# SITE-SPECIFIC EVALUATIONS OF NITRIFICATION INHIBITOR WITH FALL APPLICATIONS OF LIQUID SWINE MANURE

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#### ABSTRACT

Nitrification inhibitors (NI) can be used with animal manure to decrease N losses and increase corn (Zea mays L.) yields. However, data are limited specifying when and where applications of NI can be profitable. The objective was to identify site-specific conditions for using Instinct (an encapsulated form of nitrapyrin) with fall-injected liquid swine manure (LSM). Eleven on-farm trials with Instinct added to LSM were conducted in 2009 and 15 trials in 2010. The manure was applied at optimal manure rates in at least three field-long strips with and without NI. The corn stalk nitrate test (CSNT) and digital aerial imagery of the corn canopy were used to assess the late-season corn N status. Yield responses (YR) to NI were calculated by dividing fields into 50m long cells, and hierarchical models were used to estimate the probability of economic YR at different field-level factors (rainfall and total N rate) and within-field level factors (soil organic matter and drainage). On average, NI produced no YR in relatively normal 2009 and a 0.15 Mg ha<sup>-1</sup> YR in extremely wet 2010. In both treatments, NI did not increase N uptake and ammonia concentrations in the soil, but CSNT showed that about half of samples were deficient in 2009 and about 65% in 2010. In 2010, fields receiving >90 cm of rainfall from March through August were about 65% more likely to have economic YR (> 0.12 Mg ha<sup>-1</sup>) than fields receiving <90 cm rainfall. In both years, within-field variability in YR was about four times greater than amongfield variability, but within field-level factors had no significant effects. These studies suggest that the NI effects may have not lasted long enough to produce economic YR across all fields and its applications may be limited to fields that most likely receive above normal spring and summer rainfalls.

**Keywords:** nitrification inhibitor, liquid swine manure, late-season digital aerial imagery, the late-season corn stalk nitrate test, and hierarchical statistical models.

#### INTRODUCTION

In Iowa, nitrification inhibitors (NI) have been used historically with anhydrous ammonia to retard nitrification of ammonium-N and thereby reducing potential N and yield losses. Because many factors affect the conversion of ammonium to nitrate, the efficacy (timing and length of inhibition) and economic performance of commonly used nitrification inhibitors in corn (*Zea mays* L.) production can be extremely variable.

One of the most commonly used NI during the last 40 years was nitrapyrin (2-chloro-6-(tricloromethyl) pyridine), with a commercial name "N-Serve". Numerous field studies were focused on the efficacy of this NI. Some studies reported positive yield responses (YR) while other reported no benefits in N fertilizer savings or yields (Cerrato and Blackmer, 1990; Hoeft, 1984; Parkin and Hatfield, 2010; Quesada et al., 2000; Randall and Vetsch, 2005; Wolt, 2004).

A myriad of factors are usually involved in determining the efficacy and the likelihood of YR from using nitrapyrin. For example, a study in Iowa in relatively wet year showed a two-fold decrease in the percentage of nitrification of fall-applied anhydrous ammonia from NI when soils had a pH <6 than in soils with high pH > 7 and high free carbonate content (Kyveryga et al., 2004). However in the same study, the effect of NI was much smaller than the effect of soil pH on nitrification. In addition, positive YR to NI were observed mostly in high pH, calcareous soils (Ellsworth, 2001), where nitrification of anhydrous ammonia was much higher.

Liquid swine manure contains a considerable amount of ammonia or ammonium-N, and about 25% of fields planted to corn in Iowa receive annual LSM applications. Liquid swine manure management is more challenging because manure is usually applied in the fall before the soil temperatures are low enough to substantially retard nitrification. As a result, large losses of N are commonly observed after excessive spring rainfalls. To reduce these losses, NI might be used with fall-injected LSM.

While it is a proven NI, nitrapyrin efficacy is often reduced because of its volatilization losses, fixation by soil organic matter, and a relatively short time of inhibition activity. Instinct<sup>TM</sup> is an encapsulated form of nitrapyrin. Compared with nitrapyrin, Instinct<sup>TM</sup> is designed to be protected from volatilization losses, from permanent fixation by soil organic matter or clay minerals, and to enable the slow release of NI with the potentially longer inhibition action.

Little is known about site-specific factors that may affect the efficacy and economic performance of Instinct<sup>TM</sup> used with LSM. Precision agriculture technologies used by farmers in many on-farm evaluation trials from a specified soil area and management practices can provide a sufficient amount of information to identify these site-specific factors.

The objective of this study was to utilize precision farming technology in on-farm conditions to identify field and within-field level factors at which the use of  $Instinct^{TM}$  (an encapsulated form of nitrapyrin) with LSM might be profitable in spatially variable fields in Iowa.

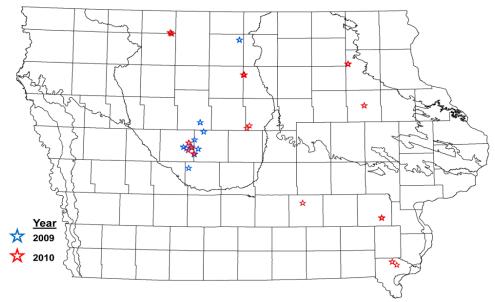


Figure 1. Locations of on-farm evaluations trials testing site-specific benefits of Instinct<sup>TM</sup> nitrification inhibitor with fall-injected liquid swine manure (LSM) across Iowa.

## **MATERIALS AND METHODS**

Instinct<sup>TM</sup> nitrification inhibitor was evaluated in 11 on-farm trials in 2009 and in 15 trials in 2010 (Fig. 1). Each trial had two alternated treatments of injected liquid swine manure (LSM) with and without Instinct<sup>TM</sup>. The treatments were replicated 3 to 5 times and were applied in a systematic order to avoid application errors and help facilitate the estimation of yield differences between the two treatments in a common way in each trial (Fig. 2).

Liquid swine manure was applied in rates considered optimal for corn. For corn after soybean (C-S), the average N rate applied with manure was about 190 kg of total N ha<sup>-1</sup> in 2009 and about 220 kg of total N ha<sup>-1</sup> in 2010 The manure applications rates were determined based on total N (organic plus mineral) content in the manure. Three or four manure samples were collected during the manure applications and sent to a laboratory for analysis. In about 60% of the trials, farmers applied additional commercial N at a rate of about 45 kg N ha<sup>-1</sup> to avoid potential large N shortages from excessive rainfall in April 2009 and June 2010 (Fig. 3). All trials were C-S, except three which were in continuous corn in each year. The manure was injected to a depth of 15-20 cm with disks or coulters to avoid ammonia losses. Instict<sup>TM</sup> was mixed with the manure and applied at a rate of 1 L ha<sup>-1</sup> (35 oz acre<sup>-1</sup>).

The manure strips were applied the full length of each field (Fig. 2). The width of the treatments was determined by the width of manure applicators and a number of applicator passes applied in each strip. The majority of the treatments were from 10 to 30 m wide.

Nitrogen status of corn was evaluated using three N diagnostic tools: the late-spring soil nitrate test (Binford et al., 1992a), the late-season corn stalk nitrate test (CSNT) (Binford et al., 1992b), and late-season digital aerial imagery of the corn canopy (Kyveryga et al., 2012).

The sampling areas for CSNT within each trial were selected using the visible color (red, green, and blue only) digital aerial imagery of the corn canopy. The areas were selected to capture a wide range of variability in the canopy reflectance to better relate canopy characteristics to yield differences between the two treatments.

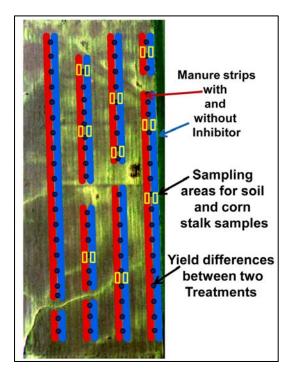


Figure 2. Treatment layout, locations of yield differences between two treatments and areas for collecting soil and corn stalk samples within a trial to evaluate site-specific benefits of Instinct<sup>TM</sup> nitrification inhibitor applied with injected liquid swine manure (LSM) to corn.

The imagery was collected by a commercial aircraft in mid or late August. Each aircraft had four (blue, green, red and near-infrared) 12-bit digital cameras with CCD (charge coupled display) array of 1600 x 1200. The imagery was taken from a height of about 2400 m above the ground producing imagery with about 1 m spatial resolution. Twenty to 30 individual images were taken from each field and the individual images were ortho-mosaiced into one composed image for the entire field. Composite images were GIS ready and georeferenced. For more information about DAI processing, normalization and enhancement see Kyveryga et al., (2012).

Soil and corn stalk samples were collected at nine locations for each treatment in each trial (Fig. 2). Soil samples were collected only in 2010. The sampling depth was 30 cm. Soil samples were collected when corn plants were at V6-7 growth stage. A composite soil sample in each sampling areas consisted of three sets of eight individual soil cores taken in different positions between three pairs of corn rows. Corn stalk samples were collected about 3 weeks after corn grain reached the black-layer stage (physiological maturity) or just before the harvest. Samples were collected from a 10 m length of two adjacent rows, avoiding plants that were irregularly spaced, damaged or barren. Both soil and stalk samples were analyzed for  $NO_3$ -N.

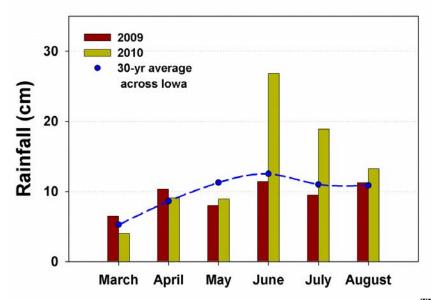


Figure 3. Average monthly rainfall for on-farm trials evaluating Instinct<sup>TM</sup> nitrification inhibitor with injected liquid swine manure in 2009 and 2010.

#### **Image Analysis**

Digital aerial imagery was analyzed to identify differences in reflectance of the corn canopy between the two treatments with and without NI. The reflectance values from each band were extracted from an area of about half the width of the manure treatments. The area for extracting the reflectance was identified by drawing a line in the middle of a treatment area and creating a buffer about half of the treatment width. The reflectance values for all replications in each trials were extracted using the Zonal Statistics Tool of Spatial Analysis in ArcGIS Desktop 9.3.1 software (Environ. Syst. Re. Inst., Redlands, CA). Only green reflectance values were used in the analysis because green band is the most sensitive to change in corn chlorophyll concentration at the end of corn growing season.

#### **Data Processing and Statistical Analysis**

Manure strips were harvested with grain combines equipped with GPS and yield monitors that recorded yield observations every 1 sec. The yield data were cleaned by deleting observations that were located < 50 m from the beginning and end of the strips, and from flooded areas, waterways, and buffer strips. Individual yield observations were aggregated into 50 m long grid cells along each pair of the treatments. Yield responses (YR) were calculated as differences in aggregated yields between treatments with and without NI. Each trial had from 50 to 300 individual YR (Fig. 2). Yield response observations that were two standard deviations above or below the mean YR for a trial were also eliminated.

Variable	Posterior regional mean	Posterior average SD	
		Regional	Within field
	]	Mg ha <sup>-1</sup>	
<u>Year</u> 2009	0.0 (-0.11, 0.10)	0.15	0.62
2010	0.15 (0.03, 0.30)	0.20	0.52
	2010		
<u>Soil drainage</u> Well	0.09 (-0.03, 0.22)	0.14	0.46
Poor	0.18 (0.02, 0.37)	0.30	0.51
Growing		<u>2010</u>	
<u>season rainfall</u> <90 cm	0.0 (-0.15, 0.14)	0.13	0.47
>90 cm¶	0.62 (0.22, 1.05)	0.22	0.22

Table 1. Posterior regional means, average regional (among fields) and within field standard deviations (SD) for corn yield response (YR) to Instinct<sup>™</sup> nitrification inhibitor evaluated with injected liquid swine manure (LSM) in 11 on-farm trials in 2009 and 15 trials in 2010.

In parenthesis are 90% credible intervals.

¶ A YR > 0.13 Mg ha<sup>-1</sup> was considered profitable from applications of NI.

Monthly rainfall estimates for each trial were obtained from the Iowa Environmental Mesonet (Iowa Environmental Mesonet). Soil characteristics such soil organic matter (SOM), slope, and soil drainage class were derived from digital soil maps available from the Iowa Cooperating Soil Survey (Iowa Cooperating Soil Survey, 2003).

Hierarchical models with Bayesian statistics were used to identify effects of field-level factors (average monthly and cumulative rainfall) and within-field factors (SOM, slope or soil drainage class) on YR to NI. Hierarchical analysis deals with random variability observed at different levels, and in Bayesian statistics prior distributions are assigned to parameters of distributions to represent knowledge of possible values of these parameters before the data are observed (Gelman et al., 2004; Gelman and Hill, 2007). The observed data are then used to update that knowledge using posterior distributions of parameters. In other words, Bayesian statistics helps to answer the question: "What are underlying probabilities of observing a range of YR to NI in new situations after collecting and observing data from on-farm trials?" More details about using Hierarchical models for analyzing data collected from on-farm trials see Kyveryga et al., (2010).

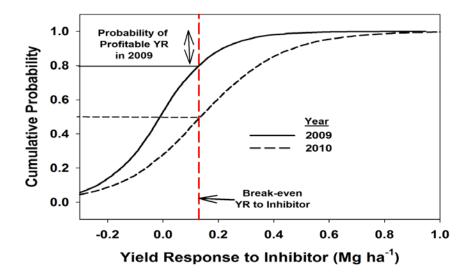


Figure 4. Posterior predictive distributions of average field-level yield response (YR) to Instinct<sup>TM</sup> nitrification inhibitor in on-farm trials evaluating site-specific benefits of Instinct<sup>TM</sup> nitrification inhibitor (NI) used with injected liquid swine manure (LSM) in 2009 and 2010.

We used posterior predictive distributions of field level means to predict a mean YR for a field that was not studied or observed. We also estimated the probability of profitable YR to NI using posterior predictive distributions. Posterior distributions were obtained by using Markov Chain Monte Carlo simulation method with 10,000 runs. A mean YR >0.13 Mg ha<sup>-1</sup> was considered profitable from application of NI. Ninety percent credible intervals for posterior means were estimated using 5<sup>th</sup> and 95<sup>th</sup> percentiles of the simulated data.

#### **RESULTS AND DISCUSSION**

### **Factors Influencing Yield Response to Inhibitor at Different Scales**

Parameters of posterior distributions of YR to NI are shown in Table 1. Posterior regional means represent expected YR across the state or for two categories of trials based on soil drainage class or site-specific cumulative growing season rainfall. Across Iowa, NI did not increase yield in 2009, a relatively normal year, but increased yield by 0.15 Mg ha<sup>-1</sup> in 2010, a relatively wet year. The posterior 90% credible interval for the field level mean YR in 2010 ranged from 0.03 to 0.30 Mg ha<sup>-1</sup>, suggesting a positive YR to NI. But the difference in YR between 2009 and 2010 was marginal because both credible intervals slightly overlapped (Table 1). Based on average posterior among and within field standard deviations, within field variability in YR in both years was from three to four times larger than among field variation (Table 1). Slightly larger within field variability was observed in 2009 than in 2010.

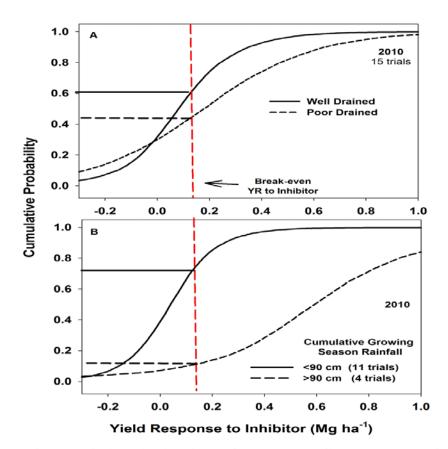


Figure 5. Posterior predictive distributions of the average field level corn yield response (YR) to Instinct<sup>TM</sup> nitrification inhibitor (NI) as affected by soil drainage and cumulative growing season (March through August) rainfalls. The probability of profitable YR to NI can be estimated as the distance from 1 on the Y axis to the intersection of the cumulative probability curve with the break-even YR line shown in red.

It is noteworthy that within field variability in a relatively responsive 2010 was about three times larger than the posterior regional mean YR, indicating relatively large uncertainty in average YR estimates. Although the observed large within field variability could be attributed to possible errors generated by yield monitors or by the method used to calculate YR within fields (Figs 1 and 2), data in Table 1 confirm results of many previous controlled small-plot studies also showing large variability and uncertainty in measuring YR to NI (Blackmer, 1986; Ellsworth, 2001).

Based on field-level posterior predictive simulations for unobserved fields, about 20% of the trials were predicted with a profitable field-level YR in conditions similar to 2009 and about 50% of trials with a profitable YR in conditions similar to 2010 (Fig. 4). Although receiving profitable YR to inhibitor was about twice as likely in 2010 than 2009, it is important to stress that a 50% probability of receiving a profitable YR in 2010 could partially be due to a random chance.

Because of large within-field variability in YR, we tested whether within-field factors such as SOM, slope or soil drainage had influenced YR. The analysis showed that none of the factors used were significant in 2009. But in 2010, poorly drained soil map units within fields had a

posterior mean YR of 0.18 Mg ha<sup>-1</sup>, which was about twice that of well drained soil map units (Table 1). This difference in YR suggests that NI may have prevented N losses by reducing denitrification or leaching within poorly drained areas within the fields studied in 2010. However, 90% credible intervals for posterior means of the two soil drainage categories largely overlapped, suggesting that the estimated difference between the two drainage classes is largely uncertain. In addition, posterior predictions for unobserved fields showed that the probability of profitable YR within poorly drained soil was only about 15% higher than that within well drained areas (Fig. 5A).

In 2010, fields with above normal cumulative growing season rainfall (>90 cm) were predicted to have the average field-level profitable YR about 65% more often than those with below normal growing season rainfall (Table 1 and Fig. 5B). However, more studies of the predicted effects are needed because only four trials located in the southeastern corner of Iowa (see Fig.1) had excessive growing season rainfall in 2010. Classifying the 2010 trials using cumulative spring rainfall (below normal, <25 cm, and above normal, >25 cm) also showed that the same four trials were about 65% more likely to have profitable YR with above than with below normal spring rainfall. Both observations indicate that a combination of excessive spring and summer rainfall created ideal conditions for N losses and the relatively high chance of NI to produce a profitable YR.

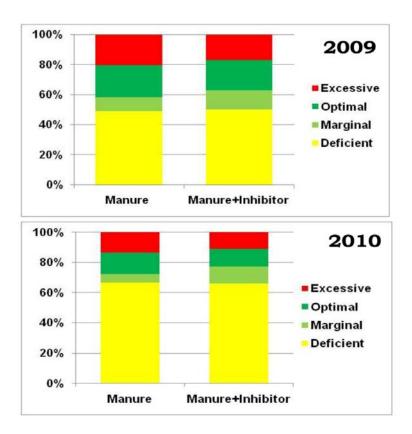
From analysis of CSNT sufficiency categories for the two treatments, NI did not affect corn N uptake (Fig. 6). But more than half of corn stalk samples were tested as deficient in 2009 and about 65% as deficient in 2010 from both treatments, suggesting favorable conditions for detecting a positive YR to NI. In comparison, other on-farm trials with the relatively same manure N rates and additional commercial N (about 56 kg N ha<sup>-1</sup>) produced an average YR of about 0.5 Mg ha<sup>-1</sup> in 2009 and of about 0.7 Mg ha<sup>-1</sup> in 2010 (data not shown).

Year/variable	LSM	LSM + Inhibitor		
	Late Spring Soil Nitrate Test mg kg <sup>-1</sup>			
<u>2010</u>				
NH <sub>4</sub> -N	5	6		
NO <sub>3</sub> -N	23	23		
	Green band reflectance			
2009	108 (20)	108 (20)		
2010	77** (21)	73(21)		

Table 2. Effect of Nitrification Inhibitor (NI) applied with injected liquid swine manure (LSM) in fall on soil mineral-N concentrations measured in early June of 2010 and on green reflectance of late-season digital aerial imagery of the corn canopy measured in 2009 and 2010

# In parenthesis are standard deviations (SD).

\*\*Statistically different (p < 0.05) based on 14-paired comparisons of trial-level mean reflectance values.



# Figure 6. Percentage of stalk samples tested in different corn stalk nitrate test (CSNT) categories. In both years, geometric means of stalk NO<sub>3</sub>-N values for the two treatments were the same.

In 2010, soil mineral-N measured in early June was not affected by adding NI to LSM (Table 2). The positive effects of NI should have been reflected in higher concentrations of soil  $NH_4$ -N in the manure strips with NI. We speculate that in the majority of the trials, the inhibitor effects may have passed by the time of the soil sampling in early June.

Contrary to the effects of NI on soil mineral N, the inhibitor, on average, significantly decreased the green reflectance of the corn canopy by about 4% compared with the control, partially confirming the observed positive YR in 2010 (Table 2). In both years, NI did not change within strip variability in green reflectance of the corn canopy.

Several factors including soil moisture, soil temperature, and soil drainage can influence the efficacy of NI. The interactions of these and other factors along with farmers'site-specific N management practices often create unique conditions at which the probability of YR to NI can be relatively high. These conditions can be grouped in at least four major requirements: (i) relatively large N losses and potential large N saving from using NI, (ii) a low supply of soil-derived N, (iii) a relatively low N rates applied with LSM or commercial fertilizer, and (iv) using sensitive experimental methods for detecting positive or economic YR to NI. If one of these requirements is not met, the likelihood of detecting positive YR to NI is becoming relatively low. Thus, the described methodology, which is based on using precision farming technologies in on-

farm evaluations, can be used to identify individual factors and their interactions at different scales where YR to NI can produce both potential economic and environmental benefits.

## CONCLUSIONS

In the relatively normal year (2009), Instinct<sup>TM</sup> had no effect on yield or corn N uptake. In the relatively wet year (2010), the inhibitor produced: (i) a positive YR, with a 50 % chance of a profitable YR (that suggest by a random chance); (ii) slightly decreased the green reflectance of the corn canopy;(iii) did not increase late-season corn N

uptake or (iv) decrease early season soil nitrate concentrations. In 2010, fields with abovenormal spring (>25 cm) or cumulative growing season rainfall (> 90 cm) were about 65% more likely to have profitable YR to NI than those with below normal spring or growing season rainfall.

In both years, more than half samples for CSNT showed deficient corn N status for both treatments with and without Instinct<sup>TM</sup> but some trials had manure N rates applied slightly above recommended.

Because we found no difference in soil mineral N measured in early June of 2010, NI effects might be limited during winter or early spring, suggesting the low likelihood of economic benefits from its use across all fields receiving fall-injected LSM in Iowa.

Despite large spatial variability in YR to NI, we found no significant effects of soil properties on YR. In 2010 only, within field areas having poor soil drainage tended to have a larger YR than well drained areas.

The proposed on-farm methodology and statistical data analysis enable researchers to continually search for factors that influence YR to NI at the field and within field levels.

#### ACKNOWLEDGEMENTS

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