insecticide seed treatment applied at Fargo, ND, in 2013 and 2014.

Cultivar	2013	2014
90Y41	Gaucho (a.i. imidacloprid)†, Trilex (a.i. trifloxystrobin and metalaxyl)†	Gaucho (a.i. imidacloprid)†, Trilex (a.i. trifloxystrobin and metalaxyl)†
04403	Poncho/Votivo (a.i. pyraclostrobin and metalaxyl)‡	Poncho/Votivo (a.i. pyraclostrobin and metalaxyl)‡
12R06	Apron Maxx RTA (a.i. mefenoxam and fludioxonil)§	Apron Maxx RTA (a.i. mefenoxam and fludioxonil)§
6088	Apron Maxx RTA (a.i. mefenoxam and fludioxonil)§	N/A
0906R2	N/A	Acceleron (a.i. pyraclostrobin and metalxyl)‡

†Bayer Crop Science LP, Research Triangle Park, NC.
‡Monsanto Co., St. Louis, MO.
§Syngenta Crop Protection, Greensboro, NC.

The plots were planted four rows wide (row spacing 35.6 cm) and about 6 m long. The previous crop was wheat. Management dates and observation dates are found in Table 21. Stand counts were recorded for a length of 1.5 m. in the two middle rows of each plot. A vigor score, scale of 1-9 with 9 being the best, were determined twice throughout the growing season. An IDC score, 1-5 with 1 as no chlorosis, was given to each plot in early July along with a SPAD reading to determine greenness. SPAD readings were taken with a handheld SPAD meter (Spectrum Technologies, Aurora, IL). SPAD meter readings are on a scale from 0 to 99.9 (although readings over 50.0 are considered less accurate) with higher readings being greener. SPAD readings were taken from the upper-most fully developed leaf and readings were averaged over six plants from the inside rows.

Table 21. Dates of field measurements or applications at NW22 Experiment Station, Fargo, ND.

	Date	
	Year	
Measurement/Application	2013	2014
Soybean seeded	16 May	23 May
SPAD reading 1	7 June	4 June
Vigor score 1	7 June	7 July
Applied fertilizer	11 June	4 June
IDC score	1 July	23 June
SPAD reading 2	11 July	10 July
Vigor score 2	11 July	18 July
Second Fertilizer application	17 July	10 July
SPAD reading 3	29 July	29 July
SPAD reading 4	13 Aug.	19 Aug.
Maturity notes	18 Sept.	9 Sept.
Plant height recorded	24 Sept.	15 Sept.
Soybean harvested	2 Oct.	7 Oct.

Soil samples were obtained in 2014 from 16 plots containing the cultivar 0906R2 with the fertilizer application treatments 0 and 56 kg ha^{-1} N (urea). The soil samples were tested by the NDSU Soils lab, for pH value, calcium carbonate equivalent (CCE), and the amount of salts in the soil or soil electrical conductivity (EC).

Two plants from each plot were collected just prior to harvest and the height of the lowest pod (from the soil), pods per plant, seeds per pod, and seeds per plant were determined.

Plots were harvested using a Wintersteiger Classic plot combine (Wintersteiger Ag, Ried, Austria) after physiological maturity at harvestable seed moisture levels. The samples were cleaned using a Clipper seed cleaner (Ferrell-Ross, Bluffton, IN) and weighed for yield. Test weight, 1000 kernel weight, seed oil and protein content were measured with a DA 7200 NIR analyzer (Perten instruments, Hagersten, Sweden). Moisture was measured using a GAC 2100 moisture tester (DICKEY-John Corp., Minneapolis, MN) and observations were corrected to 13% moisture.

Income estimation analysis was conducted by using the Feb. 2015 average soybean base price for Minneapolis of \$0.35 kg^{-1} multiplied by the kg ha^{-1} grain yield. For plots that received N applications the price of urea or ESN was subtracted from the grain yield price. Costs of fertilizer used were \$0.50 kg^{-1} for urea and \$0.64 kg^{-1} for ESN (December, 2014 Dakota Ag, Kindred, ND). Labor and fuel costs were not included into this income estimation.

Statistical analysis was conducted using standard procedures for a factorial experiment in a randomized complete block design with a split-plot arrangement. All variables were analyzed using analysis of variance with SAS (SAS Institute Inc., Cary, NC). PROC MIXED procedure and Type 3 ANOVA tests were used to analyze treatment data. Data was first analyzed as separate experiments for each year. Data was combined for a combined statistical analysis with the addition of the random factor of year. Mean square tables can be found in the appendix. Fixed effects in the analysis were drainage, N application, and cultivar with all other factors considered random effects. All means were separated using a paired t-test at the 5% level of significance, except grain yield which used a 10% level of significance. Analysis of correlation between yield and SPAD readings for treatments was conducted using simple linear analysis in SAS as well as use of scatter plots and trend lines in Microsoft Excel (Microsoft, Redmond, WA).

Note: Mention of trade names, proprietary products, or vendors does not constitute a guarantee or warranty for the product by NDSU and does not imply its approval to the exclusion of other products or vendors that may be suitable.

Results and Discussion

Weather and Environment

Weather can vary widely from year to year, and although 2013 and 2014 were fairly similar in terms of temperature, there were differences in rainfall recorded (Table 22). In May and June of 2013 above normal rainfall fell in Fargo causing some periodic overland flooding at the Fargo NW22 experimental site. This overland flooding early in the season appeared to have increased the severity of IDC symptoms in 2013 (Table 28). During July and August of 2013, below average rainfall was recorded, and cracks were visible in the soil between each plot (Figure 2). This below average rainfall occurred during the reproductive growth of the soybean plant, possibly contributing to low grain yields (Table 24). In July and August of 2014, there was slightly higher rainfall than in 2013, which allowed for higher grain yields (Table 24).

Figure 2. Cracks between plots at NW22 experiment station from lack of moisture.

Table 22. Monthly minimum, maximum, and mean temperatures and mean rainfall for Fargo, ND, in 2013 and 2014.

Month	Minimum air temp.			Maximum air temp.			Mean air temp.			Mean rainfall		
	2013	2014	Historical†	2013	2014	Historical†	2013	2014	Historical†	2013	2014	Historical†
	°C									mm		
May	8	8	7	20	20	21	14	14	14	141	50	71
June	14	14	13	25	25	25	20	20	19	199	140	99
July	16	15	15	28	27	28	22	21	22	26	34	71
August	15	16	14	28	26	27	22	21	21	12	37	65
September	12	10	9	24	22	22	18	16	15	106	51	65
October	3	3	2	12	15	13	7	9	8	112	8	55

†Data represents a 30-yr average from 1981-2010 (NDAWN, 2015).

Tile

There were no significant differences between tile treatments for grain yield, 1000 kernel weight, test weight, oil and protein content, or any growth observations such as early and late vigor or plant height. In 2013, soybean grain yield was 2 280 kg ha^{-1} in non-tiled versus 2 414 kg ha^{-1} in tiled, and in 2014, yield was 3 571 kg ha^{-1} in non-tiled and 3 652 kg ha^{-1} in tiled treatments. Although the statistical analysis shows that the yield increase due to tile was not significant, the increased yield trend (5.6% in 2013 and 2.2% in 2014) is consistent with previous research at the same experiment station (Kandel et al., 2013).

Tile had a significant effect on IDC severity in 2014 with an IDC score of 2.6 without tile and a score of 2.0 with tile. Early in each season, when rainfall was heavy (Table 22, Figures 3 and 4), the tile treatment had a lower water table (Figures 3 and 4). The removal of excess water early in the growing season may have reduced stress levels during regular IDC appearance resulting in a lower IDC score in the tile treatment. During the second half of the growing season the water tables for each tile treatment were about the same.

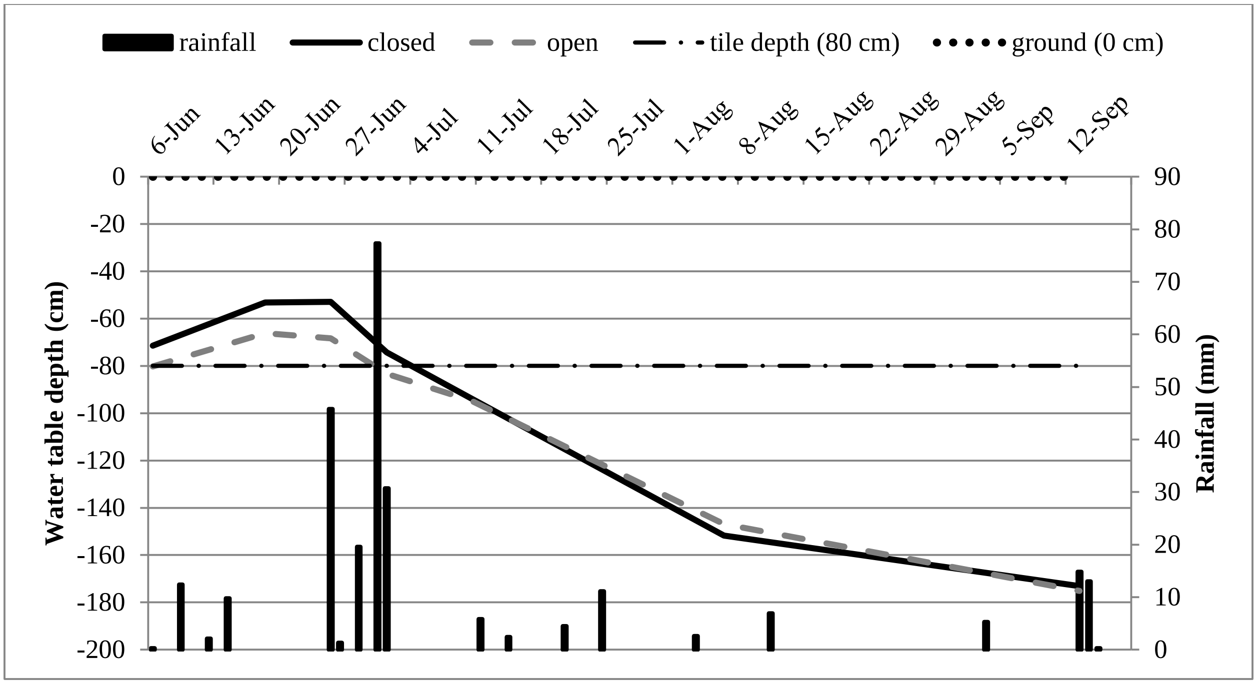

Figure 3. Depth of water table for drainage treatments as affected by rainfall at Fargo, ND, in 2013. Tiled treatments are represented by open tile lines and non-tiled by closed tile lines.

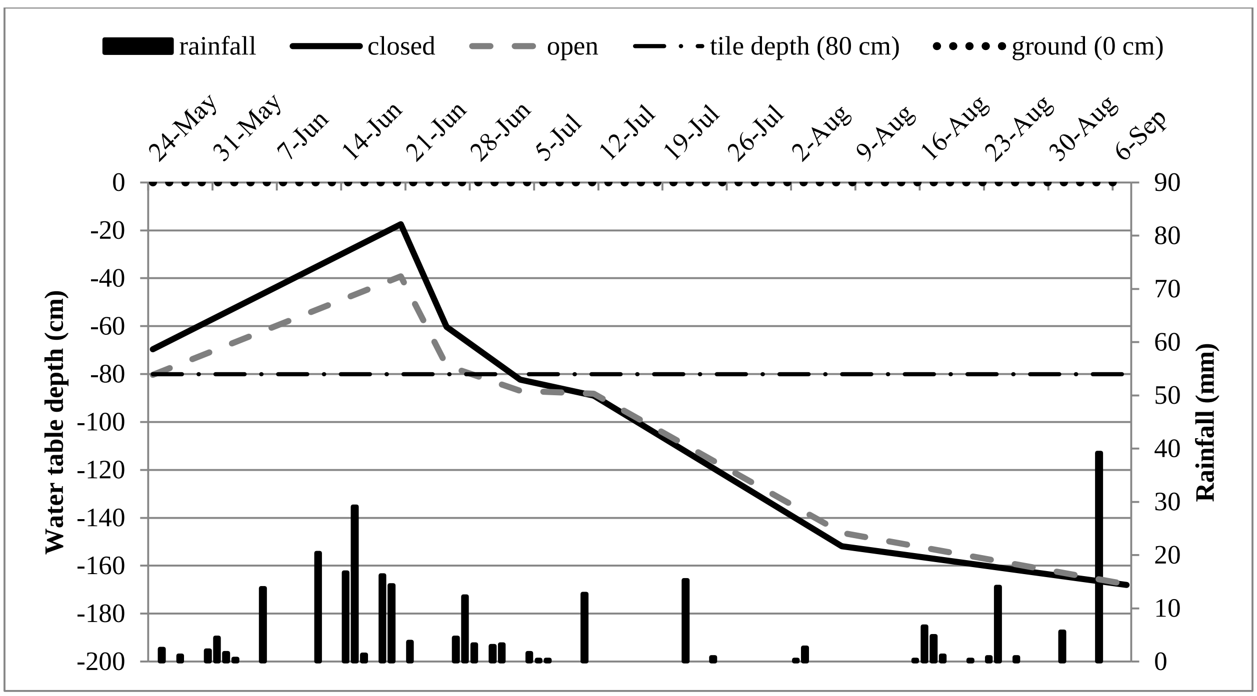

Figure 4. Depth of water table for drainage treatments as affected by rainfall at Fargo, ND, in 2014. Tiled treatments are represented by open tile lines and non-tiled by closed tile lines.

There was a trend for higher yield with tile for each N treatment compared to the non-tiled counterpart, although there were no significant differences between fertilizer application and tile (Table 23).

Table 23. Yield of fertilizer treatments on Non-tiled and Tiled ground across cultivars Fargo, ND, 2014.

N applied	Non-tile	Tile
----kg ha^{-1}-----	-----------------------kg ha^{-1}--------------------	
0	3 412	3 506
28-28 (urea split)†	3 592	3 823
56 (urea)	3 557	3 692
56 (ESN)	3 619	3 631
56 (urea at R2)‡	3 557	3 605
84 (urea)	3 648	3 671
LSD (0.05)	ns	ns

†Treatment has two applications, 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2.
‡Treatment applied at R2, all other treatments applied at emergence.
ns = not significant.

Nitrogen Fertilizer

Yield

There was no yield increase due to fertilizer application in 2013, but in 2014 most N treatments yielded significantly more than the control (no additional fertilizer) and combined across all years all N treatments yielded significantly higher than the control (Table 24).

Table 24. Harvested yield for N applications across cultivars and tile treatments at Fargo, ND.

N applied	2013	2014	Combined
----kg ha^{-1}-----		-----------------------------kg ha^{-1}-----------------------------	
0	2 291	3 462b‡	2 876c
28-28 (urea split)†	2 384	3 708a	3 046a
56 (urea)	2 420	3 625a	3 025ab
56 (ESN)	2 325	3 625a	2 979ab
56 (urea at R2)§	2 361	3 581ab	2 971b
84 (urea)	2 410	3 659a	3 035ab
LSD (0.10)	ns	133	70

†Treatment has two applications, 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2.
‡Within columns, means followed by the same letter are not significantly different at ($P \leq 0.10$).
§Treatment applied at R2, all other treatments applied at emergence.
ns = not significant.

In 2014 grain yield was higher with application of fertilizer; all treatments except 56 kg ha^{-1} of N at R2 were significantly higher yielding compared with the control. The combined mean grain yields were significantly higher with each N application compared to the control. However, the grain yields with N applications were not significantly different from each other (Table 24). These results indicate that N fertilizer increased grain yield; however, these results did not show a N rate or time-of-application response. The increase in yield from application of N in 2014 conflicts with the research of Schmitt et al. (2001). They not only showed no increase from application of N, but also that polymer-coated urea and late application can increase yield. In 2014 the late application of 56 kg ha^{-1} at R2 did not yield differently from the control, while the other treatments yielded significantly higher.

Financial Return

Nitrogen application to soybean has not been recommended in the past in ND (Franzen, 2013) as soybean can form symbiotic relationships with rhizobacteria to fix N from the atmosphere. There was a significant increase in yield (Table 25), but there were no significant differences in financial returns after cost of fertilizer was accounted for. In Table 25 with the split application in the combined analysis there was about a $30.00 increase ha^{-1}, however this was not significantly different ($P = 0.30$) than the control. Addition of fertilizer can increase yield and may have the potential to increase a grower’s profit, but also has potential for causing financial losses if the cost of the fertilizer is greater than the increased revenue (like ESN in this study). The economics will depend on the price of fertilizer N and soybean, which both fluctuate.

Table 25. Adjusted soybean income ha^{-1} estimates for N applications across cultivars and tile treatments at Fargo, ND.

N applied	2013	2014	combined
kg ha^{-1}	------------------------U.S. $ ha^{-1}‡	--------------------------------	
0	792	1196	994
28-28 (urea) split†	796	1254	1025
56 (urea)	798	1225	1013
56 (ESN)	758	1217	987
56 (urea) at R2§	788	1210	999
84 (urea)	779	1223	1001
LSD (0.05)	ns	ns	ns

†Treatment has two applications, 28 kg at emergence and 28 kg at R2.
‡Prices are based on the Minneapolis February average soybean base price \$0.35 kg^{-1} (USDA, 2015) and fertilizer costs of \$0.50 kg^{-1} for urea and \$0.64 kg^{-1} for ESN (December, 2014 Dakota Ag, Kindred, ND).
§Treatment applied at R2, all other treatments applied at emergence.
ns = not significant.

Test Weight

Although no significant differences between N treatments were found for seeds per pod, pods per plant, or 1000 kernel weight, the 28 kg and 56 kg ESN treatment did have a significantly higher test weight compared to the control in 2014 (Table 27). Although test weight, or bulk density, is no longer part of U.S. grades for soybeans as of 2007, it is routinely measured since a higher test weight is a general indicator of better grain quality (Bern and Brumm, 2009). With the 28 kg split and the 56 kg of ESN treatments (with the highest test weights in 2014), it appears that a steady supply of N throughout plant development may increase grain test weight (Table 26).

Table 26. Plants per ha^{-1} and post-harvest data for N treatments across cultivars and tile treatments at Fargo, ND, 2013.

N applied	Population	TW†	TS	TP	SP	KWT†	Yield	Revenue	PC†	PC ha^{-1}†	OC†	OC ha^{-1}†
---kg ha^{-1}-----	Plants ha^{-1}	kg m^3	----------number------			gram	kg ha^{-1}	$	%	kg	%	kg
0	249770	719.6	62.1	25.1	2.5	133	2291	792	30.9	708	17.9	410
28 (urea) split‡	261058	722.7	61.6	24.6	2.5	135	2384	796	30.9	738	18.0	428
56 (urea)	252172	715.3	62.8	25.1	2.5	136	2420	808	31.0	751	17.8	430
56 (ESN)	248809	726.8	65.6	26.5	2.5	132	2325	768	31.1	720	17.9	415
56 (urea) at R2§	254333	720.0	62.4	25.1	2.5	134	2361	788	31.0	729	17.9	422
84 (urea)	248329	730.5	62.1	24.9	2.5	135	2411	791	31.1	748	17.8	430
LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

†TW = test weight, TS = total seeds $plant^{-1}$, TP = total pods $plant^{-1}$, SP = seeds pod^{-1}, KWT = 1000 kernel weight, PC = % protein content, PC ha^{-1} = kg ha^{-1} of protein, OC = oil content, OC ha^{-1} = kg ha^{-1} of oil.
‡Treatment has two applications, 28 kg at emergence and 28 kg at flowering.
§Treatment applied at flowering, all other treatments applied at emergence.
ns = not significant.

Table 27. Plants per ha^{-1} and post-harvest data for N treatments across cultivars and tile treatments at Fargo, ND, 2014.

N applied	Population	TW†	TS	TP	SP	KWT†	Yield	Revenue	PC†	PC ha^{-1}†	OC†	OC ha^{-1}†
kg ha^{-1}	Plants ha^{-1}	kg m^3	----------number----------			gram	kg ha^{-1}	$	%	kg	%	kg
0	225754	735.3	54.8	23.0	2.4	137	3462	1196	31.4	1086	18.5	635
28 (urea) split†	198855	740.1	58.9	25.3	2.3	136	3708	1254	31.5	1168	18.2	674
56 (urea)	212785	738.4	58.0	24.5	2.4	135	3625	1225	31.5	1143	18.3	658
56 (ESN)	207261	739.8	56.3	23.8	2.4	134	3625	1217	31.4	1140	18.3	661
56 (urea) at R2‡	226714	735.8	54	22.7	2.4	136	3581	1210	31.4	1125	18.4	657
84 (urea)	199336	737.6	55.8	23.1	2.4	138	3659	1223	31.4	1149	18.2	665
LSD (0.05)	21116	3.7	ns	ns	ns	ns	ns	ns	ns	52	ns	ns

†Treatment has two applications, 28 kg at emergence and 28 kg at flowering.
‡Treatment applied at flowering, all other treatments applied at emergence.
§TW = test weight, TS = total seeds $plant^{-1}$, TP = total pods $plant^{-1}$, SP = seeds pod^{-1}, KWT = 1000 kernel weight, PC = % protein content, PC ha^{-1} = kg ha^{-1} of protein, OC = oil content, OC ha^{-1} = kg ha^{-1} of oil, ns = not significant.

Plant Height

Soybean plant height in 2014 was significantly influenced by N application (Figure 5). The treatments that received urea at emergence were significantly taller than the control treatment, but treatments with 56 kg urea split and early application or 84 kg urea had similar height (Figure 5). The application of 56 kg of N as urea at R2 or 56 kg of ESN were not significantly different in height from the control which suggests that the N in ESN was not available early in plant development and contributing to vegetative growth (Figure 5). While increased height can be a positive sign of vegetative growth and possibly higher yield, it also can lead to lodging and potentially lose yield in doing so.

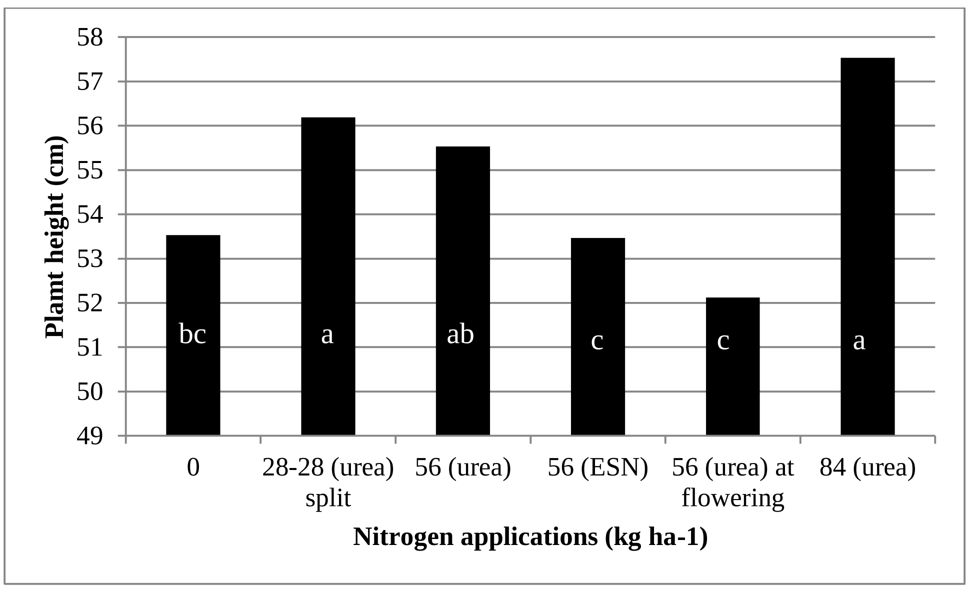

Figure 5. Height of soybean plants for N treatments across cultivars and tile treatments at Fargo, ND, in 2014. Within the bars, means followed by the same letter are not significantly different at ($P \leq 0.05$).

IDC

Application of N is generally not recommended due to economics, and it has been shown in the past to aggravate IDC. Increase in IDC score was confirmed in Fargo in both 2013 and 2014, as higher N application significantly increased the severity of chlorosis early in the season

(Table 28). High soybean yields with N application across years (Table 24) suggests that the plants were generally able to recover from the IDC symptoms.

Table 28. IDC scores for N application across cultivars and tile treatments at Fargo, ND.

N applied	2013	2014	Combined
--kg ha^{-1}------	---------------------------------------1-5†-----------------------------		
0	2.2c‡	2.0c	2.1d
28-28 (urea) split§	2.7b	2.4b	2.6b
56 (urea)	3.0a	2.5b	2.8a
56 (ESN)	2.5bc	2.1c	2.3c
56 (urea) at R2¶	2.2c	2.0c	2.1cd
84 (urea)	2.9ab	2.8a	2.9a
LSD (0.05)	0.25	0.18	0.19

†Treatment has two applications, 28 kg at emergence and 28 kg at R2.
‡Treatment applied at R2, all other treatments applied at emergence.
§Based on the visual scale from Goos and Johnson (2008), with 5 being most chlorotic
¶Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).

Soil test results and IDC scores for selected treatments within the cultivar 0906R2 are provided in Tables 29 and 30. The application of 56 kg urea resulted in a significantly higher IDC score. The percent calcium carbonate equivalent (CCE) and electrical conductivity (EC) or soluble salts were not significantly different among the treatments (Table 29). The tile drainage treatment alleviated the effects of IDC.

Table 29. Soil test results and IDC score for two N treatments applied to cultivar 0906R2 across tile treatments at Fargo, ND, in 2014.

N applied	IDC	pH	CCE (0-31 cm)	CCE (31-61 cm)	EC (0-31 cm)	EC (31-61 cm)
kg ha^{-1}	--1-5†--		--------% CaCO3--------		---------mmhos /cm------	
0	1.9b‡	8.2	3.4	7.7	0.7	1.2
56 (urea)	2.6a	8.2	1.7	4.3	0.7	1.2
LSD (0.05)	0.5	ns	ns	ns	ns	ns

†Based on the visual scale from Goos and Johnson (2008), with 5 being most chlorotic.
‡Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
ns = not significant.

Table 30. Soil test results and IDC score for tile treatments with cultivar 0906R2 across N treatments at Fargo, ND, in 2014.

Tile drainage	IDC	pH	CCE (0-31 cm)	CCE (31-61 cm)	EC (0-31 cm)	EC (31-61 cm)
	--1-5†--		--------% $CaCO_3$---------		---------mmhos /cm----------	
Closed	2.5b‡	8.2	2.7	8.1	0.7	1.5
Open	1.9a	8.2	2.4	3.9	0.7	0.9
LSD (0.05)	0.2	ns	ns	ns	ns	ns

†Based on the visual scale from Goos and Johnson (2008), with 5 being most chlorotic.
‡Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
ns = not significant.

Cultivar

Cultivars 90Y41 and 04403 shared the same maturity. However, 90Y41 has a more erect growth type compared to the intermediate/bushy growth type of 04403. This difference in growth type allowed for a more vigorous plant and showed significantly more vigorous growth in 2014, which may have allowed for the cultivar 04403 to yield higher than 90Y41 (Table 31).

Table 31. Grain yield in 2014 for cultivars across N application and tile treatments at Fargo, ND.

Cultivar	Maturity	Yield	Early vigor
		-kg ha^{-1}-	--1-9†--
90Y41	0.4	3 109c‡	6.1b
04403	0.4	3 692b	6.5a
12R06	0.6	3 642b	6.1b
0906R2	0.8	3 998a	6.7a
LSD (0.05)		129	0.3

†Visual score (1-9) with 1 as poor plant vigor and 9 indicating best plant vigor.
‡Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).

Cultivar 0906R2 had the highest yield at 3 998 kg ha^{-1} and also had one of the highest early vigor scores. Later-maturing cultivars tend to yield more than early-maturing, although 04403 and 0906R2 had statistically similar early vigor, the grain yield was significantly different (Table 31).

Table 32. 1000 kernel weight, seeds plant^{-1} and grain yield for cultivars across N application and tile treatments at Fargo, ND, in 2013.

Cultivar	1000 KWT	Seeds plant $^{-1}$	Yield
	-----g-----		-----kg ha^{-1}-----
90Y41	146a†	51.5b	2 345
04403	130b	66.2a	2 364
12R06	131b	64.5a	2 396
6088	132b	67.9a	2 361
LSD (0.05)	0.3	9.4	ns

†Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
ns = not significant.

Cultivar choice can play a large part in grain yield, as found in 2014 (Table 31). In 2013, there were no significant differences for grain yield among cultivars but there was a significant difference in 1000 kernel weight (Table 32).

Cultivar 90Y41 had both significantly higher kernel weight and significantly fewer seeds per plant in 2013. The lower seed number per plant could have allowed the plant to allocate more energy towards filling seeds as there were less to fill, causing a heavier kernel weight (Table 32).

Optical Sensors

SPAD readings were taken throughout the season using the uppermost developed leaf. There appeared to be inconsistencies between treatments comparing different dates and over the years 2013 and 2014. There were consistent significant SPAD differences between cultivars in 2013 (Tables 33 and 34). Cultivar 90Y41 had a significantly higher SPAD reading throughout the season and the highest reading in the last observation (Table 33 and 34). This suggests that there were many differences between cultivars, but could make it difficult to pick up differences in N applications.

From the SPAD observations made (Tables 35 and 36) it appears that there are significant differences in the SPAD readings among treatments, however when the SPAD data was correlated with the yield data there was no significant correlation. No significant interactions with cultivar and fertilizer were observed. The color differences between cultivars as well as the method of obtaining the observations could possibly make a difference in the reading. It does not appear that SPAD readings would be an appropriate method of estimating plant N needs for soybean.

Table 33. SPAD readings for cultivars across N application and tile treatments at Fargo, ND, in 2013.

Cultivar	SPAD† 1	SPAD 2	SPAD 3	SPAD 4
90Y41	37.6a‡	34.0ab	43.1a	48.8a
04403	36.1ab	32.5c	36.9c	45.3b
12R06	34.8bc	33.1bc	37.8c	44.0c
6088	34.3c	35.0a	39.0b	45.4b
LSD (0.05)	1.6	1.2	1.0	0.7

†SPAD readings (0-99.9) with higher readings representing darker green.
‡Within columns, means followed by the same letter are not significantly different at (P≤0.05).

Table 34. SPAD readings for cultivars across N application and tile treatments at Fargo, ND, in 2014.

Cultivar	SPAD† 1	SPAD 2	SPAD 3	SPAD 4
90Y41	23.0c‡	32.6a	38.6	48.7a
04403	26.3a	30.7b	34.1	45.1b
12R06	21.9c	30.3b	39.3	44.3c
0906R2	24.7b	30.4b	32.3	45.1b
LSD (0.05)	1.5	0.8	ns	0.7

†SPAD readings (0-99.9) with higher readings representing darker green.
‡Within columns, means followed by the same letter are not significantly different at (P≤0.05).
ns = not significant.

Table 35. In season observations for N applications across cultivar and tile treatments at Fargo, ND, in 2013.

N applied	SPAD 1	SPAD 2	SPAD 3	SPAD 4	Vigor 1	Vigor 2	IDC	Height
---kg ha^{-1}-----	-------------------------------0-99.9†------------------------------				-----------1-9‡-----------		--1-5§--	--cm--
0	35.4bc¶	32.5b	39.0	46.0	6.5	7.0	2.2c	48
28 (urea) split††	35.3bc	33.7ab	39.2	45.5	6.5	7.7	2.7ab	49
56 (urea)	36.5ab	34.4ab	38.8	45.5	6.6	6.6	3.0a	49
56 (ESN)	33.9c	33.9ab	38.6	46.2	6.4	6.7	2.5bc	49
56 (urea) at R2‡‡	34.9bc	32.5b	39.8	46.2	6.3	7.0	2.2c	48
84 (urea)	38.2a	34.8a	39.8	46.0	6.9	6.4	2.9a	49
LSD (0.05)	2.0	1.4	ns	ns	ns	ns	0.3	ns

†SPAD readings (0-99.9) with higher readings representing darker green.
‡Visual score (1-9) with 1 as poor plant vigor and 9 indicating best plant vigor.
§IDC = iron deficiency chlorosis. Based on the visual scale from Goos and Johnson (2008), with 5 being the most chlorotic.
¶Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
††Treatment has two applications, 28 kg at emergence and 28 kg at flowering. Observations SPAD1, SPAD2, Vigor 1, and IDC were done before the second N application.
‡‡Treatment applied at flowering. Observations SPAD1, SPAD2, Vigor 1, and IDC were done before N application.
ns = not significant.

Table 36. In season observations for N applications across cultivar and tile treatments at Fargo, ND, in 2014.

N applied	SPAD 1	SPAD 2	SPAD 3	SPAD 4	Vigor 1	Vigor 2	IDC	Height
kg ha^{-1}	-------------------------------0-99.9†------------------------------				----------1-9‡--------------		--1-5§--	--cm--
0	24.2a¶	30.2	34.5	46.0a	6.3ab	7.5c	2.0c	53.5bc
28 (urea) split††	24.9a	30.7	34.3	46.3a	6.5a	7.7abc	2.4b	56.2a
56 (urea)	24.0a	31.5	34.3	46.0a	6.5a	7.8ab	2.5b	55.5ab
56 (ESN)	24.5a	31.9	34.6	45.8a	6.4a	7.6bc	2.1c	53.5bc
56 (urea) at R2‡‡	24.7a	30.2	35.4	45.7ab	5.9b	7.5c	2.0c	52.1c
84 (urea)	21.8b	31.6	43.2	44.8b	6.6a	7.9a	2.8a	57.5a
LSD (0.05)	1.9	0.9	ns	0.9	0.4	0.2	0.2	2.0

†SPAD readings (0-99.9) with higher readings representing darker green.
‡Visual score (1-9) with 1 as poor plant vigor and 9 indicating best plant vigor.
§IDC = iron deficiency chlorosis. Based on the visual scale from Goos and Johnson (2008), with 5 being the most chlorotic.
¶Within columns, means followed by the same letter are not significantly different at ($P \leq 0.05$).
††Treatment has two applications, 28 kg at emergence and 28 kg at flowering. Observations SPAD1, SPAD2, Vigor 1, and IDC were done before the second N application.
‡‡Treatment applied at flowering. Observations SPAD1, SPAD2, Vigor 1, and IDC were done before N application.
ns = not significant.

Together with Researchers from the NDSU Department of Agricultural and Biosystems Engineering we conducted research on this experiment using multiple active optical sensors including OptRx (Ag leader Technology, Inc., Iowa, USA) and SPAD meter with the objective of determining N need for potential in-season applications. Using OptRx we found significant differences between tiled and non-tiled plots in early and mid-season, later in the season significant differences were found between N application treatments and varieties. A significant correlation existed between Normalized Difference Red Edge values using the OptRx sensors and the final yield for cultivar 90Y41 compared to other varieties. The study shows that the OptRx sensor has the potential to detect N treatment differences later in the season, however further research is needed to confirm the use of sensors for variable in-season N application in soybeans. In depth details of the study can be found in the paper by Maharlooei et al. (2014).

Conclusion

No interactions existed for yield between tile treatments, cultivar, and N fertilizer treatments. Tile drainage had a significant effect on severity of IDC, but no significant differences were found for grain yield; however tile continued a trend of increasing yield from previous research done at this experiment location (Brodshaug, 2011; Hoppe, 2013). It appears that additional research is needed.

Application of N in Fargo over two years indicated that soybean growth and grain yield increased when N was applied. Yield increase due to N application appeared to rely on weather conditions. The weather in 2013 started out fairly wet, and as the soil in this location has a low permeability, the water drained slowly. The moist conditions early in the season may have affected the root nodule activity, and the dry conditions later in the season may have kept the plant from taking up the N fertilizer causing overall lower yields in 2013. In 2014 although there was sufficient moisture later in the season during pod fill for the plant to take up the N fertilizer, it too was a wet year and may have had delayed or interrupted root nodule activity. Although weather is highly variable, soil type can be an important decision maker when contemplating adding fertilizer N to soybean.

Testing additional N rates may be beneficial as it was found in the combined analysis that grain yield was not significantly different between applied N treatments. Application timing resulted in significant yield differences in the combined analysis. The common rate that was applied were multiple treatments of 56 kg ha^{-1} of N. There was only one treatment with a different rate, namely 84 kg ha^{-1} of N. It may be possible to achieve similar yield results with a lower N rate, using similar timings of application. Differences in financial return between treatments were not significant, but more research should be conducted on the economics of N application on soybean. Application of N negatively affected IDC as expected, although the plants appeared to outgrow the stress as grain yields with application of N were higher than the control which had the lowest IDC score.

Cultivars differed in grain yield. It may be possible that certain cultivars allow for different responses to N fertilizer; however no significant interactions occurred between cultivar and N treatments. Choosing the correct cultivar for the environment is an important decision and the first step towards achieving optimal yield. There are many factors out of the growers control during a season. However, there are factors such as cultivar selection, fertilizer use, and field management practices, such as tile drainage, that allow a grower to have some control over the outcome of the season.

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APPENDIX

Table A1. Average height of Lariat pinto bean for N treatments at Prosper, ND.

N applied	2013	2014	Combined
--kg ha^{-1}--		-cm-	
0	44.5	44.3	44.4
28 (urea)†	39.3	49.8	44.5
28 (ESN)†	42.5	45.3	43.9
56 (urea)	44.5	49.3	46.9
56 (ESN)	43.3	47.3	45.3
56 (other) ‡	43.5	47.5	-
84 (urea)	41.5	50.8	46.1
LSD (0.05)	ns	ns	ns

†Split application of 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2.
‡The urea product Agrotain was applied in 2013 and Super-U was applied in 2014.
ns = not significant.

Table A2. Average number of nodules of Lariat pinto bean for N treatments at Prosper, ND.

N applied	2013	2014	Combined
--kg ha^{-1}--		-nodule count-	
0	22.2	30.1	25.9
28 (urea)†	31.0	30.7	31.0
28 (ESN)†	28.1	25.8	26.9
56 (urea)	29.6	29.6	29.6
56 (ESN)	34.8	21.7	28.3
56 (other) ‡	49.6	24.7	-
84 (urea)	49.4	27.3	38.3
LSD (0.05)	ns	ns	ns

†Split application of 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2.
‡The urea product Agrotain was applied in 2013 and Super-U was applied in 2014.
ns = not significant.

Table A3. Average number of nodules of Lariat pinto bean for N treatments at Park River, ND.

N applied	2013	2014	Combined
--kg ha^{-1}--		-nodule count-	
0	21.7	17.6	19.8
28 (urea)†	16.5	19.1	17.8
28 (ESN)†	17.6	20.8	19.1
56 (urea)	12.3	19.5	15.9
56 (ESN)	15.7	19.8	17.5
56 (other) ‡	18.2	25.7	-
84 (urea)	20.6	19.2	19.9
LSD (0.05)	ns	ns	ns

†Split application of 28 kg ha^{-1} at emergence and 28 kg ha^{-1} at R2.
‡The urea product Agrotain was applied in 2013 and Super-U was applied in 2014.
ns = not significant.

Table A4. Mean squares for combined analysis of dry beans at Prosper, ND, in 2010, 2012, 2013 and Park River, ND, in 2013.

SOV	df	nodule	vigor	height	Kernel weight	Yield loss	Yield
Cultivar (C)	2	2205***	59***	312**	508008***	484678	1428092*
Inoculant (I)	2	168	1	43	257	81718	123892
C x I	4	88	2	26	1247	42658	148419
Fertilizer (F)	1	127	9*	140	7134*	1422	249395
C x F	2	83	2	15	1207	57037	87081
I x F	2	112	1	27	304	12800	51915
C x I x F	4	26	2	30	1366	3160	337054
Residual	255	101	2	59	1188	54402	354240

* ** *** Significant at (P≤0.05), (P≤0.01), and (P≤0.001), respectively.

†SOV = source of variation, df = degrees of freedom.

Table A5. Probability level of significance of dry beans at Prosper, ND, in 2010.

SOV	df	nodule	vigor	height	kernel weight	yield loss	yield
	----------Probability>F----------						
Cultivar (C)	2	0.02	<0.001	<0.001	<0.001	0.001	0.01
Inoculant (I)	2	0.26	0.17	0.87	0.23	0.20	0.99
C x I	4	0.18	0.87	0.68	0.59	0.91	0.42
Fertilizer (F)	1	0.59	0.27	0.85	0.21	0.98	0.48
C x F	2	0.59	0.64	0.29	0.40	0.58	0.18
I x F	2	0.58	0.77	0.19	0.93	0.63	0.88
C x I x F	4	0.83	0.75	0.58	0.47	0.30	0.13
Residual	51	-	-	-	-	-	-

†SOV = source of variation, df = degrees of freedom.

Table A6. Probability level of significance of dry beans at Prosper, ND, in 2012.

	----------Probability>F----------						
SOV	df	nodule	vigor	height	kernel weight	yield loss	yield
Cultivar (C)	2	0.03	<0.001	<0.001	<0.001	0.01	<0.001
Inoculant (I)	2	0.64	0.92	0.77	0.23	0.41	0.74
C x I	4	0.86	0.73	0.88	0.45	0.25	0.38
Fertilizer (F)	1	0.03	0.95	0.69	0.69	0.21	0.69
C x F	2	0.52	0.57	0.95	0.70	0.61	0.80
I x F	2	0.59	0.78	0.83	0.83	0.90	0.56
C x I x F	4	0.87	0.08	0.12	0.85	0.57	0.09
Residual	51	-	-	-	-	-	-

†SOV = source of variation, df = degrees of freedom.

Table A7. Probability level of significance of dry beans at Prosper, ND, in 2013.

		---Probability>F---					
SOV	df	nodule	vigor	height	kernel weight	yield loss	yield
Cultivar (C)	2	0.01	0.02	<0.001	<0.001	0.44	0.09
Inoculant (I)	2	0.24	0.23	0.33	0.94	0.18	0.15
C x I	4	0.53	0.01	0.27	0.78	0.98	0.72
Fertilizer (F)	1	0.92	0.002	0.14	0.14	0.63	0.62
C x F	2	0.91	0.26	0.18	0.21	0.44	0.06
I x F	2	0.66	0.38	0.88	0.92	0.31	0.18
C x I x F	4	0.53	0.01	0.75	0.30	0.99	0.70
Residual	51	-	-	-	-	-	-

†SOV = source of variation, df = degrees of freedom.

Table A8. Probability level of significance of dry beans at Park River, ND, in 2013.

		---Probability>F---					
SOV	df	nodule	vigor	height	kernel weight	yield loss	yield
Cultivar (C)	2	<0.001	<0.001	0.08	<0.001	0.09	0.47
Inoculant (I)	2	0.02	0.51	0.25	0.30	0.39	0.05
C x I	4	0.20	0.38	0.56	0.12	0.66	0.66
Fertilizer (F)	1	0.02	0.20	0.003	0.003	0.53	0.99
C x F	2	0.42	0.24	0.53	0.79	0.96	0.17
I x F	2	0.15	0.75	0.80	0.08	0.76	0.19
C x I x F	4	0.76	0.66	0.46	0.94	0.30	
Residual	51	-	-	-	-	-	-

†SOV = source of variation, df = degrees of freedom.

Table A9. Probability level of significance of Lariat pinto bean at Prosper, ND, in 2010, 2012, 2013 and Park River, ND, in 2013.

		---Probability>F---		
SOV	df	nodule	kernel weight	yield
Inoculant (I)	2	0.34	0.81	0.22
Fertilizer (F)	1	0.19	0.004	0.71
I x F	2	0.32	0.73	0.26
Residual	115	-	-	-

†SOV = source of variation, df = degrees of freedom.

Table A10. Mean squares for the ANOVA for in-season observations measured, at Fargo, ND, in 2013.

		Mean square							
SOV†	df†	SPAD 1	SPAD 2	SPAD 3	SPAD 4	Vigor 1	Vigor 2	IDC†	Height
Drainage (D)	1	196.9	13.3	0.6	1.9	18.2	54.8*	9.2	153
Rep(D)	3	100.5	35.9	17.9	1.2	16.4	2.8	4.3	352
Cultivar (C)	3	100.2***	58.3***	351.4***	200.7***	12.2***	3.5	2.5***	457***
D x C	3	2.6	16.8	0.6	2.0	0.7	2.9	0.3	16
Fertilizer (F)	5	69.6***	29.4**	7.6	3.7	1.3	6.0	4.1***	9
D x F	5	32.0	10.2	6.4	1.6	2.7*	6.1	0.4	58
C x F	15	26.3	6.9	5.8	2.3	1.0	6.7	0.2	24
D x C x F	15	11.2	9.5	5.6	2.0	0.6	5.3	0.1	29
Residual	138	16.2	8.1	5.6	2.7	1.2	5.8	0.2	32

* ** *** Significant at (P≤0.05), (P≤0.01), and (P≤0.001), respectively.

†SOV = source of variation, df = degrees of freedom, IDC = iron deficiency chlorosis score.

Table A11. Mean squares for the ANOVA for in season observations measured at Fargo, ND, in 2014.

		Mean square							
SOV†	df†	SPAD 1	SPAD 2	SPAD 3	SPAD 4	Vigor 1	Vigor 2	IDC†	Height
Drainage (D)	1	718	4	373	1	15.2	1.4	14.91*	380
Rep(D)	3	59	7	497	6	2.6	0.7	0.85	423
Cultivar (C)	3	180***	57***	559	184***	3.6***	2.5***	0.71**	1453***
D x C	3	12	2	518	1	0.8	0.4	0.29	37
Fertilizer (F)	5	41*	17***	398	8*	2.3**	0.8**	3.33***	131***
D x F	5	11	6	517	7*	0.5	0.1	0.04	19
C x F	15	15	3	506	3	0.6	0.2	0.07	14
D x C x F	15	19	1	419	2	0.4	0.2	0.19	12
Residual	138	15	4	477	3	0.6	0.2	0.13	17

* ** *** Significant at (P≤0.05), (P≤0.01), and (P≤0.001), respectively.

†SOV = source of variation, df = degrees of freedom, IDC = iron deficiency chlorosis score.

Table A12. Mean squares for the ANOVA for in season observations measured at Fargo, ND, in 2013 and 2014.

		Mean square							
SOV†	df†	SPAD 1	SPAD 2	SPAD 3	SPAD 4	Vigor 1	Vigor 2	IDC†	Height
Drainage (D)	1	831.7*	16.3	170.6	3.2	33.4	37.1	23.7	3692
Rep(D)	3	36.9	29.9	293.6	1.6	10.2	19.4	3.7	1527
Cultivar (C)	3	140.8	65.6	618.6	383.6**	6.2	0.6	1.2	3305*
D x C	3	3.8	5.2	268.4	0.9	1.4*	2.1	0.5	981
Fertilizer (F)	5	7.8	42.2**	239.7	3.6	2.9*	2.4	7.1**	1695
D x F	5	18.2*	7.4	278.7	4.1	1.7	3.2	0.2	1190
C x F	15	10.6***	3.9	270.9	2.2	0.8	4.0	0.1	1269
D x C x F	15	13.1***	5.3	207.8	2.3	0.3	2.6	0.1	1185
Residual	275	15.5	5.8	242.0	2.9	0.9	3.0	0.2	1241

* ** *** Significant at ($P \leq 0.05$), ($P \leq 0.01$), and ($P \leq 0.001$), respectively.

†SOV = source of variation, df = degrees of freedom, IDC = iron deficiency chlorosis score.

Table A13. Mean squares for plants per ha^{-1} and postharvest observations at Fargo, ND, in 2013.

		Mean square											
SOV	df	Plants ha^{-1}	TW	TS	TP	SP	KWT	Yield	$	PC	PC ha^{-1}	OC	OC ha^{-1}
Drainage (D)	1	20160214222	419	481	111	0.01	97	509997	60791	0.4	44435	1.1	19707
Rep(D)	3	14504697935	521	2256	337	0.01	1196	1808969	216015	0.1	174277	0.6	60035
Cultivar (C)	3	3839467843	2156	2685**	258*	1.2***	2789***	18212	2186	12.7***	15733***	21.3	7376
D x C	3	1095117517	654	236	27	0.01	21	91351	10949	0.7	6918	0.1	3737
Fertilizer (F)	5	736796013	833	65	14	0.01	48	74330	5003	0.2	8051	0.2	2089
D x F	5	2123075874	425	367	41	0.01	45	67707	8093	0.1	6329	0.3	2705
C x F	15	997840500	513	351	61	0.03*	67	67879	8117	0.5	7210	0.2	2241
D x C x F	15	1290761447	1106	395	66	0.01	44	91834	10998	0.3	8941	0.2	3482
Residual	138	2175695922	847	544	84	0.02	76	101565	12132	0.4	9130	0.2	3508

* ** *** Significant at ($P \leq 0.05$), ($P \leq 0.01$), and ($P \leq 0.001$), respectively.

†SOV = source of variation, df = degrees of freedom, TW = test weight, TS = total seeds $plant^{-1}$, TP = total pods $plant^{-1}$, SP = seeds pod^{-1}, KWT = 1000 kernel weight, PC = % protein content, PC ha^{-1} = kg ha^{-1} of protein, OC = oil content, OC ha^{-1} = kg ha^{-1} of oil, ns = not significant.

Table A14. Mean squares for plants per ha^{-1} and postharvest observations at Fargo, ND, in 2014.

		Mean square											
SOV†	df†	Plants ha^{-1}	TW†	TS	TP	SP	KWT†	Yield	$	PC†	PC ha^{-1}†	OC†	OC ha^{-1}†
Drainage (D)	1	11458353459	119.5	14.1	5.2	0.003	0.004	390160	45924	0.5	49537	0.02	13167
Rep(D)	3	1524423592	238.3	733.4	111.5	0.048	1084.375	1016544	121342	1.6	114801	0.01	32032
Cultivar (C)	3	7179704785**	1296.3***	1674.3*	306.4*	0.390***	164.912	6550672***	783032***	5.9***	724388***	47.5***	85000***
D x C	3	821444301	73.7	25.1	4.6	0.033	204.669	65616	7735	0.2	8454	0.004	1951
Fertilizer (F)	5	4874457470*	127.9*	114.7	32.7	0.019	58.334	225181*	11714	0.1	24563*	0.3*	5274
D x F	5	3365395038	89.3	259.8	50.5	0.052	60.469	54681	6538	0.2	7207	0.04	1543
C x F	15	955483157	69.9	364.0	61.9	0.017	181.651	141730	16971	0.4*	14842	0.13	4920
D x C x F	15	811104966	63.1	251.0	39.5	0.022	181.346	95414	11350	0.2	10379	0.07	2568
Residual	138	1838512509	57.2	394.8	61.4	0.026	166.983	102606	12265	0.2	10978	0.10	3215

* ** *** Significant at ($P \leq 0.05$), ($P \leq 0.01$), and ($P \leq 0.001$), respectively.

†SOV = source of variation, df = degrees of freedom, TW = test weight, TS = total seeds $plant^{-1}$, TP = total pods $plant^{-1}$, SP = seeds pod^{-1}, KWT = 1000 kernel weight, PC = % protein content, PC ha^{-1} = kg ha^{-1} of protein, OC = oil content, OC ha^{-1} = kg ha^{-1} of oil, ns = not significant.

Table A15. Mean squares for plants per ha^{-1} and postharvest observations at Fargo, ND, in 2013 and 2014 combined.

		Mean square											
SOV†	df†	Plants ha^{-1}	TW†	TS	TP	SP	KWT†	Yield	$	PC†	PC ha^{-1}†	OC†	OC ha^{-1}†
Drainage (D)	1	310086062093	47.7	330.0	82.0	0.01	50.6	897704	106388	0.002	93809	0.73	32634
Rep(D)	3	10279816807	310.5	1657.1	260.8	0.03	2278.9	2611712	311783	1.059	269246	0.30	84264
Cultivar (C)	3	8013398956	2460.8	3723.1	511.9*	1.37	1584.4	3282251	392169	16.541*	395106	65.19*	32759
D x C	3	1224886835	336.6	174.9	25.3	0.03	92.8	90392	10766	0.307	10502	0.07	2933
Fertilizer (F)	5	2465650339	596.7	83.7	18.8	0.01	73.9	247918*	10622	0.181	27036*	0.32	6190*
D x F	5	1470449771	279.6	124.8	23.4	0.02	45.8	31306	3721	0.219	3956	0.14	1166
C x F	15	789423285	303.5	147.9	31.6	0.03	155.3*	83580	9991	0.424	8107	0.17	3166
D x C x F	15	792365332	594.4	306.8	46.9	0.01	124.8	99059	11808	0.263	9999	0.10	3198
Residual	275	2007104216	447.8	469.4	72.5	0.02	123.9	102099	12200	0.292	10078	0.17	3357

* ** *** Significant at ($P \leq 0.05$), ($P \leq 0.01$), and ($P \leq 0.001$), respectively.

†SOV = source of variation, df = degrees of freedom, TW = test weight, TS = total seeds $plant^{-1}$, TP = total pods $plant^{-1}$, SP = seeds pod^{-1}, KWT = 1000 kernel weight, PC = % protein content, PC ha^{-1} = kg ha^{-1} of protein, OC = oil content, OC ha^{-1} = kg ha^{-1} of oil, ns = not significant.

Table A16. Mean squares for seeds per pod at Fargo, ND, in 2013.

		Mean square			
SOV†	df†	one	two	three	four
Drainage (D)	1	0.4	42.7	7.1	0.001
Rep(D)	3	0.4	38.3	133.7	0.02
Cultivar (C)	3	6.7**	578.3***	789.3***	0.66***
D x C	3	1.1	1.6	15.1	0.05
Fertilizer (F)	5	1.9	11.0	7.7	0.16
D x F	5	0.2	5.9	29.6	0.03
C x F	15	1.6	19.7	23.2	0.18*
D x C x F	15	0.8	15.9	20.5	0.12
Residual	138	1.3	18.8	32.7	0.09

* ** *** Significant at (*P*≤0.05), (*P*≤0.01), and (*P*≤0.001), respectively.

†SOV = source of variation, df = degrees of freedom.

Table A17. Mean squares for seeds per pod at Fargo, ND, in 2014.

		Mean square			
SOV†	df†	one	two	three	four
Drainage (D)	1	0.2	18.4	3.7	0.11
Rep(D)	3	5.0	9.4	46.0	0.04
Cultivar (C)	3	8.3*	181.4***	153.2***	0.27
D x C	3	1.7	9.0	6.0	0.09
Fertilizer (F)	5	3.6	16.3	6.3	0.08
D x F	5	4.5	20.8	9.8	0.03
C x F	15	3.1	12.8	16.5	0.19
D x C x F	15	3.1	16.5	10.9	0.23
Residual	138	2.3	16.3	24.2	0.14

* ** *** Significant at (*P*≤0.05), (*P*≤0.01), and (*P*≤0.001), respectively.

†SOV = source of variation, df = degrees of freedom.