

**Corn production with Instinct nitrification inhibitor applied with urea-ammonium  
nitrate solution and liquid swine manure**

by

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**DEDICATION**

I dedicate this thesis to my wife and daughter, because without their continuous love, encouragement, support, and patience this aspiration may never have been fulfilled. I also dedicate this thesis to my family, whose continuous words of encouragement kept reminding me how significant of an achievement earning an advanced degree is. Finally, I would like to dedicate this to Baxter, who was at my side during the late nights of writing. He always knew how to distract and calm me when I was stressed and frustrated.

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## CHAPTER 1. GENERAL INTRODUCTION

In recent years, producers in Iowa have had increased interest about including nitrification inhibitors with N fertilizer or manure applied to corn as a N management practice to reduce potential N losses and improve N use efficiency. This interest is a result of  $\text{NO}_3$  concentrations in local drinking water systems, and to a larger extent issues related to N loading from the Mississippi River to the Gulf of Mexico, which create eutrophication events during the summer months that impact fishing industries in states along the Gulf Coast Region. The states in the Upper Mississippi River Basin, which includes Iowa, have been identified as primary N contributors to the Mississippi River due to current agricultural practices of row crop production and applying N for corn production, including application of N well before plant N uptake. Increasing the duration that applied N is in the soil increases the potential for N to undergo processes like nitrification and denitrification. These processes convert  $\text{NH}_4$ , otherwise fixed to the soil cation exchange capacity, to soluble  $\text{NO}_3$  or gaseous forms of N. Nitrification is the first process applied  $\text{NH}_4$  must undergo, and is a two phase microbial process dependent upon the amount of available  $\text{NH}_4$ , soil pH, temperature, and moisture. The first phase of nitrification is the oxidation of  $\text{NH}_4$  by *Nitrosomonas sp.* bacteria to  $\text{NO}_2$ , while the second phase is performed by *Nitrobactor sp.* bacteria which further oxidize  $\text{NO}_2$  to  $\text{NO}_3$ . The conversion of  $\text{NO}_2$  to  $\text{NO}_3$  is rapid, and buildup of  $\text{NO}_2$  in soil is not common.

Corn can absorb both  $\text{NH}_4$  and  $\text{NO}_3$ . Ammonium and  $\text{NO}_3$  are assimilated to amino acids and proteins in the plant, but  $\text{NH}_4$  is the preferred form as it requires less energy than  $\text{NO}_3$  to produce those metabolites. Nitrate is usually absorbed in greater quantities than  $\text{NH}_4$ , because by the time of rapid plant N uptake applied  $\text{NH}_4$  is typically

converted to  $\text{NO}_3$ . Additionally,  $\text{NO}_3$  is a negatively charged molecule, and is highly water soluble, which improves mobility to the plant with water movement and uptake by roots. Nitrogen not absorbed by plants or immobilized by soil microorganisms can be lost as  $\text{NO}_3$  with water as it is leached downward through the soil profile to subsurface tile drainage and surface water, or to groundwater, thus contaminating drinking water sources. Nitrate may also be lost as  $\text{N}_2$  and the greenhouse gas  $\text{N}_2\text{O}$  through the process of denitrification if anaerobic conditions exist with saturated soil conditions. Losses of  $\text{NO}_3$  are greatly influenced by year to year variations in weather patterns, and can fluctuate within seasons. A major part of  $\text{NO}_3$  loss in tile flow occurs in the spring, and an increased loss to surface and groundwater typically occurs during years with above normal precipitation; while losses are reduced during years with normal to below normal precipitation.

Nitrification inhibitors provide corn producers an option to help control the conversion of applied  $\text{NH}_4$  to  $\text{NO}_3$ ; therefore during periods when water in the soil is in excess, they reduce the potential for N losses while improving corn yield and N use efficiency. Nitrapyrin [2-chloro-6-(trichloromethyl) pyridine], the active ingredient in N-Serve (Dow AgroSciences, Indianapolis, IN), has been the most popular and one of the effective inhibitors commercially available. It primarily inhibits *Nitrosomonas sp.* bacteria, and its effectiveness is influenced by soil texture, organic matter content, temperature, and moisture. Using a nitrification inhibitor provides the producer an opportunity to apply  $\text{NH}_4$  containing fertilizers and manure at times when economic factors, labor costs, and soil conditions are more favorable with lowered risk of

significant N losses in the spring that could be detrimental to both the environment and corn production.

Historically, nitrapyrin has been sold as N-Serve, and is primarily applied with anhydrous ammonia (AA). In 2009, Dow introduced a reformulated version of nitrapyrin called Instinct (Dow AgroSciences, Indianapolis, IN). Unlike N-Serve, the active nitrapyrin ingredient in Instinct is encapsulated into a water compatible microcapsule to help address the rapid volatilization losses that occur when nitrapyrin is surface broadcast applied, and improve the ease of use with liquid ammoniacal and urea based fertilizers. The microcapsule is suggested to retain nitrapyrin and avoid losses when surface applied for up to 10 d. Instinct may be injection or surface applied, and if surface applied must be incorporated with light tillage or with at least 1.27 cm of rainfall or overhead irrigation within 10 d after application to prevent nitrapyrin volatilization losses. The ability to surface broadcast Instinct provides a potential solution to prevent N losses from sources like liquid swine manure (LSM), urea ammonium-nitrate (UAN) solution, or urea that can be surface applied or injected into soil. In Iowa, UAN solution represents approximately 27% of fertilizer N consumption and dry urea 9%.

Iowa leads all states in swine production, with approximately one-third of the total U.S. production. Liquid swine manure is a viable source of available N for corn production, and is typically applied in the fall due to manure storage constraints, better soil conditions for equipment traffic, and increased available labor. Fall application, however, increases the time for conversion of LSM  $\text{NH}_4$  (average 82% of total LSM-N as  $\text{NH}_4\text{-N}$ ) to  $\text{NO}_3$  and thus increases the chance of spring N losses. Although UAN is applied in the spring, it can have significant N loss potential before corn uptake. The N

in UAN is comprised of 50% urea and 25%  $\text{NH}_4$ , which both can be quickly converted to  $\text{NO}_3$ , and 25% as  $\text{NO}_3$ . Using Instinct with LSM, UAN, or urea fertilizer could improve corn grain production by slowing nitrification and subsequently reducing N losses. Since Instinct is a new nitrification inhibitor formulation, research is needed to evaluate the agronomic aspects it may have for corn production.

This thesis includes two field studies designed to evaluate the effect Instinct has on corn production when used with fall applied LSM and spring applied UAN. The first project evaluated the effect of Instinct spring preplant applied with UAN fertilizer on corn production and optimum N rate across six N rates and two application methods. The second study evaluated the effect of Instinct fall applied with LSM on applied  $\text{NH}_4$  retention in the soil and corn production at two fall application times and three Instinct rates. A comparison was also made in the second study between AA and LSM without a nitrification inhibitor at the two fall application times. Both studies occurred at a different site in central Iowa across three years.

This research project has provided corn producers in Iowa with information about effects of fall applied LSM and spring applied UAN on corn production, and the potential for the nitrification inhibitor Instinct to improve N management and corn production. Of importance, the results of this research have provided data that will help crop advisors and producers determine if the use of Instinct is an economically feasible management practice for corn production in Iowa. These results will also help producers decide if use of Instinct with LSM is a better management practice compared to timing of LSM application. Lastly, the data will help determine if Instinct is an effective nitrification

inhibitor that could help to prevent N losses that would be detrimental to the environment.

### **THESIS ORGANIZATION**

This thesis is presented across four chapters. The first chapter provides a general introduction of the thesis research. Chapters 2 and 3 are manuscripts describing the efforts and outcomes of each study with the intention of being published in Agronomy Journal. The titles of the manuscripts are “Corn Response to Spring Applied Urea-Ammonium Nitrate Solution Placement with Instinct Nitrification Inhibitor” and “Corn Response to Instinct Nitrification Inhibitor Fall Applied with Liquid Swine Manure”. The final chapter (chapter 4) provides general conclusions for the research conducted in this thesis.

## CHAPTER 2. CORN RESPONSE TO SPRING APPLIED UREA-AMMONIUM NITRATE SOLUTION PLACEMENT WITH INSTINCT NITRIFICATION INHIBITOR

A paper to be submitted to Agronomy Journal

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### ABSTRACT

The use of nitrification inhibitors with fertilizer N is an attempt to improve corn (*Zea mays* L.) N use efficiency while reducing environmental and economic concerns associated with N losses. The objective of this study was to evaluate if the encapsulated formulation of nitrapyrin [2-chloro-6-(trichloromethyl) pyridine], marketed as Instinct nitrification inhibitor, would influence corn growth and production when applied with spring preplant urea-ammonium nitrate (UAN) solution. A three year field study, with a soybean [*Glycine max* (L.) Merr]-corn rotation, was conducted in a randomized complete block design with four replications of a factorial combination consisting of UAN at six incremental N rates (0 to 225 kg N ha<sup>-1</sup>), broadcast-incorporated and injection applied, and with 2.56 L ha<sup>-1</sup> (0.56 kg a.i. ha<sup>-1</sup>) and without Instinct. In one of three years, and for means across years, Instinct applied with UAN had a negative effect, with reduced early growth plant height and lower mid-vegetative canopy normalized difference vegetative index (NDVI) compared to UAN without Instinct. Corn grain yield, in two of three years and across years, also had a lower across N rate mean yield with the Instinct application.

The economic optimum N rate (EONR) with Instinct was 32 kg N ha<sup>-1</sup> higher than without Instinct, applied either broadcast or injected. Because Instinct did not provide positive effects on corn growth and yield, and resulted in some negative responses, the study results indicated that Instinct use with spring preplant applied UAN solution would not be expected to enhance N supply to corn.

**Abbreviations:** AONR, agronomic optimum nitrogen rate; EONR, economic optimum nitrogen rate; NDVI, normalized difference vegetative index; UAN, urea-ammonia nitrate solution.

## INTRODUCTION

Improving corn N use efficiency has received extensive attention by producers and environmentalist. When the price of N dramatically increases, producers want improved fertilizer return with limited N losses, so there are gains in grain yield and increased profit. The loss of N is commonly associated with leaching or denitrification of fertilizer N after it is converted to NO<sub>3</sub> through the nitrification process by *Nitrosomonas sp.* and *Nitrobactor sp.* bacteria commonly present in soil. Nitrate is water soluble, and is carried by water as it leaches through the soil profile. It can also be lost to the atmosphere when conditions in the soil become anaerobic, promoting denitrification. Producers may apply additional N to help compensate for these N losses if it will improve grain yield and profit. Losses of soil NO<sub>3</sub> are also an environmental concern as NO<sub>3</sub> moves out of the soil to surface and groundwater which can impair aquatic life and drinking water sources.



Libra et al. (2004) calculated a budget for N inputs and outputs across Iowa, and estimated both to be approximately 3.6 million metric tons equally. The input from commercial fertilizers accounted for an estimated 0.9 million metric tons, and of that 90% was used for agricultural purposes. It was also estimated that only 5% of the total N inputs for Iowa were lost through streams, but this still accounted for 20% of the N load from the Mississippi River watershed to the Gulf of Mexico. These data were estimated during a period of below normal precipitation, and N loading may be greater when there are periods of above normal precipitation (Goolsby et al., 1999; Libra et al., 2004). In response to the water quality concerns raised in the 2008 Gulf Hypoxia Action Plan, Iowa developed the Iowa Nutrient Reduction Strategy (2013) to reduce total N loading from within the state to the Mississippi River by 45% (Iowa Nutrient Reduction Strategy, 2013; Lawrence, 2013). This strategy addresses both point (i.e. wastewater treatment facilities) and nonpoint (i.e. agricultural land) sources of N, and provides management methods to help reduce N loading. It is estimated that of the 20% of N that originates from Iowa, 93% of that comes from nonpoint sources (Iowa Nutrient Reduction Strategy, 2013). The Iowa Nutrient Reduction Strategy established a goal to reduce the state's N load to the Mississippi River by 45%, with 41% of the overall load reduction from nonpoint sources.

One suggested method for reducing N loading from nonpoint sources is the inclusion of a nitrification inhibitor when applying fall anhydrous ammonia for corn production. Previous work has also suggested the use of nitrification inhibitors could reduce  $\text{NO}_3$  losses and improve corn N use efficiency in tile drained fields in Iowa (Baker and Johnson, 1981) and Ohio (Owens, 1987). What is not as well-known is if using a

nitrification inhibitor would improve N use efficiency or reduce losses with spring applied N fertilizers, especially with fertilizers other than anhydrous ammonia.

Nitrapyrin [2-chloro-6-(trichloromethyl) pyridine] is the active ingredient in the most commonly used nitrification inhibitor, N-Serve (Dow AgroSciences LLC, Indianapolis, IN). It was introduced by C. A. I. Goring of Dow Chemical Company in 1962, and reported to have high efficacy to genera *Nitrosomonas sp.* bacteria responsible for the oxidation of  $\text{NH}_4$  to  $\text{NO}_2$  (Goring, 1962a). Nitrapyrin can persist in the soil for 4 to 10 weeks after application (Nelson and Huber, 1992), with persistence influenced by soil temperature, organic matter, soil pH, rate of diffusion, volatilization, and sorption (Hoeft, 1984). Research studying nitrapyrin degradation indicates that soil temperature has the greatest influence on the hydrolysis rate and duration of inhibition (Herlihy and Quirke, 1975; Hendrickson and Keeney, 1979; Touchton et al., 1979c).

Extensive research has been conducted with nitrapyrin to evaluate the effectiveness on retention of N for corn production. A meta-analysis of published nitrapyrin research found a mean 7% corn grain yield increase when averaged across 189 observation and 158 location-years (Wolt, 2004). Specifically for Iowa, yield response with nitrapyrin has been inconsistent. One study found that spring applied anhydrous ammonia with nitrapyrin had a significantly negative effect on grain yield in 2 of 12 site-years, and no effect the other years (Blackmer and Sanchez, 1988). Cerrato and Blackmer (1990b) found nitrapyrin spring applied with  $(\text{NH}_4)_2\text{SO}_4$  significantly increased yield in only 2 of 72 site-years, and its use was not cost effective. In a seven year study, preplant N applied broadcast and incorporated with nitrapyrin consistently and significantly increased yields each year (Christensen and Huffman, 1992). Across a 10

year study, Quesada (2002) found no consistent corn yield response when nitrapyrin was spring preplant injection applied with anhydrous ammonia, UAN, or aqua ammonia in a continuous corn or soybean-corn rotation. In a more recent study Parkin and Hatfield (2010) reported a significant corn yield increase of 1.1 and 0.35 Mg ha<sup>-1</sup> when anhydrous ammonia with nitrapyrin was fall applied during a two year study in Central Iowa.

Due to these inconsistencies in yield response Randall and Sawyer (2008) concluded that further research was required to evaluate the efficacy of new nitrification inhibitors in corn production. In 2009, Instinct nitrification inhibitor (Dow AgroSciences LLC, Indianapolis, IN) was introduced as an encapsulated formulation of nitrapyrin. The encapsulation permits broadcast application with reduction of nitrapyrin volatile loss potential, which means Instinct could remain on the soil surface for up to 10 d after application before the need for light incorporation by tillage if there was not at least 1.27 cm rainfall or irrigation water within that time period. Prior research (Goring, 1962b; Redemann et al., 1964; Briggs, 1975; McCall and Swann, 1978) reported rapid volatilization of surface applied nitrapyrin if not immediately incorporated after broadcast application.

Published research on the use of Instinct applied with N fertilizer is limited. In a laboratory study Instinct was ineffective in controlling nitrification of NH<sub>4</sub> across soils, soil moisture levels, and when compared to another known effective inhibitor (dicyandiamide) (Ferrel, 2012). The researchers indicated the results were likely due to delayed release of nitrapyrin from the capsule (Ferrel, 2012). In another laboratory study, Goos (2011) found Instinct was effective in slowing nitrification when applied with urea. A field study in Indiana found Instinct band injected with UAN sidedress (corn at the V4

to V6 growth stage) significantly reduced nitrification three to six weeks beyond that when Instinct was not applied, while also reducing N<sub>2</sub>O emissions by 44% (Omonode and Vyn, 2013).

Just like with N-serve, corn yield responses to Instinct have been variable. In a summary of published and unpublished studies conducted in Nebraska, Illinois, Iowa, and Minnesota, Franzen (2011) indicated no benefits or increases in yield with use of Instinct. In a study conducted in Indiana, Instinct band injected with UAN in the spring at planting, or sidedressed when corn was at the V6 growth stage, had grain yield significantly increased as N rate increased, but there was no overall significant yield response to the Instinct (Burzaco et al., 2014). In a Kansas study to determine the effect of N rate applied as UAN with various nitrification inhibitors, including Instinct, on no-till short season corn, there was no corn grain yield response to nitrification inhibitors during a year of below normal rainfall and above normal air temperature (Sweeney and Ruiz Diaz, 2014). Due to the limited research of Instinct applied with UAN fertilizer, especially in Iowa, and inconsistent responses, additional research is needed to evaluate Instinct nitrification inhibitor in corn production. The objective of our study was to investigate if the nitrification inhibitor Instinct had a positive effect on corn growth and production across varying N rates when applied spring preplant with UAN fertilizer.

## **MATERIALS AND METHODS**

### **Site Description and Experimental Design**

A three year study was conducted from 2010 to 2012 at the Iowa State University Agricultural Engineering and Agronomy Research Farm (42°01' N, 93°46' W)

approximately 10-km from Boone, Iowa. The soils were typical for those found in Central Iowa (Table 1). Previous crop for all study sites was soybean. In the spring of 2010, and the fall prior to treatment application in 2011 and 2012, soil samples were collected from the 0- to 15-cm depth across each study site for routine analyses (Table 1). Soil test levels for P and K at each site were Optimum to High for corn production (Sawyer et al., 2011) (Table 1). Phosphorus, K, and lime were applied to eliminate potential effects of soil test variation. Monammonium phosphate ( $13 \text{ kg N ha}^{-1}$  and  $29 \text{ kg P ha}^{-1}$ ) and potash ( $84 \text{ kg K ha}^{-1}$ ) were applied across the study area in the fall of 2009. For the 2011 site, potash ( $67 \text{ kg K ha}^{-1}$ ) was fall applied and triple superphosphate ( $21 \text{ kg P ha}^{-1}$ ) spring applied. Triple superphosphate ( $56 \text{ kg P ha}^{-1}$ ), potash ( $140 \text{ kg K ha}^{-1}$ ), and lime ( $2.2 \text{ Mg ha}^{-1}$ ) were applied in the fall of 2011 for the 2012 site. In all years pre-emergence herbicide was applied prior to or shortly after planting. Growing season monthly air temperature, precipitation, and historic weather data was collected from an automated weather station located near the research site, and reported by the Iowa Environmental Mesonet Network (Arritt and Herzmann, 2014).

The experimental design was a complete factorial arrangement in a randomized complete block design with N rate, application method, and N fertilizer treated with or without Instinct as factors. Each plot had a length of 15-m and a width of 4.6-m (6 rows). Fertilizer N, as UAN (32% N), was preplant applied at six rates (0, 45, 90, 135, 180,  $225 \text{ kg N ha}^{-1}$ ). Application methods were coulter injection in a vertical band to approximately a 15-cm depth on 152-cm spacing approximately midway between future corn rows before final tillage and surface broadcast with incorporation by disking and field cultivation to a 10-cm depth for seedbed preparation. Instinct was added to the

applicator tank at the recommended  $2.56 \text{ L ha}^{-1}$  ( $0.56 \text{ kg nitrapyrin a.i. ha}^{-1}$ ) label rate and thoroughly mixed prior to treatment application. When no N was to be applied, water was used as the carrier to apply Instinct. Treatment application dates were 4 May 2010, 9 and 10 May 2011, and 26 Apr. 2012. Tillage for incorporation of surface broadcast treatments and seedbed preparation occurred on 5 May 2010, 10 May 2011, and 15 May 2012. Corn was planted on 6 May 2010, 11 May 2011, and 16 May 2012 at 79500, 79000, and 86500 seeds  $\text{ha}^{-1}$ , with Pioneer 35F44, Fontonella 6510, and Pioneer 0446XR corn hybrids, respectively. All corn production practices used were typical of those in Central Iowa for a soybean-corn rotation.

### **Soil and Plant Sampling**

Late-spring test for soil  $\text{NO}_3$  (LSNT) samples were collected on 7 June 2010, 16 June 2011, and 4 June 2012 to determine background  $\text{NO}_3\text{-N}$  levels for each site. Samples were collected at a depth of 0- to 30-cm when corn height was 15- to 30-cm from plots receiving no N with and without Instinct broadcast applied and incorporated. Soil samples were collected by starting in a specific row, with five 2-cm diameter cores collected at 15-cm increments perpendicular across the corn row direction between two rows and from two plot locations. All ten cores were combined, mixed, and a subsample collected for  $\text{NO}_3\text{-N}$  analysis (Blackmer et al., 1989). The LSNT results were well below the critical LSNT level of  $25 \text{ mg NO}_3\text{-N kg}^{-1}$  (Blackmer et al., 1997) each year (Table 1).

All soil samples were analyzed at the Iowa State University Soil Testing Laboratory. Samples were dried at  $40^\circ\text{C}$  and ground to pass through a 2-mm sieve (Gelderman and Mallarino, 2011). Soil test P and K were determined using the

Mehlich-3 extraction procedure, with P determined colorimetrically and K with atomic absorption (Frank et al., 2011; Warncke and Brown, 2011). Soil pH was measured in a 1:1 soil to water suspension (Watson and Brown, 2011). Organic matter was determined by dry combustion using a LECO CHN-2000 analyzer (LECO Corporation, St. Joseph, MI) (Combs and Nathan, 2011). The LSNT samples were extracted with 2 M KCl and an aliquot of extract analyzed for NO<sub>3</sub>-N using a Lachat flow injection analyzer (Lachat Instruments, Milwaukee, WI) (Gelderman and Beegle, 2011).

In each plot, early corn growth plant height (V4-V8 growth stage; Abendroth et al., 2011) was measured on ten random plants from within 12-m long segments of the two center rows. The plant height was determined by measuring from the soil surface to the extended leaf tip of the uppermost and fully developed leaf (Warrington and Norton, 1991). Corn canopy sensing was conducted using a Crop Circle ACS-210 active canopy sensor (Holland Scientific, Lincoln, NE) when corn growth reached the mid-vegetative (V10) growth stage following the procedure described by Barker and Sawyer (2010). The sensor was mounted on a mast, positioned inter-row, and hand carried through the center of each treatment plot at a constant speed (1.2 m s<sup>-1</sup>) and distance above the canopy (60 - 90 cm). Mean near-infrared (NIR) and visible (VIS) light reflectance data were recorded for each plot, and used to calculate NDVI [Eq. 1] for determination of corn canopy and N status response to treatments.

$$NDVI = \frac{NIR - VIS}{NIR + VIS} \quad [1]$$

Stalk lodging potential was determined at the R6 growth stage on ten random plants from within 12-m segments of the two center rows by pushing the stalk at the ear height to a 45° angle toward the inter-row. A stalk that broke was counted as lodged.

The stalk lodging potential would be considered lowest for values of zero, with greatest lodging potential at the maximum value of ten. Corn grain was harvested from the four middle rows of each plot with a research plot combine, with yield adjusted to 155 g kg<sup>-1</sup>.

### Statistical Analysis

Analysis of variance was performed by year and across years using PROC GLIMMIX in SAS 9.3 (SAS Institute, 2011) for early corn growth plant height, canopy NDVI, stalk lodging potential, and corn grain yield. Year, replicates, and their interactions were considered random for the across-year analysis, with treatments and interactions considered fixed effects. Treatment mean comparisons were determined using the PDIFF option, and were considered significantly different at  $P \leq 0.10$ . The LINES option was used to determine t-grouping differences for mean comparisons with Instinct and application method treatments. Corn response across fertilizer N rates and significant rate interactions were analyzed using PROC REG (SAS Institute, 2011) to investigate linear and quadratic regression [Eqs. 2 and 3], and PROC NLIN (SAS Institute, 2011) to investigate quadratic-plateau regression [Eqs. 4 and 5]. The best fit equation for N response was determined by the model with the lowest *P-Value* and the largest  $R^2$ . The quadratic-plateau was the best fit for all variables investigated.

$$y = a + bx \quad [2]$$

$$y = a + bx + cx^2 \quad [3]$$

$$y = a + bx + cx^2 \quad \text{if } x < x_o \quad [4]$$

$$y = a + bx_o + cx_o^2 \quad \text{if } x \geq x_o \quad [5]$$

For the parameters in these models,  $y$  represents the predicted corn response as plant height (cm), canopy NDVI, stalk lodging potential, or grain yield (Mg ha<sup>-1</sup>);  $x$  the



fertilizer N rate ( $\text{kg N ha}^{-1}$ );  $a$  (intercept),  $b$  (linear), and  $c$  (quadratic) coefficients; and  $x_o$  the fertilizer N rate at the quadratic-plateau join point. The agronomic optimum N rate (AONR) is the rate at the join point. By solving for  $x$  and using a  $0.0056 \$ \text{kg}^{-1}$  to  $\$ \text{Mg}^{-1}$  corn grain price ratio, the EONR for corn grain yield was calculated using equations [4] and [5] fit to N response (Cerrato and Blackmer, 1990a). Analysis was conducted across years and by year in order to investigate differences in yearly responses as a result of weather variation.

## RESULTS AND DISCUSSION

### Weather

Variations in monthly mean air temperature and precipitation between the three study years provided a good opportunity to study the effects Instinct might have on corn production. Figure 1a illustrates the mean monthly air temperature during each year of the study compared to the 30-yr mean (normal) monthly temperature. Mean air temperatures during the months when treatment application and planting occurred (April and May) in 2010 and 2011 varied little from normal, but in 2012 was  $3^{\circ}\text{C}$  above normal. June air temperatures were  $2^{\circ}\text{C}$  above normal in 2010 and 2011, and  $3^{\circ}\text{C}$  above normal in 2012. July 2010, 2011, 2012 air temperatures were 1, 3 and  $3^{\circ}\text{C}$ , respectively, above normal. Air temperatures in August 2010 and 2011 were 2 and  $1^{\circ}\text{C}$ , respectively, above normal, with little to no variation from the 30-yr mean in 2012. The September 2011 air temperature was the only year there was a difference from the 30-yr mean, which was  $1^{\circ}\text{C}$  below normal. Mean air temperatures in October 2010 and 2011 were  $2^{\circ}\text{C}$  above normal, while the air temperature in 2012 was  $1^{\circ}\text{C}$  below normal.

There was large variation around the 30-yr mean (normal) total monthly precipitation during each of the three study years (Fig. 1b). At treatment application and corn planting, total monthly precipitation in April was slightly below to near normal in 2010, 1.5 cm above normal in 2011, and 2.6 cm above normal in 2012; while the May monthly precipitation was 2.9 cm below normal in 2010, slightly below to near normal in 2011, and 5.9 cm below normal in 2012. The period of June thru September had the greatest variation in total monthly precipitation compared to the 30-yr monthly mean during all study years. In 2010, precipitation was 15.7, 5.4, 16.5, and 8.6 cm above normal for June, July, August, and September, respectively. In 2011 and 2012, monthly precipitation was below the 30-yr mean during the same period. In 2011, precipitation was slightly above to near normal in June, and 2, 2.9, and 3 cm below normal for July, August, and September, respectively, while 2012 was 5.2, 8.2, 4.6, and 3.4 cm below normal for June, July, August, and September, respectively. October 2010 and 2011 monthly precipitation was 5.3 and 4.4 cm below normal, while 2012 was slightly below to near normal.

Mean monthly air temperatures provided conditions favorable for nitrification after treatment application in all years. Also, well above normal precipitation from June thru August in 2010 provided conditions potentially favorable for significant  $\text{NO}_3$  loss by leaching or denitrification. Initial losses of N would normally occur by leaching until conditions in the soil became saturated and anaerobic promoting simultaneous denitrification and leaching. Soil saturation could have occurred rather quickly in the fine textured soils at the study site in 2010 (Table 1), and it is thought denitrification would have caused the greatest loss of  $\text{NO}_3$ . Nitrate loss potential after treatment

application in 2011 and 2012 would have been expected to be considerably lower compared to 2010. The greatest chance of N losses in 2011 would have occurred between April and June when total precipitation during those months were slightly above normal (Fig. 1b). Extremely dry soil conditions for July thru September 2011, and May thru September 2012, would have limited N losses, as well as making it difficult for corn roots to absorb N in the upper root zone (especially in 2012). Specific loss of applied N attributed to leaching or denitrification during all years of the study cannot be made since measurements for soil inorganic-N, leaching, and denitrification were not collected.

### **Early Corn Growth Plant Height**

The statistical analysis for early corn growth plant height measured at the V4-V8 growth stages is presented in Table 2. Plant height response to the main effect of N rate was significant ( $P \leq 0.10$ ) in 2010, 2011, and across years. As N rate increased plant height increased. The N rate main effect regression analysis indicated a maximum response to 66 kg N ha<sup>-1</sup> across years and at 132 and 115 kg N ha<sup>-1</sup> in the wetter 2010 and 2011 years, respectively (Table 3 and Fig. 2a). The main effect of Instinct, across N rate and application method, had no effect on plant height in any year or across years (Table 2). Nitrogen application method main effect was significant in 2010, 2011, and across years, with broadcast-incorporation of UAN being 2, 5, and 3 cm taller in 2010, 2011, and across years, respectively, than injection application (Table 4). This indicates an earlier N supply to plants with the broadcast-incorporated application, whereas roots would need some time to grow to the banded N placed between corn rows.

There were several two-way interactions between the main effects of N rate and Instinct, and N rate and application method (Table 2). There were no significant

three-way interactions. Table 5 gives the plant height and regression results for the N rate and application method interaction in 2010, 2011, and across years. Early plant growth increased as N rate increased only when broadcast-incorporated (Table 5), with height response maximizing at rates of 145, 142, and 137 kg N ha<sup>-1</sup> in 2010, 2011, and across years, respectively. In each year, at or above the maximum response rate, plant height with broadcast-incorporation of UAN was greater than when injected and no regression model fit a rate response when UAN was injected. An interaction between N rate and Instinct occurred only in 2012 (Table 2), and in that year plant height was inconsistent across N rates with and without Instinct (data not shown). Also, there was no significant regression model fit for N rate with or without Instinct.

The interaction between Instinct and UAN application method was significant for plant height in 2010, 2012, and across years (Table 2). The general plant height response was no effect to greater plant height when UAN with Instinct was broadcast-incorporated, but lower plant height when UAN with Instinct was injected (Table 6). Across all years, plant height was the same between UAN with or without Instinct when broadcast-incorporated, but plants were slightly shorter (2 cm) when injected. It is unknown why there would be a differential Instinct effect with placement, especially as there was no influence on plant height with increasing N rate with injected UAN. It is possible Instinct affected the NH<sub>4</sub>-N concentration in the UAN band, which would not necessarily cause a growth difference by itself, or the nitrapyrin in a concentrated band affected root and plant growth.

## Corn Canopy Sensing

Table 2 gives the statistical analysis for the mid-vegetative corn canopy sensing NDVI. Canopy NDVI responded to the N rate main effect each year and across years (Table 3 and Fig. 2b), with the NDVI increase indicating N responsiveness of each site. The NDVI value at the maximum N response was consistent across years, while the maximum N response rate varied (from 110 to 161 kg N ha<sup>-1</sup>), with the highest rate in 2012 indicating a higher N rate need to maximize canopy NDVI in that dry year.

Instinct application, mean across N rate and application method, had a significant ( $P \leq 0.10$ ) effect in 2010 and across years (Table 2); with canopy NDVI lower in each case when Instinct was applied (Table 4). The main effect of application method was significant in two of three years, and across years; with broadcast-incorporation having a greater canopy NDVI than injection application.

There were significant two-way interactions for N rate and Instinct, and N rate and application method in two of three years and across years; but none between Instinct and application method (Table 2). There were no significant three-way interactions. Table 7 and Figs. 3 and 4 give the NDVI regression models for each N rate interaction. While the maximum NDVI achieved was similar for UAN with or without Instinct; that was not the case for application method where maximum values were slightly lower with UAN injection (0.010 to 0.017 lower NDVI). Also, the maximum N response rate was different for each interaction. Overall, the maximum N response rate was lower without Instinct than with (63 to 110 kg N ha<sup>-1</sup> lower), and lower when UAN was injected than broadcast-incorporated (12 to 82 kg N ha<sup>-1</sup> lower). This can be seen in Fig. 3 for the N rate by Instinct interaction where at low N rates the canopy NDVI values with Instinct

were lower than without Instinct; and in Fig. 4 where the canopy NDVI values were lower with injection application compared to broadcast-incorporation, and where the maximum NDVI was achieved at a lower N rate when injected. These results are similar to that found with early growth plant height.

The mid-vegetative corn canopy NDVI response to increasing N rates are similar to other studies conducted in Iowa (Barker and Sawyer, 2010; Pantoja, 2013). Increasing fertilizer N rate increases the amount of available N for corn assimilation improving canopy biomass. Many studies have reported higher fertilizer N rates can increase leaf N concentrations (examples include Cerrato and Blackmer, 1991; Bullock and Anderson, 1998; Ziadi et al., 2009; Yin and McClure, 2013), which relates to higher canopy NDVI and indicates reduced N stress within the canopy as N rate increases. These canopy sensing results indicate the N responsiveness of each site in the study, and therefore the potential for documenting application method and Instinct treatment effects on corn growth and canopy development, especially at low N rates.

The NDVI results also show that excess N does not influence corn canopy development; that is, more than adequate N does not increase canopy size or coloration, and NDVI does not indicate excess N supply (Fig. 2). This explains the constant NDVI values at N rates greater than the maximum response rate. The increased stress at low N rates would be the result of below optimum N supply at the time of sensing with the potential for reduced corn production at those rates in these soils. Low N rates provide the potential to show effects related to nitrification inhibitor performance when weather conditions cause excess soil moisture increasing N losses which reduce the N supply below optimum levels, or when low soil moisture can reduce N mobility and plant N

availability. Canopy stress from N loss at the low N rates may have been especially possible in the wetter than normal 2010 growing season and with the wet conditions in April thru June of 2011, while dry conditions after June 2011 through the 2012 growing season could have led to reduced N mobility or root exploration due to low soil moisture.

Lack of differences in NDVI between UAN with or without Instinct at N rates greater than the maximum response (Table 7) in 2010, 2012, and across years (Fig. 3) are consistent with previous research where no ear leaf N concentration responses were reported with N-Serve applied in the spring (Touchton et al., 1979a, 1979b; Warren et al., 1980). The canopy sensing results suggest that at the high N rates soil N concentrations were at levels optimum for corn production, regardless of the use of Instinct or soil moisture content. Also, at the high N rates Instinct had no negative effect on canopy NDVI. The significant negative NDVI response to Instinct with N rates  $< 135 \text{ kg N ha}^{-1}$  is not clear. The negative effect may have been a result of increased  $\text{NH}_4\text{-N}$  (which is immobile in soil) as a result of the inhibition of nitrification physically limiting N availability, and thus increasing N stress and lower NDVI; while treatments that did not receive Instinct at the same N rates did not have that effect. Although inhibition of nitrification was not measured in this study, Omonode and Vyn (2013) reported that Instinct significantly reduced nitrification when applied with UAN in the spring. Franzen (2011) reported in unpublished field and laboratory studies that Instinct was an effective nitrification inhibitor. Also, there was no Instinct by application method interaction with NDVI, as was found for plant height measured earlier in the season.

The greater NDVI values for broadcast-incorporation compared to injection application at the high N rates found in 2010, 2011, and across years (Fig. 4) are contrary

to other studies. For example, Mengel et al. (1982) found leaf N concentrations were greater when N was injected compared to when N was broadcast-incorporated. The NDVI values measured might have been a result of the differential UAN placement in a narrow and concentrated coulter-injection zone; thus increasing distance from applied N to corn plants compared to broadcast-incorporation throughout the upper soil zone affecting early season N uptake and response to N rate. There was no difference in NDVI values between application methods with the 40 kg N ha<sup>-1</sup> N rate, but that result might have occurred as the combination of applied N and soil available N was not enough for maximum canopy development and green coloration even with the broadcast-incorporation of UAN. Another possible explanation for the NDVI difference between application methods could be a deeper UAN placement with injection compared to shallow incorporation of broadcast UAN with secondary tillage, and assuming the incorporation depth would be about one-half of the tillage depth.

### **Stalk Lodging Potential**

The potential for a corn stalk to lodge (values from 0 for no stalk lodged to 10 for all stalks lodged), as determined by pushing stalks to a 45° angle, was influenced by the N rate main effect in 2011, 2012, and across years; by the application method main effect in the same years; and by the interaction of Instinct and application method in 2012 (Table 2), mean across N rates. Instinct application, mean across N rate and application method, had no significant ( $P \leq 0.10$ ) effect on measured lodging potential (Table 2). There were no treatment interactions with N rate. Overall, and although some significant effects were found, changes in stalk lodging potential values were small and could be considered not important.



Stalk lodging potential response to the N rate main effect reached a plateau of 2.5 at 42 kg N ha<sup>-1</sup> ( $R^2 = 0.74$ ; 0.029  $P > F$ ) in 2011 and 1.4 at 40 kg N ha<sup>-1</sup> ( $R^2 = 0.84$ ; 0.01  $P > F$ ) across years (quadratic-plateau regression models not shown). In 2012 no regression model could be fit due to the only response being similar, but slightly higher, with any N rate compared to no N application.

Stalk lodging potential response to application method, mean across N rate and Instinct, was inconsistent. In 2012 lodging potential was lower with broadcast-incorporation compared to injection, but in 2011 and across years it was higher with broadcast-incorporation (Table 4). The differences, as well as the lodging potential values, however, were small. The only significant Instinct by application method interaction occurred in 2012, where the lodging value for UAN without Instinct when broadcast-incorporated was higher than with Instinct, but the opposite occurred when injected. Again, the lodging potential values and differences were small.

### **Corn Grain Yield**

Corn grain yield responded positively to N rate, mean across Instinct and application method, in all years and across years (Table 2). Like mid-vegetative canopy NDVI, these results show the sites each year were responsive to applied N and would allow opportunity to see potential Instinct or placement effects. Grain yields were highest in 2010 and 2011, and lowest in the 2012 dry year (Table 3 and Fig. 2c). The AONR and EONR were highest in 2010 and 2011, years with above normal rainfall, while lowest in 2012 (Table 3). The high EONR values in 2010 and 2011 are greater than normally found for corn following soybean (Sawyer et al., 2006), an indication of

the wet conditions those years, and provide an opportunity for Instinct to reduce N loss and lower the optimal N rate response.

Instinct application, mean across N rate and application method, was significant ( $P \leq 0.10$ ) in 2010, 2011, and across years (Table 2). Similar to canopy NDVI, UAN with Instinct in each instance had lower grain yield than UAN without Instinct; which was lower by 0.78, 0.45, and 0.36 Mg ha<sup>-1</sup> in 2010, 2011, and across years, respectively (Table 4). In the dry 2012 year, there was no Instinct treatment effect on grain yield. Application method, mean across N rate and Instinct treatment, was only significant in 2010 (Table 2), with injection application having a grain yield 0.34 Mg ha<sup>-1</sup> greater than broadcast-incorporation (Table 4).

A significant three-way treatment interaction was not found in any of the years or across years (Table 2). The interaction between Instinct and application method was significant in 2010 and 2012, but not across years. The grain yield response to Instinct was inconsistent. In 2010, UAN with Instinct yield was lower than without Instinct when broadcast-incorporated and injected, with the difference greater when injected (Table 6). In 2012, yield was the same for UAN with or without Instinct when broadcast-incorporated, but higher with Instinct than without Instinct when injected.

The interaction of Instinct and N rate was significant in 2010 and across years (Table 2). Opposite of what would be expected for use of a nitrification inhibitor in a wet year (2010); the AONR and EONR were considerably higher with Instinct than without (Table 7). While the yield at the AONR was similar, at N rates  $\leq 135$  kg N ha<sup>-1</sup> grain yield with Instinct was reduced (Fig. 5a). The across-year interaction was similar to that in 2012, where the AONR and EONR were considerably higher when Instinct was

applied and the yield at the AONR the same, but the yield at the lowest applied N rates were lower with Instinct than without (Fig. 5b). There was a significant interaction of N rate and method in 2012 only (Table 2 and Fig. 6). The effect of method across N rates was inconsistent, with only a 0.28 Mg ha<sup>-1</sup> yield difference at the AONR (Table 7). However, the AONR and EONR were considerably higher for broadcast-incorporation compared to injection of N, and in that year the EONR with injected UAN was within recommended rates for corn following soybean (Sawyer et al., 2006).

Corn grain yield response to N rate was similar to those found in other nitrification inhibitor studies when sites were responsive to N application (Touchton et al., 1979a; Chancy and Kamprath, 1982; Cerrato and Blackmer, 1990b; Quesada, 2002; Burzaco et al., 2014). As explained for canopy NDVI, increasing the N rate increased N availability and allowed for adequate uptake of N by the plant, regardless of Instinct inclusion in the UAN or application method. Losses of N at the low N rates ( $\leq 90$  kg N ha<sup>-1</sup>) would have had a larger negative effect on corn grain yield response, especially during the above normal precipitation in 2010 and normal spring precipitation in 2011 (Fig. 1b), as levels of available fertilizer N for plant uptake would be reduced creating N stress in the corn plant (Fig. 2b), thus reducing yield. The loss of N at the higher N rates ( $> 90$  kg N ha<sup>-1</sup>) would also occur during the same period, but the available N remaining for plant uptake would likely have been adequate in meeting corn requirements, thus eliminating N stress (Fig. 2b) and leading to optimum grain yield with or without a nitrification inhibitor.

The lower grain yield with Instinct at low N rates in a wet year, or higher required N application to maximize yield (AONR rate) across years, is not supported by other

published research. A study in Indiana found no significant grain yield response when Instinct was preplant applied with UAN (Burzaco et al., 2014), but not reduced yield with use of Instinct as in this study. Lack of grain yield response to Instinct at high N rates would indicate that fertilizer N was not limiting, thus no additional response with the use of Instinct would occur. Since soil samples were not collected from treatments after UAN application, it is not possible to determine the exact reason for the negative yield response from use of Instinct, especially in 2010 and 2011 when precipitation was above normal and normal, respectively.

Previous research found positive grain yield responses during years of significant N losses with use of nitrapyrin (N-Serve) on poorly drained soils similar to those in this study (Chancy and Kamprath, 1982; Christensen and Huffman, 1992; Randall and Vetsch, 2003, 2005). Negative corn grain yield responses to nitrapyrin application are not common, but have occurred. For example, in years with below normal precipitation (like 2012 in this study) a negative yield response has been reported with N-Serve (Hendrickson et al., 1978; Touchton et al., 1979b; Chancy and Kamprath, 1982). A negative yield response in a dry year may be a result of Instinct being an effective nitrification inhibitor (Omonode and Vyn, 2013) which would keep applied N as soil bound  $\text{NH}_4$  in a small zone (either shallow broadcast-incorporated or injected), and therefore reduce potential for roots to intercept the N zone; while reduced  $\text{NO}_3$  formation would decrease the chance of available N being in a larger soil volume.

Calculating the EONR helps producers determine the N rate at which they can reach optimum corn grain yields while not over applying N and reducing overall profit (Bock and Hergert, 1991). The interaction found between N rate and Instinct across

years provides an indication there would be greater costs with lower net returns if Instinct was to be applied with spring preplant UAN. Based on the calculated EONR found with Instinct application in this study, not only would there be an added cost for the Instinct product, there would also be an additional N cost to achieve the same optimum corn grain yield compared to when Instinct was not applied; that is, a higher EONR with Instinct than without (Table 7). A greater profit would occur by not using Instinct and just applying N at the recommended rate for corn following soybean.

Interestingly, the high EONR in 2012 with broadcast-incorporation compared to injection of UAN (the interaction between N rate and application method in 2012) only occurred in that dry year, thus indicates that shallow incorporation of broadcast N may have a significant negative impact on achieving optimum grain yield in a year with low growing season precipitation. This could be due to positional availability issues, or volatile N loss from UAN if not fully incorporated. Injected placement of UAN into the root zone during a period when N mobility within the soil is low may enhance N availability for plant uptake. Inclusion of Instinct in 2012, however, did not further negatively affect yield when applied with broadcast-incorporated UAN.

## **CONCLUSIONS**

This study used corn response measurements to evaluate the potential benefit from use of Instinct in spring preplant UAN across multiple N rates when broadcast-incorporated and injected. No matter the method of application, corn did not respond positively to Instinct. Early corn growth plant height, mid-vegetative canopy NDVI, stalk lodging potential, and grain yield either had no response or a negative response to

Instinct, especially at low to recommended N rates. It is not known why a negative response occurred, and although not measured, could have been due to positional N supply issues with increased  $\text{NH}_4\text{-N}$  concentration and lower  $\text{NO}_3\text{-N}$  formation (example dry 2012 year), or some unknown reaction to the Instinct product. Conditions were wetter than normal one year (2010), which should have provided an opportunity for Instinct to improve fertilizer N supply, reduce the EONR, and improve yield; but that did not occur. Across years of the study, Instinct inclusion with spring preplant UAN increased the EONR by  $32 \text{ kg N ha}^{-1}$ , which is an opposite effect expected from use of a nitrification inhibitor. Based on this research study, Instinct would not be recommended for use with spring preplant UAN.

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Table 1. Site-year soil information and initial soil test results for samples at the 0- to 15-cm depth prior to treatment application, and at the 0- to 30-cm depth for soil NO<sub>3</sub>-N in late spring where no N was applied, mean across replications.

NO <sub>3</sub> -N in late spring where no N was applied, mean across replications.							
Series	Subgroup	Texture	pH	SOM <sup>†</sup> g kg <sup>-1</sup>	STP <sup>‡</sup> -----	STK <sup>‡</sup> mg kg <sup>-1</sup>	LSNT <sup>§</sup> -----
<u>2010</u>							
Canisteo Harps	Typic Endoaquolls Typic Calciaquolls	Clay loam Clay loam	6.5	38	30 (H) <sup>¶</sup>	143 (O)	11
<u>2011</u>							
Clarion Nicollet	Typic Hapludolls Aquic Hapludolls	Loam Clay loam	6.5	38	18 (O)	199 (H)	11
<u>2012</u>							
Canisteo Clarion Nicollet Webster	Typic Endoaquolls Typic Hapludolls Aquic Hapludolls Typic Enoaquolls	Clay loam Loam Clay loam Silty clay loam	6.2	60	25 (H)	150 (O)	14

<sup>†</sup> SOM, soil organic matter.

<sup>‡</sup> Mehlich-3 soil test P (STP) and soil test K (STK).

<sup>§</sup> LSNT, late spring test for soil NO<sub>3</sub>-N.

<sup>¶</sup> Soil test interpretation category for O, optimum; or H, high (Sawyer et al., 2011).

Table 2. Statistical significance for corn responses to N rate, Instinct, and N application method.

Source	2010	2011	2012	Across years
----- $P > F$ -----				
<u>Plant height</u>				
N Rate (NR)	0.078	0.005	0.230	0.006
Instinct (I)	0.976	0.373	0.469	0.328
Method (M)	0.012	< 0.001	0.159	< 0.001
NR x I	0.833	0.224	0.043	0.476
NR x M	0.028	0.017	0.711	0.012
I x M	0.029	0.970	0.030	0.027
NR x I x M	0.887	0.849	0.205	0.674
<u>Canopy NDVI</u>				
N Rate (NR)	< 0.001	< 0.001	< 0.001	< 0.001
Instinct (I)	0.008	0.270	0.772	0.042
Method (M)	0.009	< 0.001	0.828	< 0.001
NR x I	0.074	0.913	0.094	0.050
NR x M	0.001	0.008	0.945	0.001
I x M	0.307	0.144	0.229	0.484
NR x I x M	0.905	0.883	0.412	0.931
<u>Stalk lodging potential</u>				
N Rate (NR)	0.194	0.001	0.098	0.001
Instinct (I)	0.974	0.575	0.497	0.899
Method (M)	0.138	0.004	0.080	0.031
NR x I	0.158	0.507	0.114	0.502
NR x M	0.431	0.868	0.767	0.949
I x M	0.715	0.779	0.012	0.150
NR x I x M	0.874	0.807	0.267	0.945
<u>Grain yield</u>				
N Rate (NR)	< 0.001	< 0.001	< 0.001	< 0.001
Instinct (I)	< 0.001	0.032	0.235	0.003
Method (M)	0.020	0.865	0.223	0.469
NR x I	< 0.001	0.122	0.412	0.064
NR x M	0.872	0.275	0.023	0.731
I x M	0.025	0.906	0.032	0.979
NR x I x M	0.813	0.377	0.786	0.744

Table 3. Quadratic-plateau regression model parameters for corn plant responses to N rate, mean across Instinct treatment and application method, when significant (Table 2).

Year	Regression parameters					EONR <sup>§</sup> kg N ha <sup>-1</sup>	YEONR <sup>§</sup> Mg ha <sup>-1</sup>	R <sup>2</sup>	P > F
	a	b	c	Join point <sup>†</sup> kg N ha <sup>-1</sup> <u>Plant height</u>	Plateau <sup>‡</sup>				
2010	79	0.043	-1.617 x 10 <sup>-4</sup>	132	82	--	--	0.84	0.065
2011	53	0.082	-3.538 x 10 <sup>-4</sup>	115	58	--	--	0.98	0.002
Across years	79	0.076	-5.809 x 10 <sup>-4</sup>	66	81	--	--	0.90	0.031
<u>Canopy NDVI</u>									
2010	0.697	7.868 x 10 <sup>-4</sup>	-3.342 x 10 <sup>-6</sup>	118	0.743	--	--	1.00	< 0.001
2011	0.692	7.513 x 10 <sup>-4</sup>	-3.400 x 10 <sup>-6</sup>	110	0.734	--	--	1.00	< 0.001
2012	0.736	2.415 x 10 <sup>-4</sup>	-7.480 x 10 <sup>-7</sup>	161	0.755	--	--	1.00	0.002
Across years	0.708	5.682 x 10 <sup>-4</sup>	-2.241 x 10 <sup>-6</sup>	127	0.744	--	--	1.00	< 0.001
<u>Grain yield</u>									
2010	7.47	0.068	-1.818 x 10 <sup>-4</sup>	186	13.75	171	13.71	1.00	< 0.001
2011	9.01	0.056	-1.498 x 10 <sup>-4</sup>	186	14.17	167	14.11	0.99	0.001
2012	8.52	0.036	-1.130 x 10 <sup>-4</sup>	158	11.35	133	11.27	0.99	0.001
Across years	8.37	0.052	-1.435 x 10 <sup>-4</sup>	181	13.09	162	13.03	1.00	< 0.001

<sup>†</sup> Nitrogen rate at which the quadratic equation joins the plateau value.

<sup>‡</sup> Units of measure are cm for plant height and Mg ha<sup>-1</sup> for grain yield.

<sup>§</sup> EONR, economic optimum N rate; YEONR, yield at the economic optimum N rate.

Table 4. Corn plant responses to Instinct, mean across N rate and application method, and to application method, mean across N rate and Instinct treatment, when significant (Table 2).

	2010	2011	2012	Across years
<u>Plant height (cm)</u>				
<u>Method</u>				
Broadcast	82a <sup>†</sup>	59a	105a	82a
Injected	80b	54b	104a	79b
<u>Canopy NDVI</u>				
<u>Instinct Application</u>				
No Instinct	0.735a	0.725a	0.750a	0.737a
Instinct	0.729b	0.723a	0.749a	0.734b
<u>Method</u>				
Broadcast	0.735a	0.728a	0.750a	0.738a
Injected	0.729b	0.719b	0.749a	0.733b
<u>Stalk lodging potential</u>				
<u>Method</u>				
Broadcast	1.1a	2.7a	0.5b	1.4a
Injected	0.8a	1.8b	0.8a	1.1b
<u>Grain yield (Mg ha<sup>-1</sup>)</u>				
<u>Instinct Application</u>				
No Instinct	12.14a	12.74a	10.46a	11.78a
Instinct	11.36b	12.29b	10.61a	11.42b
<u>Method</u>				
Broadcast	11.58b	12.50a	10.61a	11.56a
Injected	11.92a	12.54a	10.46a	11.65a

<sup>†</sup> Means with the same letter within the same column of each main treatment effect and corn plant response are not significantly different ( $P \leq 0.10$ ).

Table 5. Early corn growth plant height response for the N rate and application method interaction, mean across Instinct treatment, when significant (Table 2).

N rate kg N ha <sup>-1</sup>	2010		2011		Across years	
	Broadcast	Injected	Broadcast	Injected	Broadcast	Injected
	----- cm -----					
0	78	81	54	52	79	78
45	81	81	57	54	81	80
90	83	80	61	54	83	79
135	84	81	61	53	84	80
180	84	81	61	55	82	80
225	83	79	63	52	84	78
	<u>Regression response</u>					
<i>P</i> > <i>F</i>	0.004	NS <sup>†</sup>	0.014	NS	0.026	NS
Model	QP <sup>‡</sup>	NS	QP	NS	QP	NS
<i>R</i> <sup>2</sup>	0.98	NS	0.94	NS	0.91	NS
Max <sup>§</sup>	145	---	142	---	137	---

<sup>†</sup> NS, not significant.

<sup>‡</sup> QP, quadratic plateau response model.

<sup>§</sup> Nitrogen rate, kg N ha<sup>-1</sup>, at which the quadratic equation joins the plateau value.



Table 6. Corn response for the application method and Instinct interaction, mean across N rate, when significant (Table 2).

Method	Instinct application	Plant height cm	Canopy NDVI	Stalk lodging potential	Grain yield Mg ha <sup>-1</sup>
<u>2010</u>					
Broadcast	No Instinct	81ab <sup>†</sup>	0.736a	1.2a	11.81b
	Instinct	83a	0.733a	1.1a	11.35c
Injected	No Instinct	81bc	0.733a	0.8a	12.47a
	Instinct	80c	0.726b	0.9a	11.36c
<u>2011</u>					
Broadcast	No Instinct	60a	0.728a	2.8a	12.74a
	Instinct	59a	0.729a	2.6a	12.26b
Injected	No Instinct	54b	0.722b	1.9b	12.75a
	Instinct	53b	0.717c	1.8b	12.32ab
<u>2012</u>					
Broadcast	No Instinct	104ab	0.751a	0.6b	10.68a
	Instinct	105a	0.748a	0.3b	10.55a
Injected	No Instinct	105a	0.749a	0.5b	10.25b
	Instinct	103b	0.750a	1.0a	10.67a
<u>Across years</u>					
Broadcast	No Instinct	82a	0.738a	1.5a	11.74ab
	Instinct	82a	0.737ab	1.3ab	11.38c
Injected	No Instinct	80b	0.735b	1.1b	11.82a
	Instinct	78c	0.731c	1.2ab	11.47bc

<sup>†</sup> Means with the same letter across application method and Instinct application within the same measured corn response and year are not significantly different ( $P \leq 0.10$ ).

Table 7. Corn canopy normalized difference vegetative index (NDVI) and grain yield quadratic-plateau regression model parameters for the N rate by application method interaction, mean across Instinct treatment, and N rate by Instinct interaction, mean across application method, when significant (Table 2).

Method: interaction, mean across instinct treatment, and P value by instinct interaction, mean across application method, when significant (Table 2)										
Year	Interaction	Regression Parameters					EONR <sup>§</sup> kg N ha <sup>-1</sup>	YEONR <sup>§</sup> Mg ha <sup>-1</sup>	R <sup>2</sup>	P > F
		a	b	c	Join Point <sup>†</sup> kg N ha <sup>-1</sup>	Plateau <sup>‡</sup>				
NDVI										
2010	No Instinct	0.701	9.449 x 10 <sup>-4</sup>	-5.178 x 10 <sup>-6</sup>	91	0.744	--	--	1.00	< 0.001
	Instinct	0.697	5.932 x 10 <sup>-4</sup>	-1.928 x 10 <sup>-6</sup>	154	0.742	--	--	0.96	0.007
	Broadcast	0.692	8.924 x 10 <sup>-4</sup>	-3.517 x 10 <sup>-6</sup>	127	0.749	--	--	0.99	0.001
	Injected	0.705	5.428 x 10 <sup>-4</sup>	-2.353 x 10 <sup>-6</sup>	115	0.737	--	--	0.94	0.014
2011	Broadcast	0.692	6.496 x 10 <sup>-4</sup>	-2.057 x 10 <sup>-6</sup>	158	0.743	--	--	0.99	0.001
	Injected	0.692	8.964 x 10 <sup>-4</sup>	-5.895 x 10 <sup>-6</sup>	76	0.726	--	--	0.97	0.004
2012	No Instinct	0.735	4.557 x 10 <sup>-4</sup>	-2.916 x 10 <sup>-6</sup>	78	0.753	--	--	0.92	0.022
	Instinct	0.734	2.361 x 10 <sup>-4</sup>	-6.280 x 10 <sup>-7</sup>	188	0.756	--	--	0.91	0.029
Across years	No Instinct	0.709	7.798 x 10 <sup>-4</sup>	-4.390 x 10 <sup>-6</sup>	89	0.744	--	--	1.00	< 0.001
	Instinct	0.708	4.729 x 10 <sup>-4</sup>	-1.547 x 10 <sup>-6</sup>	153	0.744	--	--	0.99	0.002
	Broadcast	0.706	5.945 x 10 <sup>-4</sup>	-2.073 x 10 <sup>-6</sup>	143	0.749	--	--	1.00	< 0.001
	Injected	0.711	5.296 x 10 <sup>-4</sup>	-2.535 x 10 <sup>-6</sup>	104	0.739	--	--	1.00	< 0.001
Grain yield										
2010	No Instinct	7.61	0.088	-3.129 x 10 <sup>-4</sup>	140	13.77	131	13.74	1.00	< 0.001
	Instinct	7.18	0.058	-1.281 x 10 <sup>-4</sup>	227	13.80	206	13.74	0.97	0.005
2012	Broadcast	9.02	0.022	-4.619 x 10 <sup>-5</sup>	225	11.59	175	11.42	0.97	0.005
	Injected	8.09	0.046	-1.657 x 10 <sup>-4</sup>	139	11.31	123	11.25	0.98	0.002
Across years	No Instinct	8.59	0.056	-1.765 x 10 <sup>-4</sup>	159	13.07	144	13.01	0.99	0.001
	Instinct	8.10	0.050	-1.275 x 10 <sup>-4</sup>	198	13.09	176	13.02	0.99	0.001

<sup>†</sup> Nitrogen rate at which the quadratic equation joins the plateau value.

<sup>‡</sup> Unit of measure for grain yield is Mg ha<sup>-1</sup>.

<sup>§</sup> EONR, economic optimum N rate; YEONR, yield at the economic optimum N rate.

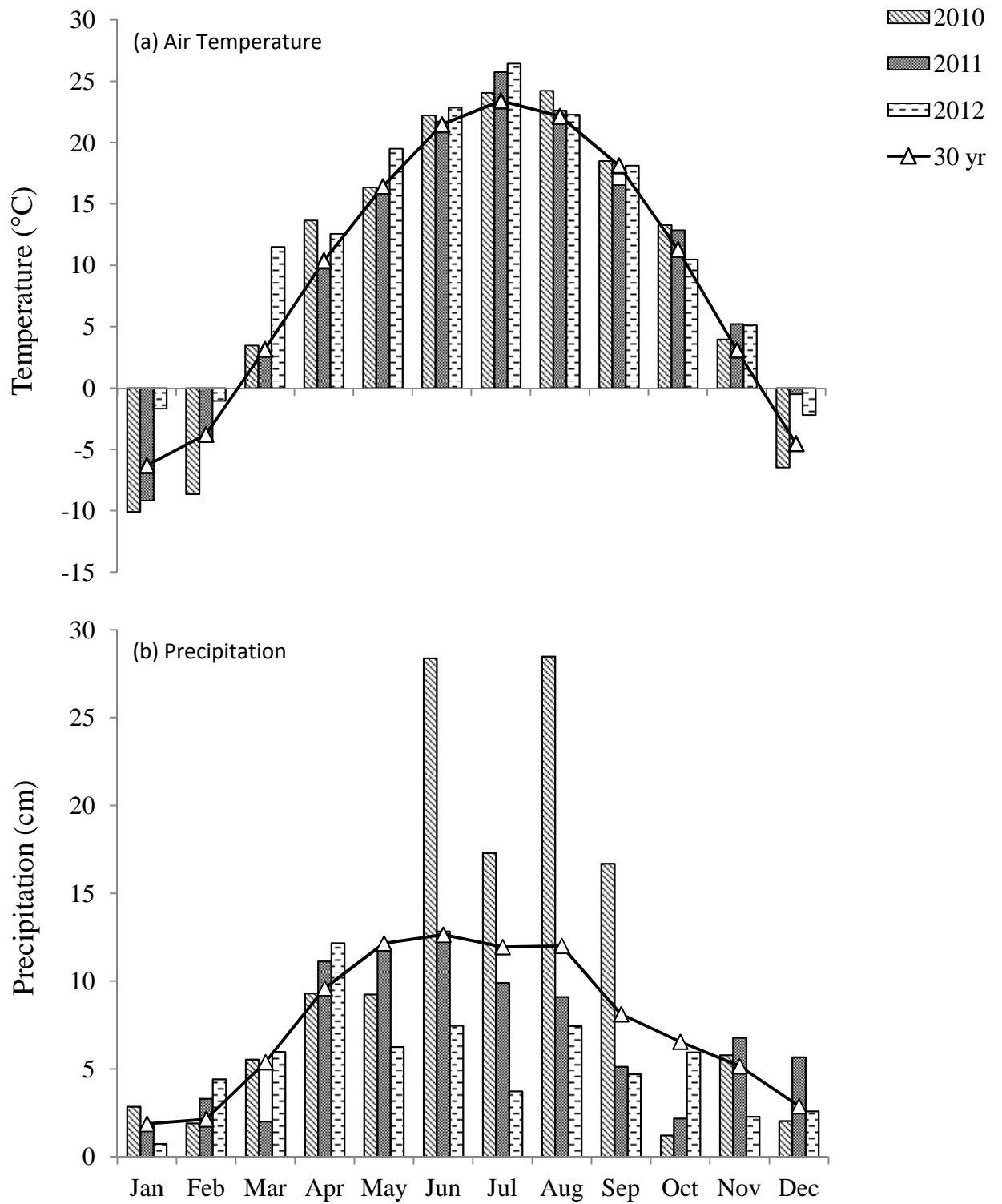


Fig. 1. Monthly mean air temperature (a) and total monthly precipitation (b) for each study year and the 30-yr mean (data from Arritt and Herzmann, 2014).

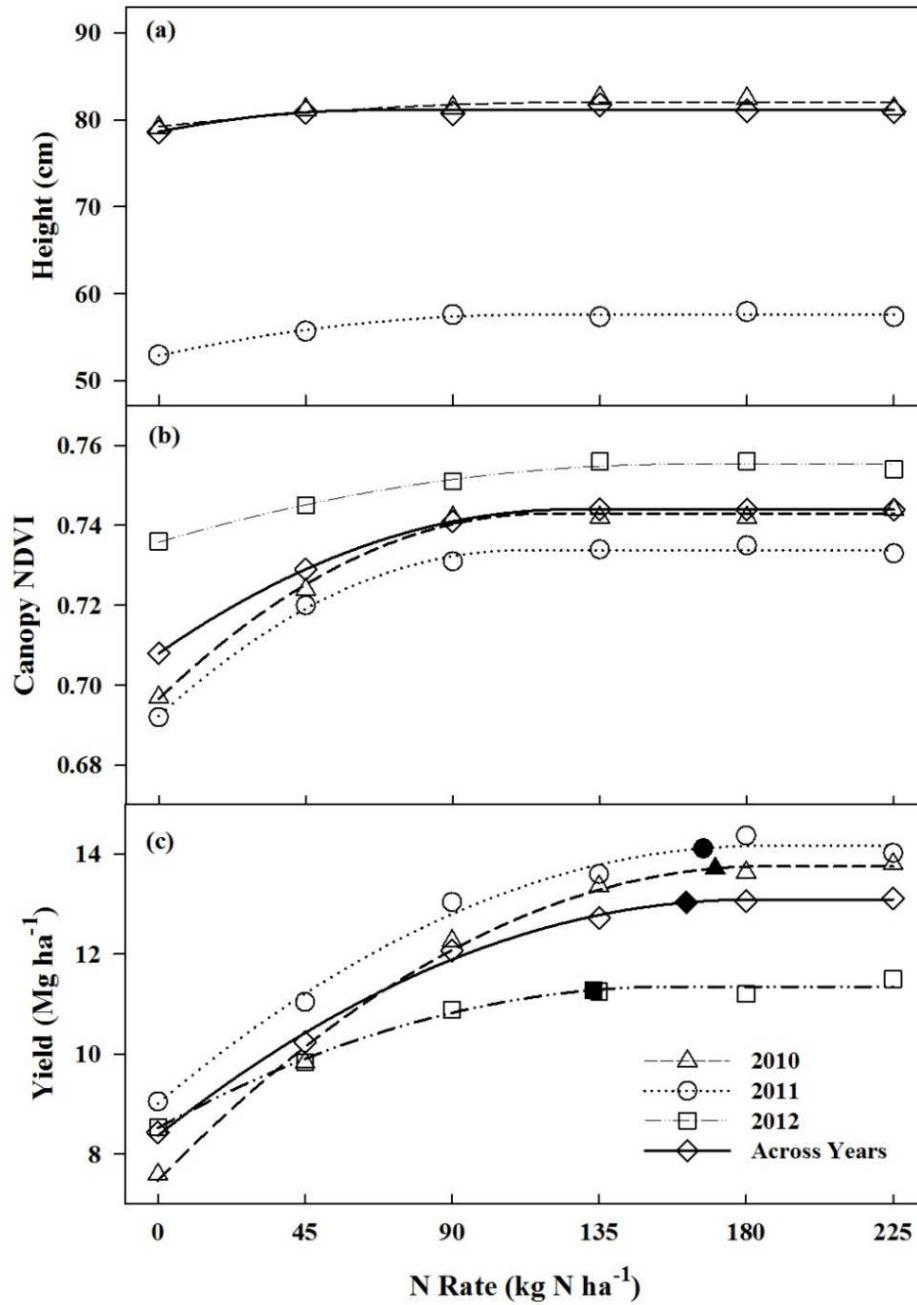


Fig. 2. Corn plant height (a), canopy normalized difference vegetative index (NDVI) (b), and grain yield (c) when N rate was significant (Table 2), mean across application method and Instinct treatment. Regression parameters are presented in Table 3. Open symbols represent means and closed symbols represent the economic optimum N rate (EONR).

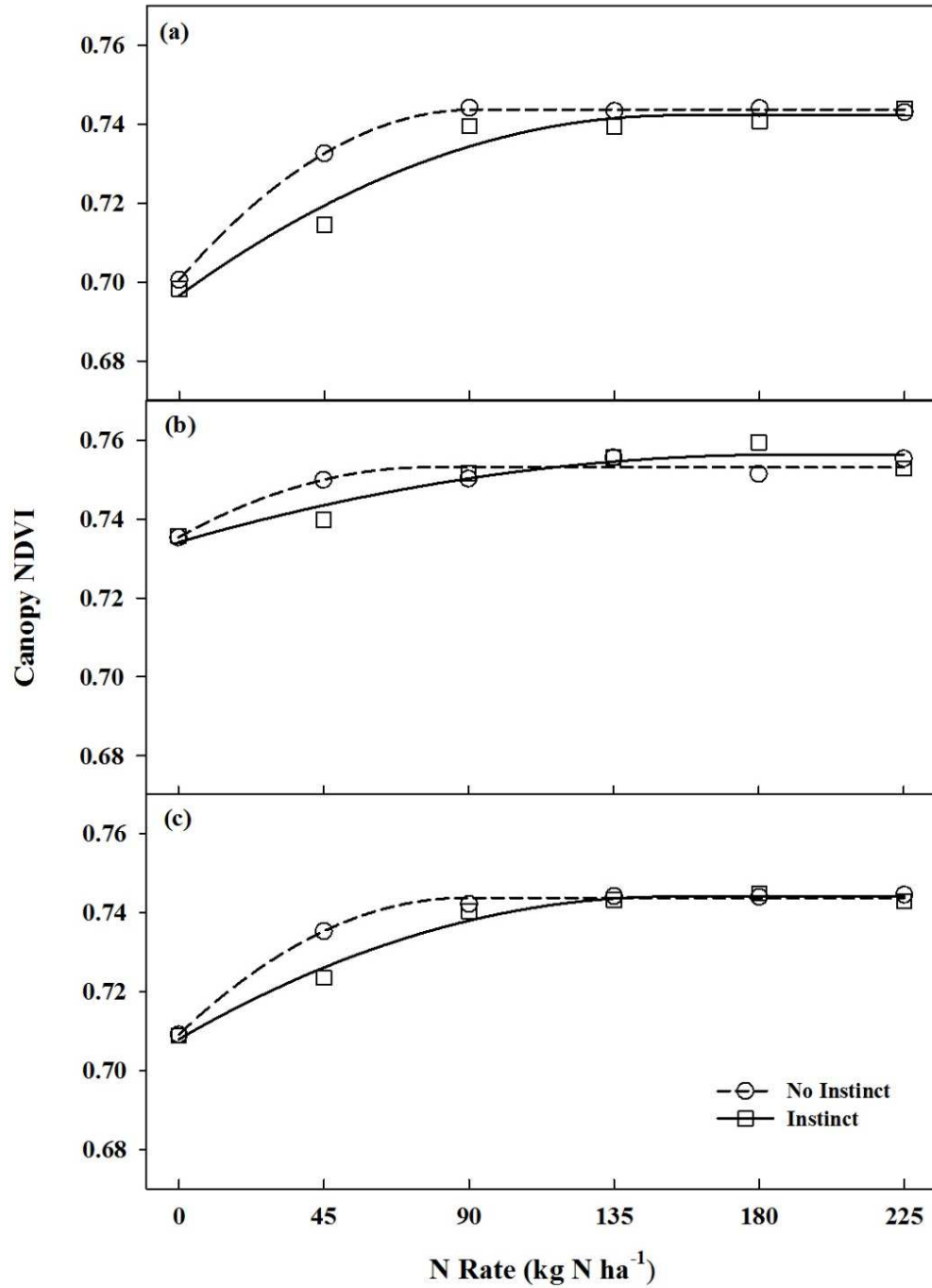


Fig. 3. Corn canopy normalized difference vegetative index (NDVI) response when the interaction between Instinct and N rate was significant (Table 2) in 2010 (a), 2012 (b), and across years (c), mean across application method. Regression parameters are presented in Table 7.

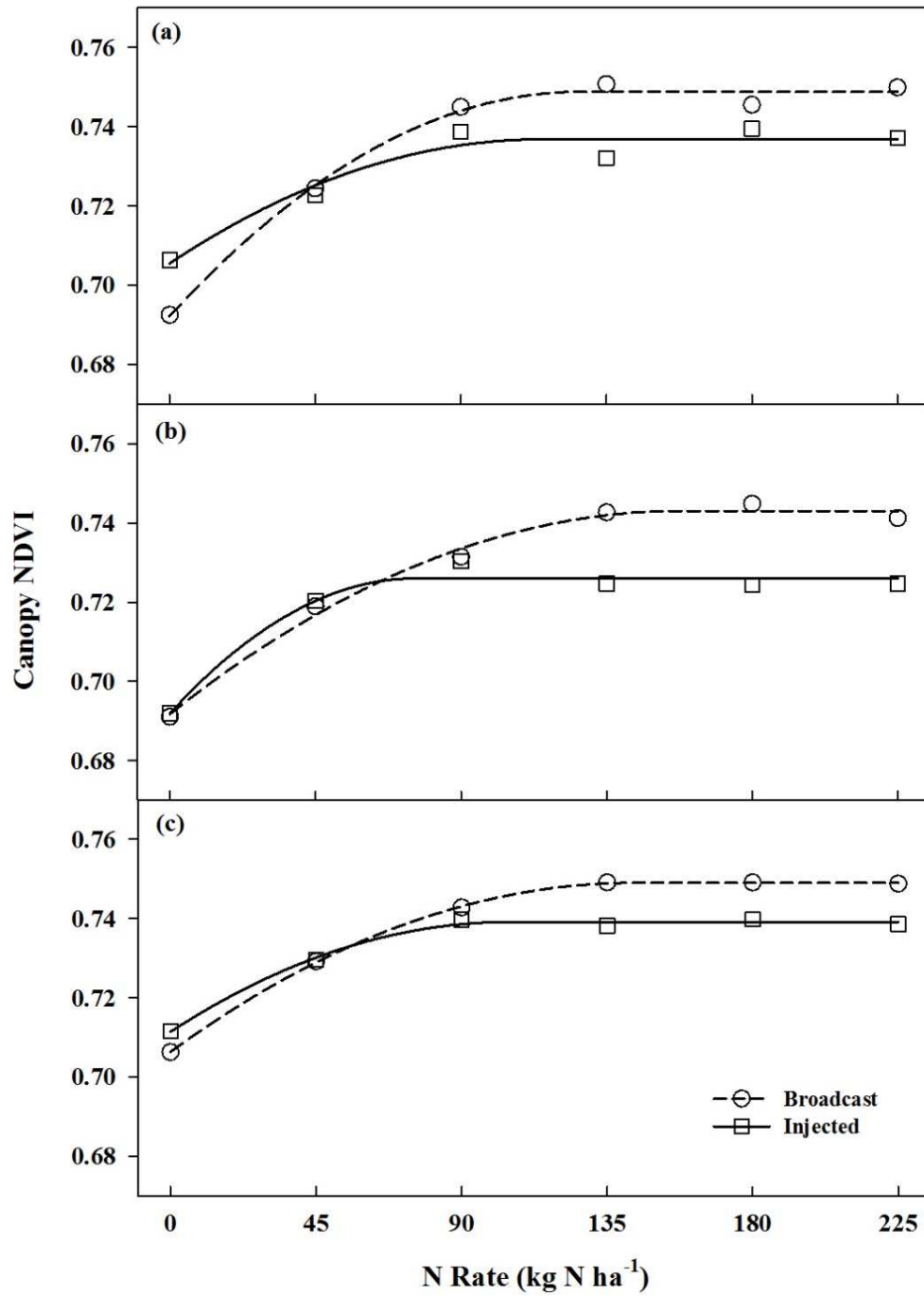


Fig. 4. Corn canopy normalized difference vegetative index (NDVI) response when the interaction between application method and N rate was significant (Table 2) in 2010 (a), 2011 (b), and across years (c), mean across Instinct treatment. Regression parameters are presented in Table 7.

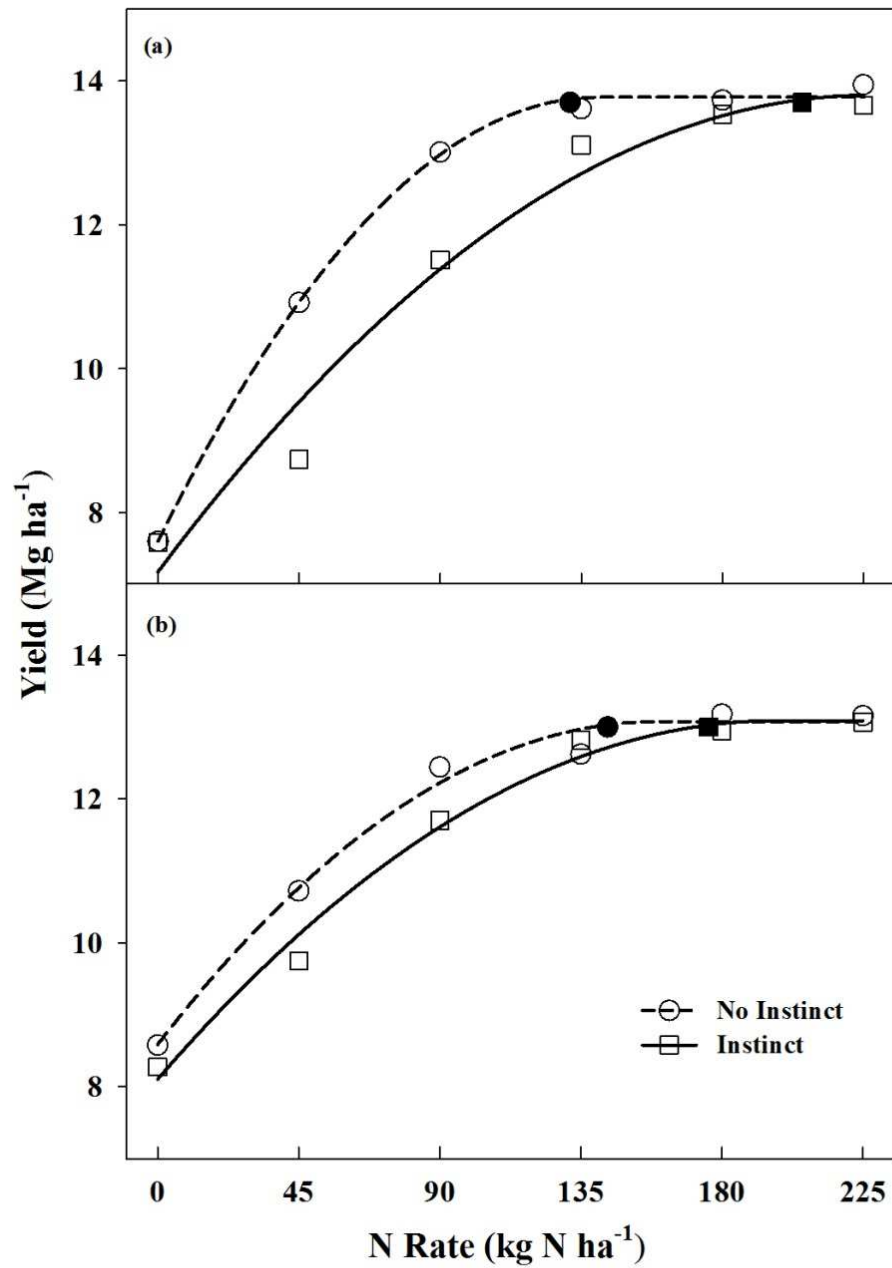


Fig. 5. Corn grain yield response when the interaction between Instinct and N rate was significant (Table 2) in 2010 (a) and across years (b), mean across application method. Regression parameters are presented in Table 7. Open symbols represent means and closed symbols represent the economic optimum N rate (EONR).

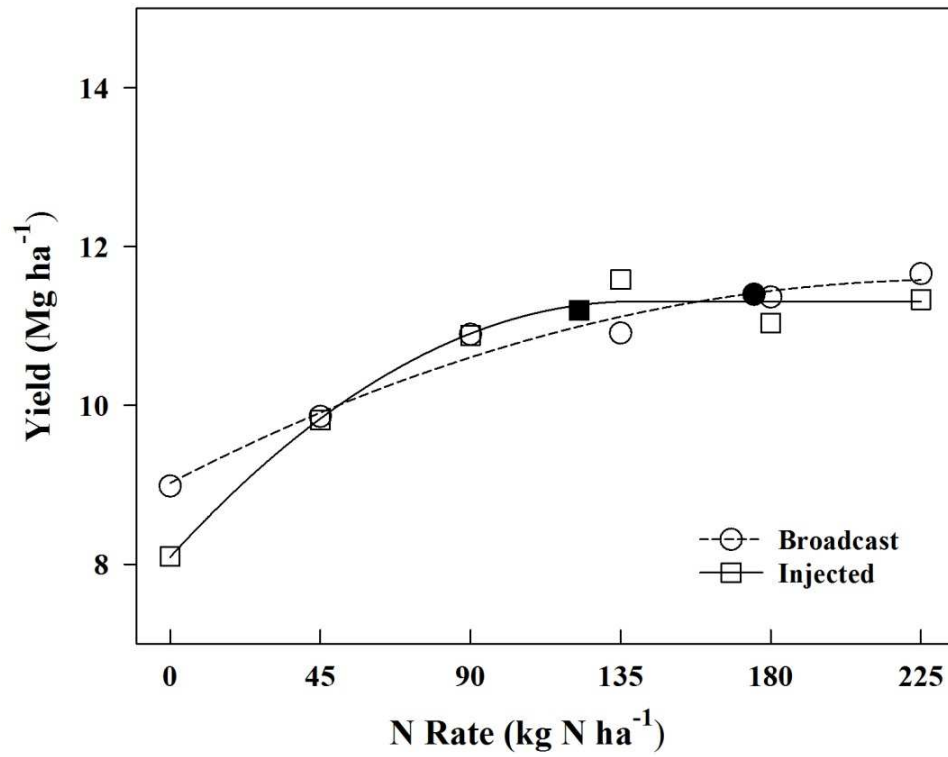


Fig. 6. Corn grain yield response when the interaction between application method and N rate was significant (Table 2) in 2012, mean across Instinct treatment. Regression parameters are presented in Table 7. Open symbols represent means and closed symbols represent the economic optimum N rate (EONR).



### CHAPTER 3. CORN RESPONSE TO INSTINCT NITRIFICATION INHIBITOR FALL APPLIED WITH LIQUID SWINE MANURE

A paper to be submitted to Agronomy Journal

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#### ABSTRACT

Fall applied liquid swine (*Sus scrofa*) manure (LSM) can lead to economic and environmental concerns due to potential losses of NO<sub>3</sub>. The objective of this study was to determine the effect of application timing and Instinct nitrification inhibitor applied with LSM on corn (*Zea mays* L.) production, and in comparison to anhydrous ammonia (AA). Treatments were a control, AA, and LSM with Instinct at three rates (0, 2.56, 5.12 L ha<sup>-1</sup>) applied near October 1 (early fall) and November 1 (late fall) at 157 kg N ha<sup>-1</sup> in a randomized complete block design with four replications. Late fall application of LSM with and without Instinct and AA, had greater fall and spring soil NH<sub>4</sub>-N concentrations in injected N bands and late spring NO<sub>3</sub>-N concentrations. Corn canopy normalized difference vegetative index (NDVI) was increased with late fall AA and LSM application, but not with Instinct added to LSM. Corn grain yield with AA was higher than LSM at both application times. Only Instinct at the low rate with LSM increased yield compared to LSM without Instinct. Also, LSM with either Instinct rate had lower yield than AA. Waiting to apply N in late fall provided better inorganic-N retention, and

with LSM higher corn yield. Based on this study, AA would be an advantageous N source compared to LSM, with or without Instinct. A decision to use Instinct with LSM must weigh cost of the inhibitor and other application options, such as later fall or spring application or use of AA.

**Abbreviations:** AA, anhydrous ammonia; LSM, liquid swine manure; LSNT, late-spring test for soil NO<sub>3</sub>; NDVI, normalized difference vegetative index.

## INTRODUCTION

Iowa leads the U.S. in swine production with more than 6000 swine operations and over 20 million head marketed annually (NASS, 2014). This has resulted in areas within the state where high concentrations of nutrients from manure are available for use in corn production. Liquid swine manure has been shown to be an excellent source of primary and secondary plant nutrients (Risse et al., 2001). When compared to inorganic-N sources, LSM has been shown to adequately supply plant available N for corn production (Park et al., 2010; Chantigny et al., 2008; Kwaw-Mensah and Al-Kaisi, 2006; McLaughlin et al., 2000; Sutton et al., 1978; Evans et al., 1977). Woli et al. (2013) concluded that LSM could provide available N for corn production due to high inorganic NH<sub>4</sub>, but must be appropriately managed in order to obtain the full agronomic benefit, similar to N fertilizers.

In Iowa, LSM is typically applied in the fall when weather and soil conditions are more favorable for application, demands for equipment and labor can be better managed, and the potential of soil compaction by heavy applicators is reduced (Bundy, 1986;

Randall et al, 1999). The concern with fall application is the increased time for nitrification of the high LSM  $\text{NH}_4$  content, increasing potential losses of N as  $\text{NO}_3$  through leaching or denitrification. Excessive losses of applied N can negatively impact corn yield, especially if early fall applied before the 10-cm soil depth decreases to  $10^\circ\text{C}$  and continues cooling (Sawyer and Mallarino, 2008; Gomes and Loynachan, 1984). Sabey et al. (1956) reported waiting to apply N until soil temperatures are  $10^\circ\text{C}$  and cooling is advantageous as the microbial oxidation rate of  $\text{NH}_4$  is limited with cold temperatures, and stops near  $0^\circ\text{C}$ . Along with yield concerns, water quality is also an issue with fall applied LSM as nitrified N is leached as  $\text{NO}_3$  to subsurface drainage, and eventually to surface waters. In an Iowa study with a corn-soybean rotation, fall applied LSM at  $135 \text{ kg N ha}^{-1}$  had significantly higher average annual flow weighted  $\text{NO}_3\text{-N}$  in tile flow with no significant difference in corn yield when compared to urea-ammonia nitrate (UAN) spring applied at  $110 \text{ kg N ha}^{-1}$  (Bakhsh et al., 2005). In another Iowa study, average annual flow weighted  $\text{NO}_3\text{-N}$  with fall applied LSM was significantly higher ( $9 \text{ mg L}^{-1}$ ) than commercial-grade 28% aqueous ammonia applied at the same N rate ( $168 \text{ kg N ha}^{-1}$ ); however, LSM out yielded the UAN in three of four years (Lawlor et al., 2011).

Use of a nitrification inhibitor with fall N application is a management practice that has potential to slow nitrification and potentially reduce N losses. Introduced by Goring (1962a), nitrapyrin [2-chloro-6-(trichloromethyl) pyridine] is the most commonly used active ingredient (a.i.) for nitrification inhibition, with specificity to the genera *Nitrosomonas* sp. bacteria which oxidize  $\text{NH}_4$  to  $\text{NO}_2$  during the nitrification process (Shattuck and Alexander, 1963; Campbell and Aleem, 1965; and Powell and Prosser,

1986). Degradation of nitrapyrin is by chemical hydrolysis, which is largely dependent on temperature (Keeney, 1980) and soil properties (Redemann et al., 1964; Briggs, 1975; Touchton et al., 1979). Nitrapyrin can slow nitrification for 4 to 10 weeks (Nelson and Huber, 1992).

Nitrapyrin use has been primarily with N fertilizers, with research focused on fall application (Hendrickson et al, 1978; Stehouwer and Johnson, 1990; Randal and Vetsch, 2005). Fewer studies have focused on the effect of nitrapyrin with liquid manure. In an Indiana study McCormick et al. (1983) applied LSM with and without 3 kg a.i. ha<sup>-1</sup> nitrapyrin (a very high nitrapyrin rate), and found that the addition of nitrapyrin delayed nitrification of the LSM NH<sub>4</sub> for 13 weeks after application; with > 50% of the initial inorganic-N recovered at the end of the experiment (24 weeks). A Minnesota study found nitrapyrin added to fall applied LSM increased soil NO<sub>3</sub>-N concentrations and corn yield in two of three site years, but the addition of nitrapyrin did not improve yield when averaged across time and rate of manure application (Randall et al, 1999). In a similar study McCormick et al. (1984) applied nitrapyrin at 0, 25, or 50 mg a.i. L<sup>-1</sup> with LSM in the fall, and found the addition of nitrapyrin increased corn yields 46% when averaged across nitrapyrin rates.

Historically, nitrapyrin has been marketed as N-Serve (Dow AgroSciences LLC, Indianapolis, IN). In 2009 a water-based, microencapsulated reformulation of nitrapyrin was introduced, and is marketed as Instinct (Dow AgroSciences LLC, Indianapolis, IN). This new reformulation of nitrapyrin was developed to address the rapid volatilization loss of nitrapyrin if not immediately incorporated after surface application (Goring, 1962b; Redemann et al., 1964; Briggs, 1975; McCall and Swann, 1978). According to

the product label, it is recommended liquid manure be injection applied, but surface application is permitted as long as incorporation occurs up to 10 d after application with light tillage or by 1.27 cm of moisture either as rainfall or overhead irrigation. Published literature on the use of Instinct is limited. In a laboratory study, Instinct was ineffective in controlling nitrification of  $\text{NH}_4$  across soils, soil moisture levels, and when compared to another known effective inhibitor (dicyandiamide) (Ferrel, 2012). The researchers indicated the negative results were likely due to a delayed release of nitrapyrin from the microcapsule (Ferrel, 2012). A field study in Indiana found Instinct band injected with sidedress UAN (corn at the V4 to V6 growth stage) significantly reduced nitrification of  $\text{NH}_4$  three to six weeks beyond when Instinct was not applied, while also reducing  $\text{N}_2\text{O}$  emissions by 44% (Omonode and Vyn, 2013). Just like with N-serve, corn yield responses to Instinct have been variable. As summarized by Franzen (2011), studies conducted in Nebraska, Illinois, Iowa, and Minnesota found no benefits or increases in yield with use of Instinct. In a two year study with fall applied LSM, Instinct resulted in no corn grain yield response during a growing season with normal precipitation, but a  $0.15 \text{ Mg ha}^{-1}$  corn grain yield increase during a growing season with above normal precipitation (Kyveryga and Blackmer, 2013). Because Instinct is a new nitrification inhibitor product, and due to limited research with LSM, additional research would be beneficial to help evaluate use of Instinct applied with LSM in Iowa corn production. The objectives of our study were to investigate i) the effect of Instinct rate with early and late fall applied LSM on soil inorganic-N and corn production; and ii) how does early and late fall applied LSM, with or without Instinct, compare to AA at the same application timing.

## MATERIALS AND METHODS

### Site Description and Experimental Design

A three year study was conducted, starting fall 2010 through 2013, at the Iowa State University Agricultural Engineering and Agronomy Research Farm (42°01' N, 93°46' W) approximately 10-km from Boone, Iowa. The soils were typical for those found in Central Iowa (Table 1). Previous crop each year was soybean [*Glycine max* (L.) Merr]. Blanket applications of P, K, and S were made to non-LSM treatments to help mask potential effects of these nutrients applied with LSM. Those applications were based on rates applied with the LSM. In all years pre-emergence herbicide was applied prior to or shortly after planting. Growing season monthly air and soil temperatures, precipitation, and historic weather data was collected from an automated weather station located near the research site, and was reported by the Iowa Environmental Mesonet Network (Arritt and Herzmann, 2014). Soil temperatures were measured at a 10-cm depth under sod.

The study contained nine treatments arranged in a randomized complete block design with four replications. Each plot had a length of 15-m with a width of 6-m (8 rows). Treatments consisted of two application times, two N sources (AA and LSM), three Instinct rates with LSM only [0, 2.56 L ha<sup>-1</sup> (0.56 kg a.i. ha<sup>-1</sup>) and 5.12 L ha<sup>-1</sup> (1.12 kg a.i. ha<sup>-1</sup>)], and a control. Instinct rates used were the recommended product label rates, and were not applied with AA. Application times were in the fall near October 1 (early) and November 1 (late). A total N rate goal of 157 kg N ha<sup>-1</sup> was used for both LSM and AA. The LSM was from a swine finishing facility with an under-building pit

located at the Iowa State University Agricultural Education and Studies 450 Farm (41°58'52.2" N, 93°39'13.39" W).

All LSM treatments were coulters injected in a vertical band to approximately a 15-cm depth on 76-cm spacing approximately midway between future corn rows. The LSM applicator was equipped with two Roper 71228 power take-off (PTO) driven positive displacement pumps (Roper Pump Company, Commerce, GA), with load cells below the storage tank to record tank weight, an Avery Weigh-Tronix scale (Avery Weigh-Tronix LLC, Fairmont, MN) and a digital readout for rate control. The LSM injection system was a Yetter 76-cm Avenger Coulters (Yetter Manufacturing Company, Colchester, IL) with a straight blade coulters, a scraper blade on the side and to the back of the coulters to keep the injection trench open, a skid shoe on the opposite side of the scraper blade next to the coulters for reduced tillage effect, LSM supply tube behind the scraper blade with outlet ports at the bottom of the coulters, and two no-till wheels angled to provide residue and soil coverage over the injection track. Anhydrous ammonia was knife injected to approximately the same depth, spacing, and placement relative to future corn rows as the LSM. The AA applicator was equipped with a Continental C-2500 Meter Matic (Continental NH<sub>3</sub> Products, West Yorktown, TX) and Impellicone (CDS-John Blue Company, Huntsville, AL) flow divider. The AA injection system (John Deere, Waterloo, IA) was a straight blade coulters, forward swept knife with a leading shoe, AA supply tube behind the knife with outlet ports at the bottom and to the side of the knife, and two wavy coulters angled to provide soil coverage over the injection track. Corn was planted in 76-cm row spacing on 11 May 2011, 16 May 2012,

and 18 May 2013 at 85200, 86500, and 86500 seed ha<sup>-1</sup> with Pioneer 34F07, 0528XR, and PO461XR corn hybrids, respectively.

### **Nitrogen Application**

Treatment applications occurred on 5 October and 5 November 2010, 4 October and 4 November 2011, and 1 October and 1 November 2012 for study years 2011, 2012, and 2013 respectively. Approximately one to two weeks prior to treatment application LSM was withdrawn from the under-building pit and stored in a tanker wagon until treatment application. At the time of manure removal from the pit, a LSM sample was collected and submitted to Minnesota Valley Testing Laboratories (MVTTL, Nevada, IA) for analysis (Table 2). Total-N, determined from the LSM pre-application sample analysis, was used to calculate the LSM application rate required to achieve the N rate goal. During application, additional samples were collected each time the manure applicator tank was filled, submitted for analysis (Table 2), and used to confirm the LSM-N rate applied. Any sample not submitted the same day as application was placed in a freezer at 0°C until submission to the lab. Before application, both LSM and AA applicators were calibrated for rate and depth in measured areas. Calibration of LSM and AA rate was based on the difference between beginning and end weight of manure or AA applied, measured using load cells located on the applicator. The weight of LSM was converted to volume using [Eq. 1].

$$\text{Volume of LSM} = \frac{\text{Weight of LSM Applied}}{\text{Weight per Volume Water}} \quad [1]$$

Liquid swine manure without Instinct was applied first, followed by LSM with 2.56 L ha<sup>-1</sup> Instinct (low rate), then 5.12 L ha<sup>-1</sup> Instinct (high rate). The volume of Instinct to add per volume of LSM was calculated [Eq. 2] prior to each application.



$$Volume\ of\ Instinct = \frac{Instinct\ Rate}{LSM\ Rate} \quad [2]$$

After the LSM application without Instinct, the applicator was filled with enough LSM for both Instinct rate applications, the weight recorded, and the volume of Instinct to add was calculated [Eq. 3] to achieve the low Instinct rate.

$$Instinct\ to\ Add = Volume\ of\ LSM \times Volume\ of\ Instinct \quad [3]$$

Upon completion of LSM application with the low Instinct rate, the weight of LSM with Instinct remaining in the applicator was recorded. Using Eq. 3, the volume of Instinct to add to achieve the high Instinct rate was calculated, as well as to account for the amount of Instinct remaining in the applicator following the LSM application with the low Instinct rate. The difference between the volume of Instinct to add to achieve the high rate and the volume of Instinct accounted for remaining in the applicator after application of the low rate was determined. This difference was the volume of Instinct to add to the volume of LSM remaining in the applicator tank in order to achieve the high Instinct rate. Instinct was added to the LSM applicator tank and thoroughly mixed for 20 min with a circulation pump prior to applications. Lines on the LSM applicator were flushed within border areas between Instinct rate applications to clear previous treated LSM material.

### **Soil and Plant Sampling**

Soil samples were collected prior to the first treatment application on 3 Oct. 2010, 3 Oct. 2011, and 28 Sep. 2012 by replicate from the 0- to 15-cm depth using a 2-cm diameter wet tip JMC T-Handle soil probe (Clements Associates Inc., Newton, IA) to determine initial soil test P and K, pH, and soil organic matter (Table 1). After the late fall application, soil samples were collected on 15 Dec. 2010, 22 Nov. 2011, and 29 Nov. 2012; and again the following spring on 1 Apr. 2011, 5 Apr. 2012, and 26 Apr. 2013.

Fall and spring soil samples were taken from within the LSM and AA injection band using a 4-cm diameter drill bit as described by Kress et al. (2003) at a depth of 0- to 30-cm, and analyzed for inorganic-N. Samples were taken between two flagged points along an injection track located at time of treatment application, and at random within the control plots. Cores from the same plot were combined, mixed, and a subsample collected for analysis. All samples were stored frozen at 0°C until analysis.

In late spring soil samples were collected to a depth of 0- to 30-cm and 30- to 60-cm when corn height was 15- to 30-cm from all plots for inorganic-N determination on 28 June 2011, 4 June 2012, and 7 June 2013. Soil samples were collected by starting in a specific row, with five 2-cm diameter cores collected at 15-cm increments perpendicular across the corn row direction between two rows, and from two plot locations. All ten cores were combined, mixed, and a subsample collected for analysis. Samples were stored in a cooler at 4°C until submitted for analysis.

All soil samples were analyzed at the Iowa State University Soil Testing Laboratory. Initial and late spring soil samples were dried at 40°C and ground to pass through a 2-mm sieve (Gelderman and Mallarino, 2011). For the initial soil samples, soil test P and K were determined using the Mehlich-3 extraction procedure, with P determined colorimetrically and K with atomic absorption (Frank et al., 2011; Warncke and Brown, 2011). Soil pH was measured in a 1:1 soil to water suspension (Watson and Brown, 2011). Organic matter was determined by dry combustion using a LECO CHN-2000 analyzer (LECO Corporation, St. Joseph, MI) (Combs and Nathan, 2011). Fall and spring soil samples collected from the LSM and AA bands for inorganic-N analyses were processed field moist by hand to pass through a 4-mm sieve, and

gravimetric moisture content determined. Samples were extracted with 2 *M* KCl, and an aliquot of extract was analyzed for NH<sub>4</sub>-N and NO<sub>3</sub>-N using a Lachat flow injection analyzer (Lachat Instruments, Milwaukee, WI) (Gelderman and Beegle, 2011).

Inorganic-N concentrations were based on oven dry weight.

Corn canopy sensing was conducted using a Crop Circle ACS-210 active canopy sensor (Holland Scientific, Lincoln, NE) when corn growth reached mid-vegetative (V10) growth stage (Abendroth et al., 2011) following the procedure described by Barker and Sawyer (2010). The sensor was mounted on a mast, positioned inter-row, and hand carried through the center of each treatment plot at a constant speed (1.2 m s<sup>-1</sup>) and distance above the canopy (60- to 90-cm). Mean near-infrared (NIR) and visible (VIS) light reflectance data were determined for each plot, and used to calculate NDVI [Eq. 4] for estimating corn canopy and N status response to treatments.

$$NDVI = \frac{NIR - VIS}{NIR + VIS} \quad [4]$$

Corn grain was harvested from the four middle rows of each plot with a research plot combine, with yield being adjusted to 155 g kg<sup>-1</sup>.

### **Statistical Analysis**

Analysis of variance (ANOVA) was performed by year and across years using PROC GLIMMIX in SAS 9.3 (SAS Institute, 2011) on N band inorganic-N, late spring soil samples, canopy NDVI, and corn grain yield. Analysis was conducted across years and by year in order to investigate differences in yearly responses as a result of weather variation. A preliminary ANOVA comparing the control to all treatments found the control for all parameters measured to be significantly lower than all treatments.

Excluding the control, treatments were arranged into two factorial groups for analysis,

and another ANOVA performed. The first factorial analysis was N source (without Instinct) and application timing; while the second factorial analysis was LSM application timing and Instinct rate. Replicate was considered random in the by year analysis. Year, replicate, and interactions were considered random in the across years analysis.

Treatments and interactions were considered fixed effects. Main effects and interactions were considered significant at  $P \leq 0.10$ . Treatment mean comparisons were determined using the PDIFF option, and were considered significantly different at  $P \leq 0.10$ . The LINES option was used to determine t-grouping differences for mean comparisons.

## RESULTS AND DISCUSSION

### Weather

Variations in monthly mean air temperature, 10-cm soil temperature, and total precipitation between the three study years provided a good opportunity to study the effects Instinct might have on corn production. Figure 1a illustrates the mean monthly air temperature during each year of the study compared to the 30-yr mean (normal). October mean air temperatures were 2°C above normal in 2010 and 2011, 1°C below normal in 2012, and near normal in 2013. The mean air temperatures for November were 1°C above normal in 2010 and 2°C above normal in 2011 and 2012. Monthly mean air temperatures were below the freezing point in December, and remained there through February 2011 and 2012; while in 2013 the mean air temperature remained below the freezing point through March, which is 4°C below normal for March. March mean air temperatures were normal in 2011 and 11°C above normal in 2012. At the time of planting in May, mean air temperature varied little from normal in 2011 and 2013, but

was 3°C above normal in 2012. June mean air temperatures varied little from normal. Mean air temperatures in July 2011 and 2012 were 3°C above normal with little variation from normal in 2013. As in June, August mean air temperatures varied little from normal. September mean air temperatures were 1°C below normal, near normal, and 2°C above normal for 2011, 2012, and 2013, respectively.

The mean monthly 10-cm soil temperatures closely reflected the mean monthly air temperatures (Fig. 1b). As with mean air temperatures, mean 10-cm soil temperatures were at or below the freezing point starting in December 2010 and 2011, and remained there through February 2011 and 2012. For study year 2013, the mean 10-cm soil temperatures did not fall to or below the freezing point until January of that year, and remained there through March. During 2012, mean monthly 10-cm soil temperatures were warmer than the mean monthly air temperatures starting in May. This did not occur in the other years until August 2011 and July 2013. The greatest difference between the mean monthly air and 10-cm soil temperatures occurred in July 2012 and 2013, and August of all years. July 2012 and 2013, mean 10-cm soil temperature were 4°C warmer than the mean air temperature. August mean 10-cm soil temperatures were 3, 4, and 5°C warmer for 2011, 2012, and 2013, respectively. Differences between the mean monthly 10-cm soil and air temperatures did not exceed  $\pm 2^\circ\text{C}$  all other months.

There was large variation around the 30-yr mean (normal) total monthly precipitation during each of the three years (Fig. 1c). Total monthly precipitation in October 2010 and 2011 was 5.4 and 4.4 cm below normal; while total October monthly precipitation in 2012 was near normal and 3.1 cm above normal in 2013. November total monthly precipitation was near normal in 2010 and 2011, and 2.9 cm below normal in

2012. January and February total monthly precipitation was near the normal low wintertime amount for all years. Total monthly precipitation for March was 3.4 cm below normal in 2011, near normal in 2012, and 1.6 cm below normal in 2013. In all years April total monthly precipitation was above normal by 1.5, 2.6, and 5.2 cm for 2011, 2012, and 2013, respectively. Near time of planting (during May), precipitation was near normal in 2011, 6 cm below normal in 2012, and 17.6 cm above normal in 2013. Except in June 2011, total monthly precipitation from June thru September of all years was below the 30-yr mean. The average difference from normal for the period of July thru September 2011 was -2.7 cm; while the period of June thru September 2012 and 2013 was -5.4 and -6.4 cm, respectively. The greatest difference from normal was -10 cm, which occurred in August 2012.

Mean air and 10-cm soil temperatures for October of all years were above 10°C (Fig. 1a and 1b). Sabey et al. (1956) and Sawyer and Mallarino (2008) suggest the application of manure should wait until the 10-cm soil temperature is 10°C and falling to reduce the rate of nitrification after manure or AA has been applied. Since mean temperatures after the early treatment application remained above 10°C, nitrification would be of greater concern than when applications were applied late fall when mean temperatures were 5°C below the 10°C threshold. The inclusion of Instinct with LSM, if Instinct were effective, should reduce the nitrification rate and fall NO<sub>3</sub> buildup, especially at the high Instinct rate, and reduce potential springtime losses when excessively wet conditions often occur. Mean 10-cm soil temperatures would not have favored nitrification or denitrification until April or May when temperatures typically begin to increase. March 2012 would have been an exception, when mean temperatures

were above 10°C, and above normal temperatures in April enhanced the potential for nitrification.

Above normal precipitation and cold temperatures in April of all years were favorable for NO<sub>3</sub> losses by leaching; while in 2013 well above normal precipitation in April and May provided conditions favorable for NO<sub>3</sub> loss by leaching in April, as explained in the previous statement, and by denitrification in May when soils were warm (Fig. 1c). Potential for NO<sub>3</sub> loss would have been expected to be considerably lower in 2012 compared to 2011 as there was near normal spring precipitation in 2011, but below normal spring precipitation in 2012. Extremely dry soil conditions for July thru September 2011, May thru September 2012, and June thru September 2013 would have limited N losses by leaching or denitrification from the soil profile. In addition, the 2012 dry growing season conditions would have limited crop growth and production, including N uptake from the upper soil profile.

### **Nitrogen Band Inorganic-N**

#### ***Application timing by N source***

Application timing, mean across N source, influenced soil NH<sub>4</sub>-N concentrations each year and across years for both fall and spring sampling (Table 3), with NH<sub>4</sub>-N concentrations greater for late than early fall application (Table 4). Across application timing, N source was only significant ( $P \leq 0.10$ ) in the fall of crop year 2011, with AA having higher NH<sub>4</sub>-N concentration than LSM. At spring sampling, N source, mean across application timing, was significant each year and across years, except for 2013, with NH<sub>4</sub>-N concentrations greater with AA than LSM. The interaction of application timing and N source was significant for spring sampling in 2011 and 2012; with greater

$\text{NH}_4\text{-N}$  concentrations for AA than LSM when early fall applied than late applied in 2011, but those differences were opposite in 2012. The greater  $\text{NH}_4\text{-N}$  concentrations with fall sampling compared to spring indicate late fall and early spring nitrification of applied  $\text{NH}_4\text{-N}$ , regardless of application timing. Also, late fall application had greater  $\text{NH}_4\text{-N}$  concentrations than early fall application at either sampling time in all years and across years, indicating delay in nitrification due to colder soils following the late application with less time for nitrification between application and fall sampling.

It is commonly suggested to delay application of N until soil temperatures are  $10^\circ\text{C}$  and falling (Sawyer and Mallarino, 2008) in order to slow nitrification and avoid large conversion to  $\text{NO}_3\text{-N}$ . Rapid nitrification can occur in early fall, and decrease as soil temperatures approach  $10^\circ\text{C}$  with complete inhibition at freezing (Sabey et al., 1956). Mean monthly air and 10-cm soil temperatures were above  $10^\circ\text{C}$  during all years at the time of early fall application, and did not reach freezing until December (Fig. 1a). This would have provided a longer period of nitrification after early fall application compared to late fall application, resulting in the lower  $\text{NH}_4\text{-N}$  concentrations measured while increasing potential for  $\text{NO}_3\text{-N}$  buildup. However, temperature alone cannot explain differences in fall and spring  $\text{NH}_4\text{-N}$  concentrations as the main N form in both sources was  $\text{NH}_4\text{-N}$ . Anhydrous ammonia can slow biological processes such as nitrification since AA is applied as  $\text{NH}_3$ . Ammonia reacts with water, which results in a high pH, while a fraction of AA remains as free ammonia, which can be toxic to microorganisms. Also, the N in LSM was not entirely  $\text{NH}_4\text{-N}$ . Some of the LSM-N is organic-N (Table 2). Therefore,  $\text{NH}_4\text{-N}$  concentration differences between sources could also be due to incomplete mineralization of organic-N present in LSM.



Nitrate-N concentrations were opposite of the  $\text{NH}_4\text{-N}$  concentrations; that is, high  $\text{NH}_4\text{-N}$  concentrations had equivalent low  $\text{NO}_3\text{-N}$  concentrations (Tables 3 and 4) with similar significant treatment effects and interactions as with  $\text{NH}_4\text{-N}$ . This would be expected as conditions suitable for nitrification would result in less  $\text{NH}_4\text{-N}$  remaining. In all years and across years  $\text{NO}_3\text{-N}$  concentrations were higher in the fall than spring when AA and LSM were early fall applied, while concentrations were higher in the spring than fall when late fall applied, regardless of N source (Table 4). Higher  $\text{NO}_3\text{-N}$  concentrations might have been expected in the fall compared to the spring when early fall applied due to potential springtime losses or  $\text{NO}_3\text{-N}$  movement below the 30-cm sampling zone, while lower concentrations would be expected in the fall compared to spring when late fall applied due to temperature effects on nitrification after the late fall application. Consistently,  $\text{NO}_3\text{-N}$  concentrations were lower with AA than LSM in both the fall and spring, especially with early fall application. As found with  $\text{NH}_4\text{-N}$ , initial AA effects may have reduced nitrification and thus  $\text{NO}_3\text{-N}$  concentrations with both application timings. Greater fall  $\text{NO}_3\text{-N}$  concentrations when N was early fall applied would increase the potential for early spring N losses or  $\text{NO}_3$  movement below the sample depth compared to when N was late fall applied, which especially may have occurred in 2013 as total monthly precipitation was well above normal in April and May (Fig. 1c) with the lowest  $\text{NO}_3\text{-N}$  concentrations of any year. There was large change in  $\text{NO}_3\text{-N}$  concentrations from fall to spring with early fall applied LSM. Such difference could be movement similar to that found by Van Es et al. (2006) with  $\text{NO}_3\text{-N}$  loss to shallow groundwater with fall manure application, and by McCormick et al. (1984) who indicated that N from LSM was more subject to overwinter losses than AA.

### ***Application timing by Instinct rate***

As noted before, application timing affected soil  $\text{NH}_4\text{-N}$  concentrations each year and across years for both the fall and spring inorganic-N band sampling (Table 5), with greater  $\text{NH}_4\text{-N}$  concentrations for late than early fall application (Table 6). At fall sampling, Instinct had little effect on soil  $\text{NH}_4\text{-N}$  concentrations at either LSM application time, and with either Instinct rate. Across years, fall  $\text{NH}_4\text{-N}$  concentrations with early application were greater with both Instinct rates compared to no Instinct, while concentrations between Instinct rates were the same with the fall sampling; however, this was not the case with late fall application (Tables 5 and 6).

At early spring sampling, Instinct had more of an effect on  $\text{NH}_4\text{-N}$  concentrations than when sampled in the fall. All years and across years, inclusion of Instinct resulted in greater  $\text{NH}_4\text{-N}$  concentrations than without Instinct, but there was no difference between the low and high Instinct rates. In two years (2011 and 2013), the interaction between Instinct rate and application timing was significant ( $P \leq 0.10$ ), but effects were inconsistent. Ammonium-N concentrations were generally increased with the low and high Instinct rates when early fall applied in 2011, and with late fall application in 2013. It is uncertain why  $\text{NH}_4\text{-N}$  concentrations would not have been influenced by Instinct when sampled in the fall, but was at early spring sampling. One explanation might be that after the late fall application there may not have been adequate time for the inhibitor to express a difference in nitrification control by the time of late fall sampling, but there should have been a difference if the inhibitor was effective when early fall applied; that is, greater late fall  $\text{NH}_4\text{-N}$  concentrations. Whatever the reason, Instinct did apparently slow nitrification as there was an increase in  $\text{NO}_3\text{-N}$  concentration at early spring

sampling. In 2013 only, did the higher Instinct rate result in higher spring  $\text{NH}_4\text{-N}$  concentration than the low Instinct rate. Compared to use of Instinct, fall LSM application timing had a larger and more consistent influence on soil  $\text{NH}_4\text{-N}$  concentrations, especially in the fall, indicating that a late fall application would be more effective in retaining  $\text{NH}_4$  within the injection band than use of Instinct.

Mean monthly air and 10-cm soil temperatures at the time of, and after, LSM application would influence duration and rate of nitrification, and could explain the significant interaction responses at the fall sampling across years; that is, higher  $\text{NH}_4\text{-N}$  concentration due to Instinct with early but not late application. This is a reason why Instinct use would be considered, to prolong  $\text{NH}_4$  retention in the soil and reduce potential  $\text{NO}_3\text{-N}$  losses when applied in early fall. Ferrel (2012) proposed that delay in nitrification inhibition with Instinct could occur as a result of delayed nitrapyrin release from the microcapsule. Also, relatively high soil organic matter (SOM) levels at each study site (Table 1) may have absorbed nitrapyrin as it was released from the microcapsule (Goring, 1962a; Briggs, 1975; Keeney, 1980) and therefore was less effective, or ineffective, until released from decomposed SOM (Laskowski and Bidlack, 1977). Either issue may have delayed the effect of Instinct on nitrification. Hence, the no  $\text{NH}_4\text{-N}$  concentration difference in late fall, but a positive effect in the early spring. Powell and Prosser (1986) also suggest that application timing of a nitrification inhibitor has a greater inhibitory affect when nitrifying bacteria are active compared to when temperatures have slowed activity.

Soil  $\text{NO}_3\text{-N}$  concentrations were generally opposite of the  $\text{NH}_4\text{-N}$  concentrations, that is, greater  $\text{NH}_4\text{-N}$  concentrations had equivalent lower  $\text{NO}_3\text{-N}$  concentrations (Tables

5 and 6), with similar application timing and Instinct effects as with  $\text{NH}_4\text{-N}$ . This would be expected as conditions suitable for nitrification, or without an inhibitor, would result in less  $\text{NH}_4\text{-N}$  remaining. Interestingly, the magnitude and consistency across years of increase in  $\text{NO}_3\text{-N}$  concentrations with late fall compared to early fall LSM application was greater than that for use of Instinct compared to no Instinct. Nitrate-N concentrations at fall band sampling in 2011, 2012, and across years,  $\text{NO}_3\text{-N}$  concentrations were lower with inclusion of Instinct at early fall LSM application, and were lowest with the high Instinct rate. Also, there was no Instinct effect when late fall applied. These are the same results as for  $\text{NH}_4\text{-N}$  concentrations where there was a positive effect of Instinct, but for  $\text{NH}_4\text{-N}$  there was no Instinct rate effect. Results were similar at spring band sampling as in the fall. In 2011, Instinct resulted in lower  $\text{NO}_3\text{-N}$  concentrations with both application times, but in 2013 Instinct resulted in lower  $\text{NO}_3\text{-N}$  concentrations with late fall application only. In all years and across years, Instinct resulted in lower  $\text{NO}_3\text{-N}$  concentrations. The lowest  $\text{NO}_3\text{-N}$  concentrations were found in 2012 and across years with the high Instinct rate. This indicates an Instinct rate effect that was not present in the soil  $\text{NH}_4\text{-N}$  concentrations. As noted for the  $\text{NH}_4\text{-N}$ , temperature can influence nitrification rate, as well as duration of active nitrification. Use of a nitrification inhibitor could change the  $\text{NH}_4\text{:NO}_3$  relation, potentially helping to reduce spring N losses. The magnitude of effect on nitrification (more  $\text{NH}_4$  and less  $\text{NO}_3$ ), however, was larger and more consistent when LSM application was delayed until late fall than with use of Instinct.

## **Late Spring Soil Inorganic-N**

### ***Application timing by N source***

Applied N was nitrified by the time of late spring sampling as  $\text{NH}_4\text{-N}$  concentrations in both sampling depths were near the control concentrations, regardless of application timing or N source (Table 7). At both depths, there were few source or timing effects (Table 3), and in those instances  $\text{NH}_4\text{-N}$  concentrations were low and differences insignificant. At the 0- to 30-cm depth in 2013, late fall AA had significantly higher  $\text{NH}_4\text{-N}$  concentrations than early fall or with LSM, but differences were minor. This may be a result of slower nitrification rates due to below normal temperatures that spring.

Nitrate-N concentrations in the 0- to 30-cm depth were below the critical concentration range of 20 to 25  $\text{mg kg}^{-1}$  for the late-spring test for soil  $\text{NO}_3$  (LSNT) (Blackmer et al., 1997) in all years, except 2012, for the late fall AA and LSM applications (Table 7). In 2012, the early fall AA and LSM application had LSNT values 2  $\text{mg kg}^{-1}$  below the 20  $\text{mg kg}^{-1}$  minimum critical range. Higher  $\text{NO}_3\text{-N}$  concentrations were also found at the 30- to 60-cm depth in 2012 for all N applications. Movement of  $\text{NO}_3\text{-N}$  below the application zone was evident by greater concentrations in the 30- to 60-cm depth in two of three years and across years. This did not occur in 2013, as the potential that the high precipitation in April and May (Fig. 1c) moved  $\text{NO}_3\text{-N}$  below the 60-cm depth or there was significant denitrification. Denitrification or leaching was also possible in 2011. Precipitation in May was well below normal in 2012, and thus may have resulted in the adequate  $\text{NO}_3\text{-N}$  concentrations that year. In all but one year, and across years,  $\text{NO}_3\text{-N}$  concentrations at both soil depths were greater with late fall

application than early fall, regardless of N source. For the 30- to 60-cm depth,  $\text{NO}_3\text{-N}$  concentrations were greater with AA than LSM in two years and across years. This did not occur with the 0- to 30-cm depth. The frequency of low  $\text{NO}_3\text{-N}$  concentrations, and the below LSNT critical level in all but one instance, indicates the fall N applications were at risk of N loss. This also indicates the N rate applied was not adequate to meet full corn N requirements. While that is a management issue for field production, it would be a benefit for the study as it allowed for expression of differences due to treatments (timing, N source, or Instinct).

#### ***Application timing by Instinct rate***

Ammonium-N concentrations in both sampling depths were near the control concentrations, regardless of LSM application timing or Instinct rate (Table 8). In 2011 and across years for Instinct rate, and across years for the interaction of Instinct rate and application timing, were there any effects on  $\text{NH}_4\text{-N}$  concentrations in the 0- to 30-cm depth (Table 5). In those cases, Instinct had only a small and inconsistent effect. There were no LSM application timing or Instinct rate effects on  $\text{NH}_4\text{-N}$  concentrations in the 30- to 60-cm depth.

Late spring  $\text{NO}_3\text{-N}$  concentrations were within the LSNT critical range in 2012 only, as described before (Table 8). Concentrations were also the highest that year in the 30- to 60-cm depth, again as described before. Instinct had only a small and inconsistent effect on soil  $\text{NO}_3\text{-N}$  concentrations in the 0- to 30-cm depth (Table 8). Use of Instinct increased concentrations across both application times in 2011, 2013, and across years, with no difference due to increased Instinct rate. However, use of Instinct did not improve  $\text{NO}_3\text{-N}$  concentrations to the LSNT critical level. Delaying LSM application to

late fall resulted in greater  $\text{NO}_3\text{-N}$  concentrations in 2012, 2013, and across years with the increase in 2012 enough to have the LSNT value within the critical range. At the 30- to 60-cm depth, late fall applied LSM with the high Instinct rate resulted in decreased  $\text{NO}_3\text{-N}$  concentrations in 2011. Across application times in 2013, only the low Instinct rate resulted in a  $\text{NO}_3\text{-N}$  concentration increase, although small. In two of three years, and across years, the late fall LSM application had higher  $\text{NO}_3\text{-N}$  concentrations in the 30- to 60-cm depth than early fall application. Again, the concentration differences were small. Kyveryga and Blackmer (2013) found no significant difference in LSNT values between LSM without and with Instinct at a single rate of  $1 \text{ L ha}^{-1}$ , a rate that was below the low rate ( $2.56 \text{ L ha}^{-1}$ ) used in this study. The higher  $\text{NO}_3\text{-N}$  concentration in the 0- to 30-cm depth at late spring sampling with the high Instinct rate in 2011, 2013, and across years, corresponds to the higher  $\text{NH}_4\text{-N}$  concentrations at the same depth found at the time of spring N band sampling. This supports that Instinct does act as a nitrification inhibitor. However, soil  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  sampling indicates that Instinct did not slow nitrification for an extended period of time, which allowed nitrification to be near, or at completion, in the spring by late spring sampling time.

### **Corn Canopy Sensing**

#### ***Application timing by N source***

Mid-vegetative corn canopy NDVI (V10 growth stage) was significant ( $P \leq 0.10$ ) for application timing in 2013 and across years, mean across N source, and N source in 2011 and 2012, mean across application timing (Table 9). Canopy NDVI at either application timing, or with either N source, was higher than the control in all years and across years (Table 10), indicating corn response to N applied as AA and LSM. In 2013

and across years, canopy NDVI was higher with late fall application than early fall application, regardless of N source. The NDVI difference due to application timing in 2013 may have been due to the extremely wet conditions during April and May of that year affecting N supply from both N sources. The across year application timing difference also reflects the consistent, but non-significant, NDVI difference between timing in 2011 and 2012. Corn canopy NDVI response to N source was inconsistent between 2011 and 2012, but was not significant in 2013 and across years. In general, differences in canopy NDVI were not large, reflecting similar N supply at the mid-vegetative growth period for both N sources.

The larger canopy NDVI when N was late fall applied corresponds to the greater  $\text{NH}_4\text{-N}$  concentrations found at fall and spring N band sampling, and greater  $\text{NO}_3\text{-N}$  concentrations at late spring sampling. In 2011, the larger canopy NDVI with AA compared to LSM corresponds to the higher  $\text{NH}_4\text{-N}$  concentrations in the fall and spring N band sampling, but does not relate to the late spring sampling results.

#### ***Application timing by Instinct rate***

Corn canopy NDVI was significantly ( $P \leq 0.10$ ) affected by application timing in 2011, 2013, and across years, mean across Instinct rate, while Instinct rate significantly affected canopy NDVI in 2011 only, mean across application timing (Table 9). Canopy NDVI with either application timing or Instinct rate was larger than the control in all years and across years (Table 10), again indicating corn response to applied N. Similar to the application timing by N source analysis, NDVI values were larger when late fall applied compared to early fall applied in two of three years and across years. In 2011, inclusion of Instinct increased canopy NDVI, with the same response for both Instinct



rates. In no other year or across years was there an effect on canopy NDVI from application of Instinct. These results indicate that Instinct, despite some influence on soil inorganic-N in the fall and spring, did not have a substantial influence on corn canopy development. It is possible that the rate of total applied N was high enough, even with soil inorganic-N differences due to Instinct, to supply adequate N at the mid-vegetative growth stage, or differences in soil  $\text{NH}_4\text{-N}$  or  $\text{NO}_3\text{-N}$  concentrations, due to Instinct application, were not substantial or consistent enough to avoid deficient N or reflect differences in canopy sensing.

Kyveryga and Blackmer (2013), however, found lower green band canopy reflectance when Instinct was added to LSM compared to LSM alone, indicating greater canopy biomass because of greater absorbance of green light by chlorophyll. Positive canopy NDVI responses to Instinct would indicate effectiveness in inhibiting nitrification long enough to prevent significant N losses prior to plant uptake, resulting in improved canopy biomass. Larger canopy NDVI values for early fall LSM with the high Instinct rate would be expected since the high rate of Instinct should have a longer inhibitory effect, but that did not occur as application of the low Instinct rate, and no Instinct, had the same canopy NDVI in two of three years. Also, there was no difference in NDVI between Instinct rates in the one year (2011) where Instinct had a significant effect on NDVI. It is unknown why the lack of Instinct rate response occurred, as well as, the general lack of Instinct effect on canopy NDVI. It could be due to delayed release of nitrapyrin from the microcapsule (as indicated by Ferrel, 2012), low, or short-term, nitrification control due to issues with nitrapyrin effectiveness (SOM interaction, degradation, volatilization), or sufficient nitrapyrin concentration as described by

previous research (Goring, 1962a, 1962b; Redemann et al., 1964; Briggs, 1975; Herlihy and Quirke, 1975; McCall and Swann, 1978; Hendrickson and Keeney, 1979; Keeney, 1980). The potential for these issues are supported by the small differences in inorganic-N measured at late spring sampling.

## **Corn Grain Yield**

### ***Application timing by N source***

No significant ( $P \leq 0.10$ ) corn grain yield responses were found for application timing, mean across N source, but significant grain yield responses were found in 2011, 2012, and across years for N source, mean across application timing (Table 9). Application of N, regardless of source, had higher grain yields than the control (Table 11). As with canopy NDVI, this provides evidence that the sites each year were responsive to applied N from AA or LSM. Mean grain yield was higher for AA than LSM in two of three years (2011 and 2013) and across years, regardless of application timing. The interaction between N source and fall application timing was significant across years, with yield being the highest with AA, but similar between early and late application. Grain yield with LSM was lower with early fall application than late fall application. These results indicate that LSM was more subject to potential N losses than AA, or the LSM-N was not as crop available. Rate of total LSM-N should not have been an issue as LSM-N rates, calculated from analyzed LSM samples collected at application (Table 2), were not below the goal rate in the two years when yield was lower (the late fall LSM-N was below the rate goal, but did not have lower grain yield). Also, although not expected, yield was similar between the early and late fall AA application.

The grain yield responses are somewhat contradictory to previous research that found LSM to be an adequate substitute to fertilizer N (Kwaw-Mensah and Al-Kaisi, 2006; McLaughlin et al., 2000; Park et al., 2010; Woli et al., 2013). Weather throughout the study did not cause large year to year variation in yields. Fall and spring N band inorganic-N, late spring inorganic-N, and canopy NDVI data collected during this study does correspond to the higher yields found with AA compared to LSM. In two of three years and across years, higher concentrations of  $\text{NH}_4\text{-N}$  were found with AA, at spring N band sampling, while there was higher  $\text{NO}_3\text{-N}$  concentrations at both N band sampling times in all years and across years with LSM. The higher  $\text{NO}_3\text{-N}$  concentration in the LSM band increased the potential for spring N losses, which was found at the late spring sampling, as  $\text{NO}_3\text{-N}$  concentration was generally greater with AA at either depth. Greater inorganic-N supplied by AA longer into the growing season was also measured with greater canopy biomass in two of three years and across years. The fraction of LSM-N as organic-N may have influenced results. Having mineralizable organic-N could allow slower release of  $\text{NH}_4\text{-N}$ ; thus, potentially avoiding early spring N losses. However, if organic-N in LSM is rapidly mineralized, then there would be no change in potential N supply or loss potential.

Economic losses would have occurred if LSM were used instead of AA. Assuming the cost per unit N is the same for AA or LSM (Leibold and Olsen, 2006), and weighted-average grain prices in 2011, 2013, and across years were 204, 271, and 240 \$  $\text{Mg}^{-1}$  (ERS, 2014), respectively, deciding to apply AA would have increased profits by 305, 298, and 240 \$  $\text{ha}^{-1}$  in 2011, 2013, and across years, respectively, compared to applying LSM alone. In studies by Kwaw-Mensah and Al-Kaisi (2006) and Park et al.

(2010), based on yield response to LSM and fertilizer, substituting manure for fertilizer would have been economically advantageous. While in this study, yield response favored application of AA fertilizer instead of LSM. Additionally, when averaged across years, deciding to delay LSM application to late fall increased the economic return of LSM by \$144 ha<sup>-1</sup>. As others have shown, delaying N application to late fall can improve profit, while better managing fall resources (Bundy, 1986; Randall et al., 1999).

#### ***Application timing by Instinct rate***

Instinct rate, mean across application times, was the only significant ( $P \leq 0.10$ ) effect on corn grain yield response, which was across years only (Table 9). Grain yield differences due to Instinct were inconsistent, with higher yield with Instinct at either rate. However, there was no difference between LSM with the high Instinct rate and LSM without Instinct (Table 11). The low Instinct rate had consistently higher grain yields, but was only significantly higher than LSM without Instinct across years. It is unknown why the high Instinct rate would have resulted in inconsistent yields compared to either the low Instinct rate or no Instinct. Also, there clearly was no advantage to using the higher Instinct rate with either the early or late fall application timing. As stated previously, AA had higher yield than LSM at both application times, including the wetter 2011 and 2013 years. Although no direct comparison was made, the higher yield with AA compared to LSM was not eliminated when Instinct was included with LSM at either rate.

A study in Minnesota found no significant yield response when LSM with or without nitrapyrin was averaged across three application times and two application rates (Randall et al., 1999). McCormick et al. (1984) found inclusion of nitrapyrin (at a rate

much higher than recommended) with fall applied LSM improved grain yields by an average of 46%. In a study where Instinct was applied at  $1 \text{ L ha}^{-1}$  (which was less than the low Instinct rate used in this study) with fall LSM, no grain yield response was found in a year with normal precipitation; while a  $0.15 \text{ Mg ha}^{-1}$  yield increase was found during a year when precipitation was above normal (Kyveryga and Blackmer, 2013). Weather throughout this study did not cause large year to year variation in yields. As explained with canopy NDVI, it is unclear why yields for LSM with the low Instinct rate were equal to LSM with the high Instinct rate; especially why this would occur with early fall application as  $\text{NH}_4\text{-N}$  concentrations at spring N band sampling and  $\text{NO}_3\text{-N}$  concentrations at late spring sampling were generally higher with the high Instinct rate.

Economically, the decision to use Instinct at the low rate with LSM compared to LSM without Instinct would have increased profit return by  $\$83 \text{ ha}^{-1}$  across years. This is assuming the added cost for Instinct at the low rate would be  $\$37 \text{ ha}^{-1}$  and mean grain price across years was  $\$240 \text{ Mg}^{-1}$  (ERS, 2014). Yield with the high Instinct rate was not statistically different than LSM without Instinct. This would have resulted in reduced across year returns by  $\$74 \text{ ha}^{-1}$  if LSM with the high Instinct rate was applied compared to LSM without Instinct, assuming the inclusion of Instinct at the high rate would be double the low rate cost. Compared to the low Instinct rate, the added cost for the additional Instinct at the high Instinct rate would be  $\$37 \text{ ha}^{-1}$  with no profit return, as there was no yield increase for the high rate compared to the low Instinct rate. Kyveryga and Blackmer (2013) concluded from their study that the probability of economic benefits would be low as a result of limited Instinct effects during winter and early spring in a year with above normal precipitation. This study found increased profitability with

inclusion of Instinct at the low rate with LSM across years when spring precipitation was normal, below normal, and above normal, indicating good probability of increased profitability for inclusion of Instinct with fall applied LSM.

## **CONCLUSIONS**

Corn response to fall AA or LSM application was greater with late fall application compared to early fall application, especially for LSM. This supports the currently suggested practice of waiting to apply N until later in the fall as soils begin to cool. Delaying fall N application has the potential to help limit spring N losses, increase grain yields, and provide for greater return to N application. This study found that of the two common fall-applied N sources, AA or LSM, AA typically had higher  $\text{NH}_4\text{-N}$  concentrations within the injected N band, lower  $\text{NO}_3\text{-N}$  concentrations in the fall and early spring, and higher corn grain yield. Use of Instinct with LSM improved spring inorganic-N concentrations and corn yield. However, the response was not consistent, and early and late fall application grain yield was not fully comparable to AA application. With a favorable grain to Instinct price relationship, Instinct at the low rate provided greater profit return than applying LSM without Instinct. The additional cost of Instinct at the high rate resulted in a negative return when compared to the low Instinct rate, since no significant yield difference was found between Instinct rates. However, due to higher yields with AA, even with Instinct added to LSM, return to N application was greater with AA. The decision on when to apply fall N, the source of N, and inclusion of a nitrification inhibitor is difficult. Weather conditions after application, especially in the spring, determine the rate of  $\text{NO}_3$  formation and if significant N losses might impact N

supply. Therefore, one practice may be more efficient than another, especially when deciding if the added cost of a nitrification inhibitor would be worthwhile.

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Table 1. Site-year soil information and initial soil test results for samples collected at the 0- to 15-cm depth prior to treatment application.

Series	Subgroup	Texture	pH	SOM <sup>†</sup> g kg <sup>-1</sup>	STP <sup>‡</sup> ----- mg kg <sup>-1</sup> -----	STK <sup>‡</sup>
<u>2011</u>						
Clarion	Typic Hapludolls	Loam	6.5	43	11 (L)	130 (L)
Nicollet	Aquic Hapludolls	Clay loam				
<u>2012</u>						
Nicollet	Aquic Hapludolls	Clay loam	6.7	53	34 (VH)	162 (Opt)
Canisteo	Typic Endoaquolls	Clay loam				
<u>2013</u>						
Clarion	Typic Hapludolls	Loam	5.6	45	13 (L)	162 (Opt)
Nicollet	Aquic Hapludolls	Clay loam				

<sup>†</sup> SOM, soil organic matter.

<sup>‡</sup> Mehlich-3 soil test P (STP) and soil test K (STK).

<sup>§</sup> Soil test interpretation category for L, low; O, optimum; or VH, very high (Sawyer et al., 2011).

Table 2. Liquid swine manure (LSM) nutrient analysis of samples collected pre-application and during application for each study year, and LSM total-N applied.

Application	LSM analysis					LSM application <sup>‡</sup>	
	Moisture	TN <sup>†</sup>	NH <sub>4</sub> -N	P	K	Rate	TN
	%	----- mg L <sup>-1</sup> -----				L ha <sup>-1</sup>	kg N ha <sup>-1</sup>
<u>Pre-application</u>							
5 Oct. 2010	98	4671	3713	838	3473	33,700	---
5 Nov. 2010	98	5269	3832	1557	3832	30,000	---
4 Oct. 2011	95	6467	4551	3114	5030	24,300	---
4 Nov. 2011	96	6826	4910	1916	4671	23,400	---
1 Oct. 2012	97	6467	5629	1557	4311	24,300	---
1 Nov. 2012	97	6467	5629	1557	4311	24,300	---
<u>During application</u>							
5 Oct. 2010	98	5150	3952	958	3713	33,700	174
5 Nov. 2010	98	4551	3593	473	3832	30,000	137
4 Oct. 2011	96	5988	4551	1677	4551	24,300	146
4 Nov. 2011	97	6347	5030	1916	4192	23,400	149
1 Oct. 2012	97	6707	5629	1557	4311	24,300	163
1 Nov. 2012	97	6347	5150	1078	4431	24,300	154

<sup>†</sup> TN, total N.

<sup>‡</sup> The TN application goal was 157 kg N ha<sup>-1</sup>.

Table 3. Fall application timing and N source statistical significance of soil inorganic-N concentrations for samples collected from the N application band and in late spring.

Source	N band sampling (0-30 cm)				Late spring sampling			
	Fall		Spring		0-30 cm		30-60 cm	
	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N	NO <sub>3</sub> -N
	----- <i>P</i> > F -----							
	<u>2011</u>							
Timing (T)	< 0.001	0.004	< 0.001	< 0.001	0.604	0.067	0.850	0.008
Source (S)	0.003	0.014	0.014	0.074	0.375	0.691	0.477	0.031
T x S	0.928	0.013	0.055	0.082	0.805	0.282	0.624	0.434
	<u>2012</u>							
Timing (T)	0.030	< 0.001	0.023	0.001	0.718	0.006	0.056	0.711
Source (S)	0.827	0.014	0.069	< 0.001	0.293	0.959	1.000	0.003
T x S	0.104	0.084	0.068	0.001	0.718	0.878	0.302	0.901
	<u>2013</u>							
Timing (T)	0.008	< 0.001	0.005	0.003	0.146	0.012	0.479	0.009
Source (S)	0.540	0.075	0.228	0.001	0.058	0.291	0.479	0.889
T x S	0.549	0.139	0.695	0.187	0.092	0.904	1.000	0.339
	<u>Across years</u>							
Timing (T)	< 0.001	< 0.001	< 0.001	0.530	0.283	< 0.001	0.727	0.004
Source (S)	0.928	< 0.001	0.033	0.001	0.122	0.646	0.366	0.003
T x S	0.281	0.003	0.975	0.224	0.167	0.676	0.444	0.353

Table 4. Fall application timing and N source main effect and interaction means for the N band sampling inorganic-N concentrations.

	2011			2012			2013			Across years		
	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean
----- mg kg <sup>-1</sup> -----												
	<u>Fall NH<sub>4</sub>-N</u>											
<u>Source</u> <sup>†</sup>												
AA	74 <sup>‡</sup>	179	127A <sup>§</sup>	32	42	37 <sup>‡</sup>	68	167	118	58	122	90
LSM	30	136	83B	12	68	40	69	212	140	41	142	91
Mean	52B	158A		22B	55A		68B	189A		49B	132A	
Control <sup>¶</sup>			5			3			4			3
	<u>Spring NH<sub>4</sub>-N</u>											
AA	55b <sup>#</sup>	112a	83A	4b	25a	14A	32	129	80	30	88	59A
LSM	13c	105a	59B	4b	7b	5B	11	89	50	9	67	38B
Mean	34B	108A		4B	16A		22B	109A		20B	78A	
Control			4			3			4			3
	<u>Fall NO<sub>3</sub>-N</u>											
AA	33b	17b	25B	25b	6c	16B	56	13	34B	40b	12c	26B
LSM	90a	16b	53A	49a	11c	30A	85	16	51A	72a	15c	44A
Mean	62A	17B		37A	9B		71A	15B		56A	13B	
Control			4			6			5			5
	<u>Spring NO<sub>3</sub>-N</u>											
AA	34b	23c	28B	23b	22b	23B	10	16	13B	22	20	21B
LSM	45a	23c	34A	26b	51a	39A	19	32	25A	30	35	33A
Mean	39A	23B		25B	37A		14B	24A		26	28	
Control			6			7			3			5

<sup>†</sup> Anhydrous ammonia, AA and liquid swine manure, LSM.

<sup>‡</sup> Main effect and interaction means without a letter within the same year are not significantly different ( $P \leq 0.10$ , Table 3).

<sup>§</sup> Main effect means with a different upper case letter within the same year are significantly different ( $P \leq 0.10$ , Table 3).

<sup>¶</sup> Control not included in the statistical analysis.

<sup>#</sup> Interaction means with a different lower case letter within the same year are significantly different ( $P \leq 0.10$ , Table 3).

Table 5. Fall application timing and Instinct rate with liquid swine manure statistical significance of soil inorganic-N concentrations for samples collected from the N application band and in late spring.

Source	N band sampling (0-30 cm)				Late spring sampling			
	Fall		Spring		0-30 cm		0-60 cm	
	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N	NO <sub>3</sub> -N
	----- <i>P</i> > F -----							
	<u>2011</u>							
Timing (T)	0.015	< 0.001	0.007	< 0.001	0.788	0.118	0.766	0.004
Instinct rate (IR)	0.735	< 0.001	0.088	< 0.001	0.046	0.001	0.540	0.286
T x IR	0.143	< 0.001	< 0.001	0.016	0.134	0.717	0.977	0.059
	<u>2012</u>							
Timing (T)	0.006	< 0.001	0.099	0.001	0.718	< 0.001	0.369	0.362
Instinct rate (IR)	0.882	0.023	0.081	0.004	0.410	0.167	0.540	0.837
T x IR	0.089	0.013	0.584	0.147	0.410	0.151	0.255	0.713
	<u>2013</u>							
Timing (T)	< 0.001	< 0.001	< 0.001	0.663	0.256	< 0.001	0.295	< 0.001
Instinct rate (IR)	0.526	0.322	0.007	0.002	0.348	0.019	0.525	0.086
T x IR	0.290	0.654	0.096	< 0.001	0.348	0.288	0.525	0.420
	<u>Across years</u>							
Timing (T)	< 0.001	< 0.001	< 0.001	0.959	0.780	< 0.001	0.428	< 0.001
Instinct rate (IR)	0.706	0.004	0.006	< 0.001	0.074	0.003	0.176	0.218
T x IR	0.037	0.017	0.121	0.292	0.074	0.945	0.740	0.146



Table 6. Fall application timing and Instinct rate with liquid swine manure main effect and interaction means for the N band sampling inorganic-N concentrations.

	2011			2012			2013			Across years		
	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean
----- mg kg <sup>-1</sup> -----												
<u>Fall NH<sub>4</sub>-N</u>												
<u>Instinct rate</u> <sup>†</sup>												
0	30 <sup>‡</sup>	136	83 <sup>‡</sup>	12c <sup>§</sup>	68a	40	69	212	140	37c	137a	87
2.56	70	85	78	34bc	35bc	35	122	215	168	75b	113a	94
5.12	72	113	93	20c	52ab	36	138	194	166	76b	119a	98
Mean	58B <sup>¶</sup>	112A		22B	52A		109B	207A		62B	123A	
Control <sup>#</sup>			5			3			4			3
<u>Spring NH<sub>4</sub>-N</u>												
0	13c	105a	59B	4	7	5B	11d	89bc	50B	9	67	38B
2.56	83ab	62b	73AB	15	24	20A	45cd	114b	80B	48	68	57A
5.12	81b	83ab	82A	10	28	19A	44d	190a	117A	45	100	73A
Mean	59B	84A		10B	19A		33B	131A		34B	78A	
Control			4			3			4			3
<u>Fall NO<sub>3</sub>-N</u>												
0	88a	14cd	51A	49a	11c	30A	85	16	51	71a	14d	43A
2.56	34b	10d	22B	32b	11c	21B	80	12	46	52b	11d	31B
5.12	22c	9d	16C	23b	13c	18B	65	10	38	38c	11d	25B
Mean	48A	11B		35A	12B		77A	13B		54A	12B	
Control			4			6			5			5
<u>Spring NO<sub>3</sub>-N</u>												
0	45a	23b	34A	26	51	39A	19bc	32a	25A	30	35	33A
2.56	22b	11c	17B	39	51	45A	22b	15cd	19B	28	26	27B
5.12	18b	11c	15B	24	31	27B	20bc	11d	15B	21	18	19C
Mean	28A	15B		30B	44A		20	19		26	26	
Control			6			7			3			5

<sup>†</sup> Instinct rate, L ha<sup>-1</sup>.

<sup>‡</sup> Main effect and interaction means without a letter within the same year are not significantly different ( $P \leq 0.10$ , Table 4).

<sup>§</sup> Interaction means with a different lower case letter within the same year are significantly different ( $P \leq 0.10$ , Table 4).

<sup>¶</sup> Main effect means with a different upper case letter within the same year are significantly different ( $P \leq 0.10$ , Table 4).

<sup>#</sup> Control not included in the statistical analysis.

Table 7. Fall application timing and N source main effect and interaction means for inorganic-N concentrations collected in late spring.

	2011			2012			2013			Across years		
	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean
----- mg kg <sup>-1</sup> -----												
	<u>0-30 cm NH<sub>4</sub>-N</u>											
Source <sup>†</sup>												
AA	2.6 <sup>‡</sup>	2.2	2.4 <sup>‡</sup>	5.0	5.3	5.1	5.0b <sup>§</sup>	8.0a	6.5A <sup>¶</sup>	4.2	5.1	4.7
LSM	2.9	2.7	2.8	4.8	4.8	4.8	4.8b	4.5b	4.6B	4.1	4.0	4.1
Mean	2.7	2.5		4.9	5.0		4.9	6.3		4.2	4.6	
Control <sup>#</sup>			2.3			4.3			4.3			3.6
	<u>30-60 cm NH<sub>4</sub>-N</u>											
AA	1.2	1.3	1.3	2.8	3.5	3.1	3.8	3.5	3.6	2.6	2.8	2.7
LSM	1.2	0.9	1.0	3.0	3.3	3.1	3.5	3.3	3.4	2.6	2.5	2.5
Mean	1.2	1.1		2.9B	3.4A		3.6	3.4		2.6	2.6	
Control			4.3			3.0			3.5			3.6
	<u>0-30 cm NO<sub>3</sub>-N</u>											
AA	3.9	6.7	5.3	17.8	25.5	22.1	7.3	10.3	8.8	9.6	14.5	12.1
LSM	4.5	5.3	4.9	18.3	26.3	22.3	6.0	9.3	7.6	9.6	13.6	11.6
Mean	4.2B	6.0A		18.0B	26.4A		6.6B	9.8A		9.6B	14.0A	
Control			2.0			12.0			4.5			6.2
	<u>30-60 cm NO<sub>3</sub>-N</u>											
AA	12.1	15.6	13.8A	16.5	16.0	16.3A	7.0	9.0	8.0	11.9	13.5	12.7A
LSM	7.5	13.2	10.4B	12.5	12.3	12.4B	6.3	10.0	8.1	8.8	11.8	10.3B
Mean	9.8B	14.4A		14.5	14.2		6.6B	9.5A		10.3B	12.7A	
Control			2.8			5.5			3.3			3.8

<sup>†</sup> Anhydrous ammonia, AA and liquid swine manure, LSM.

<sup>‡</sup> Main effect and interaction means without a letter within the same year are not significantly different ( $P \leq 0.10$ , Table 3).

<sup>§</sup> Interaction means with a different lower case letter within the same year are significantly different ( $P \leq 0.10$ , Table 3).

<sup>¶</sup> Main effect means with a different upper case letter within the same year are significantly different ( $P \leq 0.10$ , Table 3).

<sup>#</sup> Control not included in the statistical analysis.

Table 8. Fall application timing and Instinct rate with liquid swine manure main effect and interaction means for inorganic-N concentrations collected in late spring.

	2011			2012			2013			Across years		
	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean
----- mg kg <sup>-1</sup> -----												
<u>0-30 cm NH<sub>4</sub>-N</u>												
<u>Instinct rate</u> <sup>†</sup>												
0	2.9 <sup>‡</sup>	2.7	2.8A <sup>§</sup>	4.8	4.8	4.8 <sup>‡</sup>	4.8	4.5	4.6	4.1b <sup>¶</sup>	4.0b	4.1B
2.56	2.4	1.4	1.9B	5.3	4.8	5.0	4.8	5.3	5.0	4.1b	3.8b	4.0B
5.12	2.7	3.5	3.1A	5.0	5.3	5.1	4.8	5.5	5.1	4.1b	4.8a	4.4A
Mean	2.6	2.5		5.0	4.9		4.8	5.1		4.1	4.2	
Control <sup>#</sup>			2.3			4.3			4.3			3.6
<u>30-60 cm NH<sub>4</sub>-N</u>												
0	1.2	1.0	1.0	3.0	3.3	3.1	3.5	3.3	3.4	2.6	2.5	2.5
2.56	1.7	1.7	1.7	3.5	3.3	3.4	3.5	4.8	4.1	2.9	3.2	3.1
5.12	1.8	1.5	1.6	3.0	3.5	3.3	3.5	4.3	3.9	2.8	3.1	2.9
Mean	1.5	1.4		3.2	3.3		3.5	4.1		2.7	2.9	
Control			4.3			3.0			3.5			3.6
<u>0-30 cm NO<sub>3</sub>-N</u>												
0	4.5	5.3	4.9C	18.3	26.3	22.3	6.0	9.3	7.6B	9.6	13.6	11.6B
2.56	7.0	8.1	7.5B	19.3	22.5	20.9	7.5	14.0	10.8A	11.3	14.9	13.1A
5.12	8.5	10.9	9.7A	21.0	25.5	23.3	7.3	12.5	9.9A	12.3	16.3	14.3A
Mean	6.7	8.1		19.5B	24.8A		6.9B	11.9A		11.0B	14.9A	
Control			2.0			12.0			4.5			6.2
<u>30-60 cm NO<sub>3</sub>-N</u>												
0	7.5b	13.2a	10.4	12.5	12.3	12.4	6.3	10.0	8.1B	8.8	11.8	10.3
2.56	9.1b	13.2a	11.2	13.0	11.5	12.3	7.5	11.3	9.4A	9.9	12.0	10.9
5.12	9.4b	9.2b	9.3	12.0	11.8	11.9	7.3	9.8	8.5AB	9.5	10.2	9.9
Mean	8.7B	11.9A		12.5	11.8		7.0B	10.3A		9.4B	11.3A	
Control			2.8			5.5			3.3			3.8

<sup>†</sup> Instinct rate, L ha<sup>-1</sup>.

<sup>‡</sup> Main effect and interaction means without a letter within the same year are not significantly different ( $P \leq 0.10$ , Table 4).

<sup>§</sup> Main effect means with a different upper case letter within the same year are significantly different ( $P \leq 0.10$ , Table 4).

<sup>¶</sup> Interaction means with a different lower case letter within the same year are significantly different ( $P \leq 0.10$ , Table 4).

<sup>#</sup> Control not included in the statistical analysis.

Table 9. Fall application timing and N source, and fall application timing and Instinct rate with liquid swine manure, plant response statistical significance.

Source	2011	2012	2013	Across years
----- $P > F$ -----				
<u>Canopy NDVI</u>				
Timing (T)	0.175	0.236	0.018	0.005
Source (S)	0.093	0.035	0.623	0.520
T x S	0.832	0.183	0.699	0.899
Timing (T)	0.024	0.321	0.040	0.007
Instinct rate (IR)	0.003	0.590	0.683	0.223
T x IR	0.802	0.229	0.653	0.652
<u>Grain yield</u>				
Timing (T)	0.748	0.410	0.820	0.388
Source (S)	0.004	0.372	0.014	< 0.001
T x S	0.408	0.132	0.483	0.060
Timing (T)	0.327	0.215	0.850	0.352
Instinct rate (IR)	0.207	0.197	0.723	0.086
T x IR	0.161	0.223	0.741	0.117

Table 10. Fall application timing and N source, and fall application timing and Instinct rate with liquid swine manure, corn canopy normalized difference vegetative index (NDVI) main effect and interaction means.

	2011			2012			2013			Across years		
	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean
<u>Application timing by N source</u>												
<u>Source</u> <sup>†</sup>												
AA	0.732 <sup>‡</sup>	0.738	0.735A <sup>§</sup>	0.738	0.738	0.738B	0.651	0.686	0.669 <sup>‡</sup>	0.707	0.721	0.714
LSM	0.726	0.730	0.728B	0.740	0.745	0.743A	0.650	0.676	0.663	0.705	0.717	0.711
Mean	0.729	0.734		0.739	0.742		0.651B	0.681A		0.706B	0.719A	
<u>LSM application timing by Instinct rate</u>												
<u>Instinct rate</u> <sup>¶</sup>												
0	0.725	0.730	0.727B	0.740	0.745	0.743	0.650	0.676	0.663	0.705	0.717	0.711
2.56	0.733	0.741	0.737A	0.744	0.741	0.743	0.658	0.690	0.674	0.712	0.724	0.718
5.12	0.736	0.740	0.738A	0.743	0.746	0.745	0.666	0.676	0.671	0.715	0.721	0.718
Mean	0.731B	0.737A		0.742	0.744		0.658B	0.681A		0.711B	0.721A	
Control <sup>#</sup>			0.665			0.720			0.577			0.654

<sup>†</sup> Anhydrous ammonia, AA and liquid swine manure, LSM.

<sup>‡</sup> Main effect and interaction means without a letter within the same year are not significantly different ( $P \leq 0.10$ , Table 9).

<sup>§</sup> Main effect means with a different upper case letter within the same year are significantly different ( $P \leq 0.10$ , Table 9).

<sup>¶</sup> Instinct rate, L ha<sup>-1</sup>.

<sup>#</sup> Control not included in statistical analysis.

Table 11. Fall application timing and N source, and fall application timing and Instinct rate with liquid swine manure, corn grain yield main effect and interaction means.

	2011			2012			2013			Across years		
	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean
----- Mg ha <sup>-1</sup> -----												
<u>Application timing by N source</u>												
<u>Source</u> <sup>†</sup>												
AA	13.5 <sup>‡</sup>	13.3	13.4A <sup>§</sup>	13.2	12.8	13.0 <sup>‡</sup>	12.2	12.1	12.2A	13.0a <sup>¶</sup>	12.7a	12.8A
LSM	11.7	12.1	11.9B	12.0	13.1	12.6	10.9	11.2	11.1B	11.5c	12.1b	11.8B
Mean	12.6	12.7		12.6	13.0		11.6	11.6		12.2	12.4	
<u>LSM application timing by Instinct rate</u>												
<u>Instinct rate</u> <sup>#</sup>												
0	11.5	12.1	11.8	12.0	13.1	12.6	10.9	11.2	11.1	11.5	12.1	11.8B
2.56	12.1	12.4	12.3	13.2	13.0	13.1	11.6	11.2	11.4	12.3	12.2	12.3A
5.12	12.2	11.8	12.0	12.3	12.6	12.5	11.4	11.2	11.3	12.0	11.9	12.0AB
Mean	11.9	12.1		12.5	12.9		11.3	11.2		11.9	12.1	
Control <sup>††</sup>			7.5			8.0			6.8			7.5

<sup>†</sup> Anhydrous ammonia, AA and liquid swine manure, LSM.

<sup>‡</sup> Main effect and interaction means without a letter within the same year are not significantly different ( $P \leq 0.10$ , Table 9).

<sup>§</sup> Main effect means with a different upper case letter within the same year are significantly different ( $P \leq 0.10$ , Table 9).

<sup>¶</sup> Interaction means with a different lower case letter within the same year are significantly different ( $P \leq 0.10$ , Table 9).

<sup>#</sup> Instinct rate, L ha<sup>-1</sup>.

<sup>††</sup> Control not included in statistical analysis.

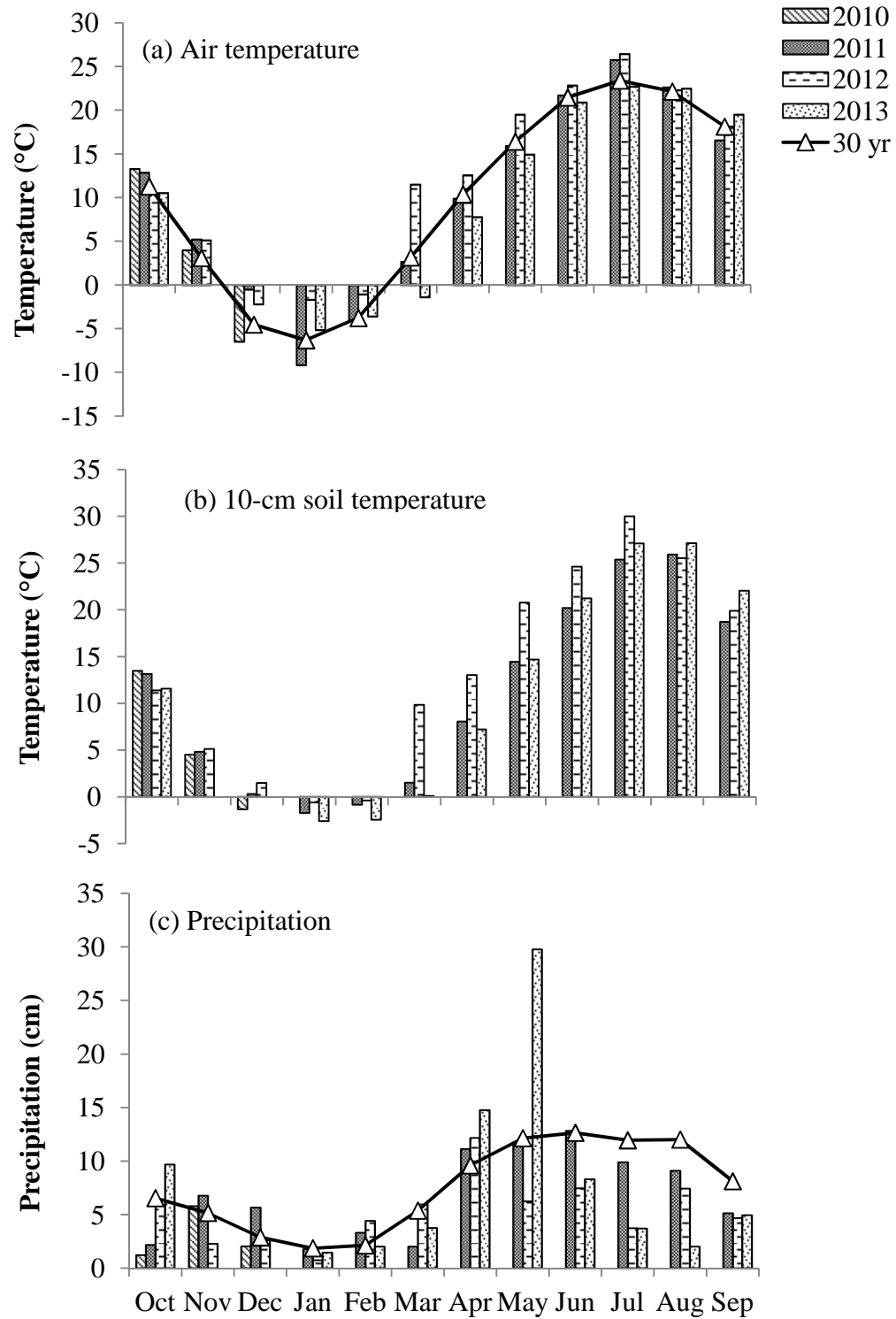


Fig. 1. Monthly mean air temperature (a), 10-cm soil temperature (b), and total monthly precipitation (c) for each study year and the 30-year mean (data from Arritt and Herzmann, 2014).

## CHAPTER 4. GENERAL CONCLUSIONS

This thesis included two, small plot field studies designed to evaluate the effect Instinct (Dow AgroSciences, Indianapolis, IN) nitrification inhibitor had on corn production when used with spring preplant applied urea-ammonium nitrate (UAN) and fall applied liquid swine manure (LSM). The first study evaluated the effect of Instinct applied with UAN fertilizer on corn production, and optimum N rate across six N rates and two application methods. The second study evaluated the effect LSM applied with Instinct at, two rates, and without Instinct had on corn production when applied at two application times in the fall. A comparison was also made in the second study with fall applied anhydrous ammonia (AA) without a nitrification inhibitor.

Corn did not respond positively to Instinct, no matter the application method, in the first study. Early corn growth plant height, mid-vegetative (V10) canopy normalized difference vegetative index (NDVI), stalk lodging potential, and grain yield either had no response or a negative response to Instinct, especially at low to recommended N rates. It is not known why a negative response occurred, and although not measured, could have been due to positional N supply issues with increased  $\text{NH}_4\text{-N}$  concentration and lower  $\text{NO}_3\text{-N}$  formation, or some unknown reaction to the Instinct product. Conditions were wetter than normal during one year of the study, which should have provided an opportunity for Instinct to improve fertilizer N supply, reduce the economic optimum N rate (EONR), and improve yield. That did not occur. Across years of the study, Instinct inclusion with spring preplant UAN actually increased the EONR by  $32 \text{ kg N ha}^{-1}$ , which is an opposite effect expected from use of a nitrification inhibitor.

Corn response to Instinct was inconsistent in the second study. Fall applied AA typically had higher  $\text{NH}_4\text{-N}$  concentrations within the injected N band, lower  $\text{NO}_3\text{-N}$



concentrations in the fall and early spring, and higher corn grain yield than fall applied LSM without Instinct, regardless of the timing of N application. Use of Instinct with fall applied LSM improved spring inorganic-N concentrations and corn yield. However, the response was not consistent, and early and late fall application grain yield was not fully comparable to AA. With a favorable yield response, and grain to Instinct price relationship, Instinct at the low rate with LSM provided greater profit return compared to LSM with the high Instinct rate and LSM without Instinct. However, higher grain yields with AA had greater return to N than LSM, even with inclusion of Instinct. This study supported the suggestion to delay application of N in the fall until soils cool to limit potential spring N losses, which can increase grain yields and provide for greater return to N application.

Overall, these studies show that use of Instinct with applied N does not guarantee a positive corn grain yield response. Additionally, these studies provide corn producers and crop advisors in Iowa data that Instinct with spring preplant UAN was not an economically feasible N management practice, but that Instinct with fall applied LSM was economically feasible when grain response and prices are favorable. Lastly, Instinct was shown to be an effective nitrification inhibitor with LSM, but delaying N application to later in the fall, or spring, was a better management practice in preventing N losses that would be detrimental to the environment.