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Assessing Management of Nitrapyrin with Urea Ammonium Nitrate Fertilizer on Corn Yield and Soil Nitrogen in a Poorly-Drained Claypan Soil

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Abstract

Use of nitrification inhibitors (NI) in agricultural production systems is considered a risk management strategy for both agricultural and environmental considerations. It can be utilized when risk of reduced nitrogen (N) fertilizer use efficiency or yield, and risk of pollution from mineral N is high which can occur in poorly-drained soils that are vulnerable to waterlogging and runoff. Field research was conducted on corn (*Zea mays* L.) from 2012 to 2015 in Missouri, USA on a poorly-drained claypan soil. Treatments consisted of two application timings of urea ammonium nitrate (UAN) fertilizer solution [pre-emergence (PRE) and V3 growth stage], two application rates (143 and 168 kg N ha^{-1}) in the presence or absence of nitrapyrin, and a non-treated control. UAN at 143 kg ha^{-1} with nitrapyrin at the V3 growth stage resulted in the highest yield (8.6 Mg ha^{-1}). Similarly, pre-emergence application of UAN 168 kg ha^{-1} with nitrapyrin resulted in greater yields (7.7 Mg ha^{-1}). UAN application rates and timings affected soil NO_3-N and NH_4-N concentrations more than the presence or absence of nitrapyrin during the growing season. A side-dress application of a lower rate of UAN with nitrapyrin at V3 was effective in poorly-drained soils when risk of N losses during the growing season due to unfavorable precipitation events and other environmental variables was high. A pre-emergence application of UAN with nitrapyrin was also effective and it may eliminate the need for split-application of N fertilizer later in the season thereby reducing the workload on growers during the growing season.

Keywords: nitrification inhibitor, urea ammonium nitrate, grain yield, fertilizer application timing

1. Introduction

Careful selection of N fertilizer sources, application rates, and application timings are common strategies to better match the crops N demand with supply. Application of N fertilizer in the spring at the time of planting or soon after emergence of the crop is a common fertilization practice for corn production in the Midwestern U.S. (Randall & Sawyer, 2008). Nitrification inhibitors (NI) are also sometimes combined with ammonium based N fertilizers, such as anhydrous ammonia (AA), urea or urea ammonium nitrate solution (UAN) to slow the conversion of ammonium to nitrate (NO_3^-) after fertilizer application. Substantial research has been conducted on the use of nitrapyrin with AA (Wolt, 2004). There is lack of research studies which have investigated the effects of a new formulation of nitrapyrin (Instinct II, Dow Agro Sciences, Indianapolis, IN) and UAN fertilizer solution on soil N, corn N status, and grain yield. Research has reported a 29 to 50% reduction in soil NO_3-N loss when UAN was combined with a urease inhibitor and a NI (Halvorson et al., 2010; Halvorson & del Grosso, 2012). However, few studies have reported significant increases in grain yields. One exception was Maharjan et al. (2017) who observed grain yield increases with application of UAN and nitrapyrin only in one out of two years when rainfall was relatively lower. The NI might not have had significant effects on yields in those studies since they were conducted with irrigated systems and NI typically works best in soils that experience saturated conditions.

In row-crop agriculture, synthetic fertilizers, such as urea, UAN, ammonium nitrate (NH_4NO_3), AA and ammonium sulfate ($(NH_4)_2SO_4$), are commonly used (Millar et al., 2010). As a N fertilizer source, UAN comes in a liquid form which makes it convenient for application and mixing with other nutrients or chemicals. Half of the N in UAN is in the urea form while the other half consists of ammonium nitrate which contains NH_4^+ and NO_3^- forms at 25% each. Research is limited on use of UAN combined with nitrification inhibitors. One study, conducted over two years in Indiana, compared UAN application rates (0, 90 and 180 kg N ha^{-1}), application timing (preemergence and sidedress at the V6 stage of corn growth) and use of nitrapyrin on corn yield, soil N_2O emissions and yield-scaled N_2O emissions (Burzaco et al., 2013). There was a 3 Mg ha^{-1} increase in yield when UAN was applied as a sidedress application with nitrapyrin at 180 kg N ha^{-1} compared to a preemergence application without nitrapyrin at 90 kg N ha^{-1}. Although nitrapyrin significantly reduced both daily and cumulative soil N_2O emissions when averaged across both years, UAN rate was the primary factor influencing corn yield, yield-scaled N_2O emissions, soil N_2O fluxes and cumulative soil N_2O emissions followed by N application timing and nitrapyrin.

The objective of this study was to determine the effects of applying a NI in a poorly-drained claypan soil on soil N, plant N status, and corn grain yield for different UAN application rates and application timings in the presence or absence of nitrapyrin.

2. Method

2.1 Site Location and Experimental Design

This research was conducted from 2012 to 2015 at the University of Missouri's Greenley Memorial Research Center (40°1′17″N, 92°11′24.9″W) near Novelty, Missouri, USA. The soil is a Putnam silt loam (fine, smectitic, mesic Vertic Albaqualfs). This soil is characterized by the presence of a poorly-drained claypan subsoil at a depth of 20 to 40 cm from the surface (Anderson et al., 1990; Jung et al., 2006; Myers et al., 2007). This claypan layer has a 100% higher clay content than the above horizon. The depth to the claypan at this particular location ranges from 46 to 60 cm (data not presented).

The field site for each growing season was different from the previous year and all sites had been in corn-soybean (*Glycine max* L.) rotation. Soybean residues on the surface of the soil were not disturbed and field sites in all years were no-till. Field sites had a slope less than one percent and plot size was 3 by 15 m. The experiment was arranged as a randomized complete block design (RCBD) with five replications. Treatments consisted of a factorial arrangement of two application timings of UAN fertilizer solution [pre-emergence (PRE) and V3 growth stage], two application rates (143 and 168 kg N ha^{-1}), and the presence or absence of nitrapyrin (0 or 0.513 kg a.i. ha^{-1} as Instinct (DowAgroSciences, Indianapolis, IN)). A non-treated control was included. Both the PRE and V3 applications were surface dribble-banded between corn rows using a CO_2 propelled hand boom.

The corn hybrids planted each year were DKC62-97 in 2012, 2013 and 2014, and DK62-08 in 2015 in 76 cm wide rows. Seeding rate was 79,000 seeds ha^{-1} in 2012 and 82,000 seeds ha^{-1} in 2013, 2014 and 2015. Field operation timeline, maintenance fertilizer, and initial soil properties are reported in Table 1. Crop protection chemical applications are listed in Table 2. Chlorophyll (SPAD) meter leaf readings (Minolta SPAD-502, Konica Minolta Optics, Inc., Tokyo, Japan) were recorded for 10 plants per plot at V8 and VT growth stages (Ritchie et al., 1992). Corn grain yields were determined with a small-plot two row combine (Wintersteiger Inc., Salt Lake City, UT) and adjusted to 155 g kg^{-1} moisture content before statistical analysis. Additional corn response measurements included harvested plant population, grain protein concentration (Foss Intratec 1241, Eden Prairie, MN), grain oil concentration, grain starch concentration, test weight and grain moisture content. The duration of the growing season in 2012, 2013, 2014 and 2015 was 144, 136, 182 and 148 days, respectively.

2.2 Soil Sampling and Analytical Procedures

Each year, soil sampling occurred prior to planting at each site using a stainless steel push probe from depth increments of 0 to 22 cm and 23 to 46 cm to characterize selected initial soil properties (Table 1). Standard soil test analytical methods were used by the University of Missouri Soil and Plant Testing Lab to analyze these samples (Nathan et al., 2006). Additional soil samples were collected from 0 to 22 and 23 to 46 cm depths at V3 and V7 corn growth stages during the season, as well as at harvest to determine soil NH_4^+-N and NO_3^--N concentrations. All soil samples were air-dried, ground in a hammer mill and passed through a stainless steel sieve with 2 mm openings. Soil NH_4^+ and NO_3^- were extracted using a 2 M KCl solution and analysis conducted using a Lachat QuikChem automated ion analyzer (Hach Corp., Loveland, CO).

2.3 Climate Information

Daily precipitation and air temperature data for each growing season were collected from an automated weather station maintained by the University of Missouri at the Greenley Memorial Research Center. Historical weather data from the same weather station were obtained from the Missouri Historical Agricultural Weather Database website (University of Missouri Extension, 2016) to calculate the average cumulative precipitation from 2001 to 2011.

2.4 Statistical Analysis

All statistical analyses were conducted using the SAS statistical program (SAS Institute, 2013). Initially, a single-factor ANOVA was performed to assess any significant difference between the non-treated control and N treatments. This was followed by a three-factor ANOVA to investigate any significant main effects and interactions. If the overall F was significant, Fisher's Protected Least Significant Difference (LSD) at $P \leq 0.10$ was used for mean separation.

Table 1. Experimental timeline, initial soil properties from 0 to 22 cm and 23 to 46 cm depth, and maintenance fertilizer details from 2012 to 2015

	2012	2013	2014	2015
Timeline				
Initial soil sample	28 Mar.	1 May	9 Apr.	22 Apr.
Planting	2 Apr.	14 May	9 Apr.	22 Apr.
PRE-treatment application	2 Apr.	14 May	9 Apr.	22 Apr.
V3-soil sample[†]	15 May	5 Jun.	12 May	18 May
V3-treatment application	15 May	5 Jun.	12 May	18 May
V7-soil sample	18 Jun.	8 Jul.	23 Jun.	29 Jun.
V8-SPAD reading	13 Jun.	8 Jul.	23 Jun.	29 Jun.
VT-SPAD reading	27 Jul.	29 Jul.	2 Jul.	15 Jul.
Plant population	9 Jul.	8 Aug.	1 Jul.	1 Jul.
Harvest	23 Aug.	26 Sep.	7 Oct.	16 Sep.
Harvest soil sample	5 Sep.	26 Sep.	20 Oct.	29 Oct.
Initial Soil Properties				
---------- 0 to 22 cm ----------				
pH	5.9	5.7	6.4	6.3
Neutralizable acidity (NA), $cmol_c\ kg^{-1}$	1.7	3.5	0.8	0.9
Organic matter (OM), %	3.3	2.1	2.4	2.3
Bray 1 phosphorus (P), $kg\ ha^{-1}$	30	37	26	53
Calcium (Ca), $kg\ ha^{-1}$	4,822	3,624	3,873	5,049
Magnesium (Mg), $kg\ ha^{-1}$	584	305	361	580
Potassium (K), $kg\ ha^{-1}$	228	182	155	293
Cation exchange capacity (CEC), $cmol_c\ kg^{-1}$	14.9	12.9	11.0	14.7
Nitrate-nitrogen (NO_3-N), $mg\ kg^{-1}$	6.6	13.1	9.2	8.0
Ammonium-nitrogen (NH_4-N), $mg\ kg^{-1}$	4.0	6.8	2.4	2.4
---------- 23 to 46 cm ----------				
pH	NA[‡]	5.1	5.5	5.2
Neutralizable acidity (NA), $cmol_c\ kg^{-1}$	NA	5.4	2.9	4.9
Organic matter (OM), %	NA	2.0	1.7	2.2
Bray 1 phosphorus (P), $kg\ ha^{-1}$	NA	20	13	13
Calcium (Ca), $kg\ ha^{-1}$	NA	3,283	3,578	4,941
Magnesium (Mg), $kg\ ha^{-1}$	NA	293	407	874
Potassium (K), $kg\ ha^{-1}$	NA	114	103	137
Cation exchange capacity (CEC), $cmol_c\ kg^{-1}$	NA	15.0	13.7	21.1
Nitrate-nitrogen (NO_3-N), $mg\ kg^{-1}$	4.3	4.9	6.4	3.4
Ammonium-nitrogen (NH_4-N), $mg\ kg^{-1}$	4.1	7.1	4.1	3.6
Maintenance Fertilizer				
Fertilizer type	N-P-K	NA[§]	N-P-K-S-Zn	NA
Application rate, $kg\ ha^{-1}$	19-90-134	NA	22-90-157-22-2	NA
Application date	12 Apr.	NA	NA	NA

Note. [†] Corn growth development stages (Ritchie et al., 1992); [‡] NA = Not available. Data were only collected from 23 to 46 cm depth for NO_3-N and NH_4-N for 2012; [§] Maintenance fertilizer was not applied in 2013 and 2015.

Table 2. Plant protection chemical application timings, rates and date from 2012 to 2015

Year	Herbicide common name	Timing	Rate (kg a.i. ha^{-1})	Date
2012				
	Simazine[†]	Fall applied	1.12	3 Oct. 2011
	Glyphosate[‡]		0.43	
	Ace. + Flu. + Clo.[§]	Post application 1	1.19	2 Apr
	Glyphosate		1.26	
	Glyphosate	Post application 2	0.43	5 Jun
	Mesotrione		0.11	
2013				
	Simazine	Fall applied	1.12	28 Nov .2012
	Acetolchlor[¶]	Post application 1	1.61	14 May
	Atrazine[#]		2.25	
	Glyphosate	Post application 2	0.43	22 May
	Mesotrione		0.11	
	Lambda-cyhalothrin[††]		0.04	
2014				
	Acetochlor + Atrazine	Post application 1	3.97	6 May
	Atrazine		0.56	
	Glyphosate		0.87	
	Glyphosate	Post application 2	0.87	11 Jun
	Topramezone[‡‡]		0.012	
	Atrazine		0.28	
2015				
	Saflufenacil[§§]	Before emergence	0.025	23 Apr
	Glyphosate		1.42	
	Acetolchlor	Post application 1	2.53	28 Apr
	Atrazine		1.68	
	Topramezone	Post application 2	0.012	6 Jun
	Glyphosate		1.08	

Note. Chemical names: [†]2-chloro-4,6-bis(ethylamino)-s-triazine; [‡]N-(phosphonomethyl) glycine; [§]Acetolchlor, 2-chloro-2'-ethyl-N-ethoxymethylacetanilide + Flumetsulam, N-(2,6-dfluorophenyl)-5-methyl-1,2,4-triazolo-[1,5a]-pyrimidine-2-sulfonamide + Clopyralid, 3,6-dichloro-2 pyradinecarboxylic acid; [¶]2-cholor-N-(2-ethyl-6-methylphenyl) acetamide; [#](2-chloro-4-ethylamino)-6-(isopropylamino)-s-triazine; [††][1a(S*),3a(Z)]-(±)-cyano-(3-phenoxyphenyl) methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate; [‡‡][3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl) phenyl] (5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone; [§§]N'-(2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl) bezoyl-N-methylsulfamide.

3. Results and Discussion

3.1 Precipitation

Precipitation in 2012 was 35% (277 mm) lower compared to average cumulative precipitation (784 mm) (Figure 1). The amount of daily precipitation started to decline shortly before UAN application at V3, and did not recover until the end of the season on 31 October. A 13-day difference was noted between harvest and harvest soil sample, and this period received 34% (135 mm) of the total precipitation for the season. Cumulative precipitation in 2013 (795 mm) did not differ from the average cumulative precipitation (784 mm). Harvest and harvest soil sampling occurred on the same day. Cumulative daily precipitation in 2014 was 9% (74 mm) higher than the average cumulative precipitation (784 mm). The daily precipitation events were relatively evenly distributed through the season. The number of days between harvest and post-harvest soil sampling were 13 and during this period there was 47 mm of precipitation. In 2015, daily cumulative precipitation was 962 mm and it was 23% (179 mm) higher than the average daily cumulative from 2001 to 2011. The time-period between harvest and harvest soil sample in 2015 was 43 d, and there was 36 mm of rainfall during that time.

3.2 Air Temperature

Average daily air temperatures from January 1 to December 31 for 2012, 2013, 2014 and 2015 are reported in Figure 2. In all four years, temperature was generally below freezing (0 °C) from early-January to late-February. Relatively small temperature differences were observed among study years for the period of early-January to late-February except for 2012 and 2013 compared to 2014 and 2015. In 2012 and 2013, air temperature in this period fluctuated between ±10 °C more than was noted in 2014 and 2015. In 2014 and 2015, the air temperature remained below 0 °C for longer intervals during that period. However, temperatures across years were similar for the time-period for which the corn crop was in the field. Temperatures started to rise above 10 °C from mid-March to late-May across years. Temperatures remained above 20 °C and below 30 °C from June to mid-October. This was followed by a decline in early-November which again ended up with several daily average temperatures below freezing in December.

3.3 Grain Yields

When corn yield data were analyzed using a single-factor ANOVA from 2012 to 2015, all the treatments had higher grain yields than the non-treated control at $P \leq 0.05$ (data not presented). Subsequently, data were analyzed in the absence of non-treated control to determine any interactions using a three-factor ANOVA, and a significant interaction at $P \leq 0.10$ among UAN application timing, rate, and nitrapyrin was noted (Table 3). A pre-emergence (PRE) application of UAN at 168 kg N ha^{-1} with nitrapyrin resulted in the highest grain yield (8.6 Mg ha^{-1}) and was 11% greater than a PRE application of UAN at 168 kg N ha^{-1} without nitrapyrin (7.7 Mg ha^{-1}) (Figure 3). The UAN at 143 kg N ha^{-1} with nitrapyrin at V3 (8.2 Mg ha^{-1}) resulted in a 7% increase over UAN at 143 kg N ha^{-1} without nitrapyrin at V3 (7.6 Mg ha^{-1}). No significant difference was noted between yields of PRE UAN at 143 or 168 kg N ha^{-1} with or without nitrapyrin.

These results are in contrast with those of Burzaco et al. (2013) who observed that both PRE and sidedress applications of nitrapyrin with UAN (0, 90 and 180 kg N ha^{-1}) did not have significant effects on corn grain yields compared to when nitrapyrin was not applied. The differences in results between the two studies may be due to the differences in tillage between the two studies since that study had conventional tillage and in this research the fields were maintained in no-till. In a subsequent meta-analysis of the research literature regarding grain yield response to spring-applied nitrapyrin, Burzaco et al. (2014) found that in 56% of the research studies grain yield response was greater than zero. Similarly, Wolt (2004) in a synthesis of the literature found 62% positive grain yield responses to spring-applied nitrapyrin.

3.4 Soil N

Soil NO_3-N and soil NH_4-N concentrations at 0 to 22 and 23 to 46 cm depths for the V3 growth stage were analyzed using a single-factor one-way ANOVA because side-dress treatment applications had not occurred at that time (Table 4). Data were combined over years due to a lack of an interaction between years and treatments. At V3, all of the PRE applied treatments with or without nitrapyrin had greater N concentrations than the non-treated control (14.2 mg kg^{-1}) for soil NO_3-N from 0 to 22 cm. All the treatments resulted in similar soil NO_3-N concentrations from 0 to 22 cm at the V3 growth stage. At V3, only PRE applied UAN at 168 kg N ha^{-1} without nitrapyrin (25.5 mg kg^{-1}) resulted in significantly higher soil NH_4-N concentration in the 0 to 22 cm depth compared to the non-treated control (5.1 mg kg^{-1}). At the V3 growth stage in the 23 to 46 cm depth, PRE applied UAN at 168 kg N ha^{-1} with nitrapyrin (10.3 mg kg^{-1}) had higher soil NO_3-N concentration compared to the non-treated control (6.2 mg kg^{-1}). Soil test N concentrations at V7 were analyzed using a three-factor ANOVA at $P \leq 0.10$.

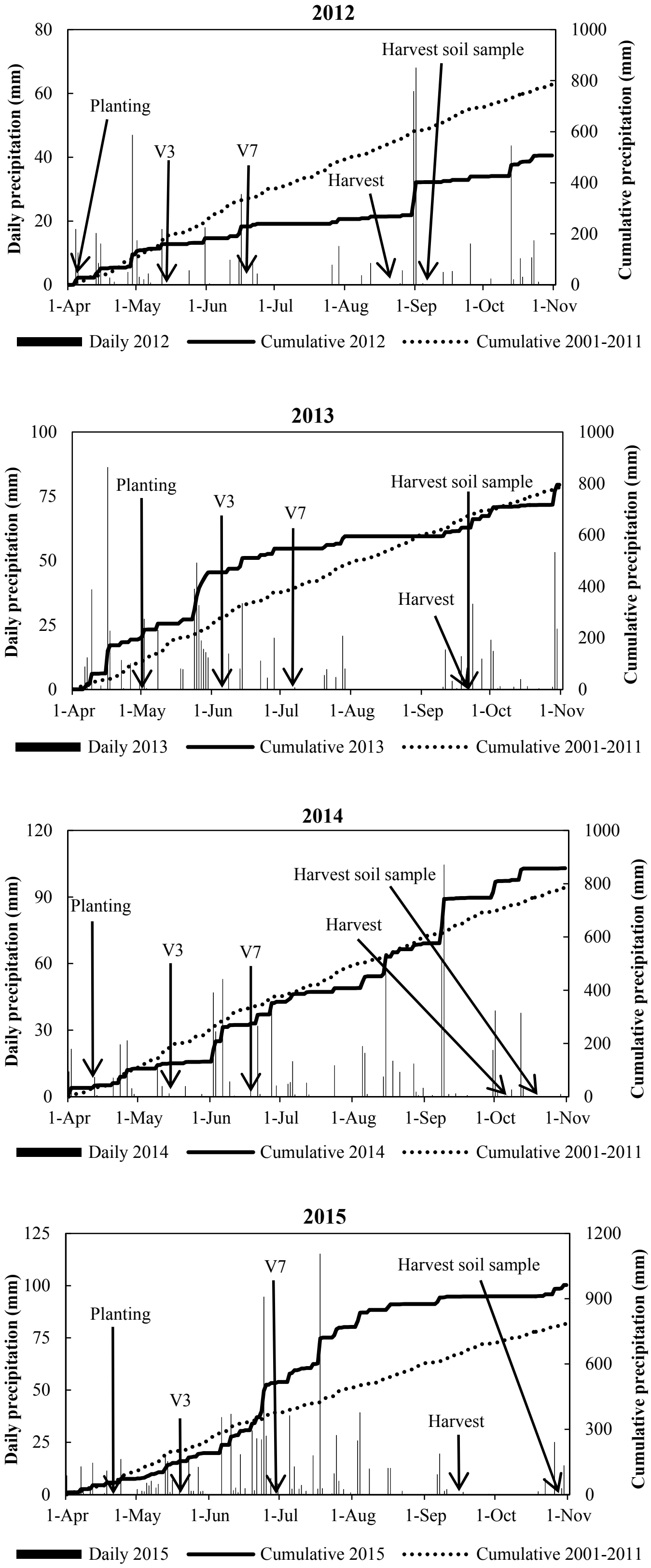

Figure 1. Precipitation history and timing of crop management practices for 2012, 2013, 2014 and 2015

Note. Bars represent daily precipitation; solid line represents cumulative precipitation over the season; and dotted-line represents cumulative precipitation from 2001 to 2011. V3 and V7 are corn growth stages (Ritchie et al., 1992).

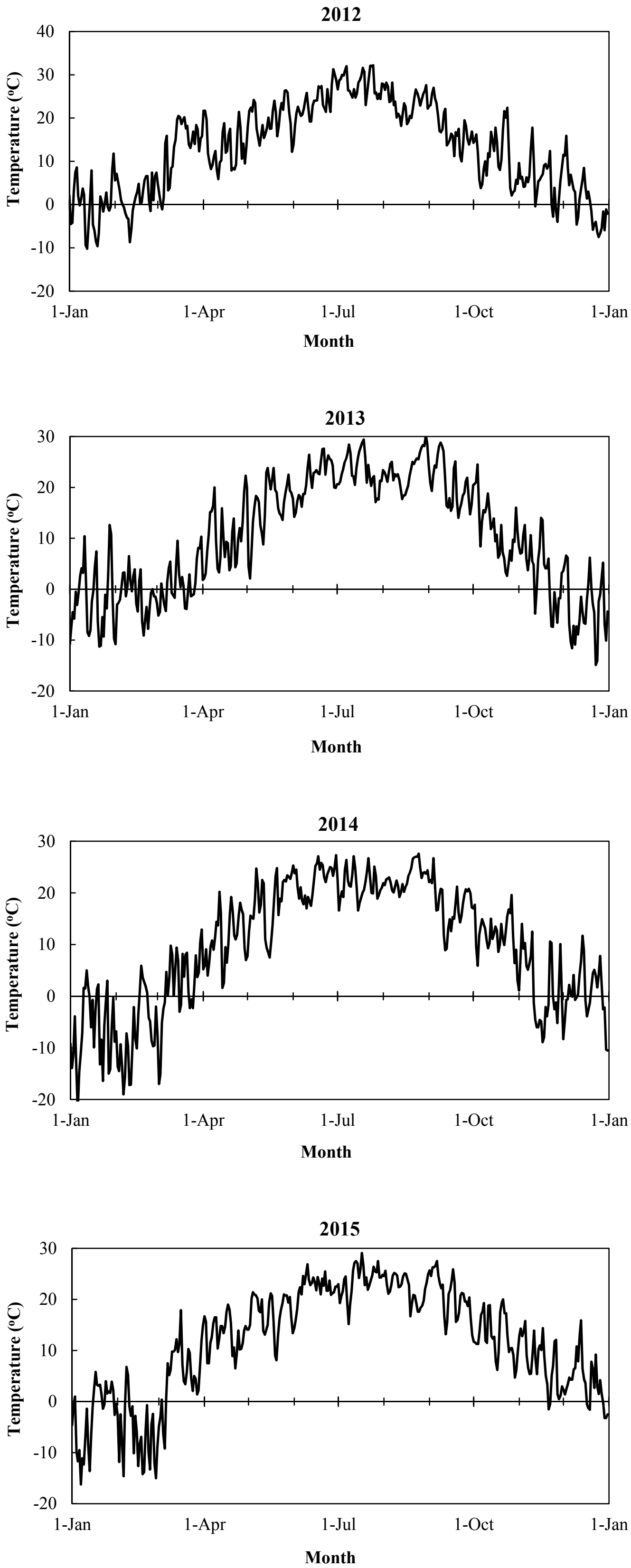

Figure 2. Daily average air temperature (ºC) for 2012, 2013, 2014 and 2015

Table 3. Three-factor ANOVA table for selected corn production and quality variables

Source	df	SPAD		Plant Population	Grain					
		V8	VT		Moisture	Test Wt.	Oil	Protein	Starch	Yield
		---- Pr > F ----								
Year	3	<.0001	<.0001	0.0229	<.0001	0.0065	<.0001	<.0001	<.0001	<.0001
Year (Rep)	4	0.1199	0.0036	0.0642	0.0069	0.5357	0.6273	0.2272	0.6617	0.9639
Timing	1	0.0896	0.4302	0.4601	0.0104	0.1285	0.5837	0.2854	0.6112	0.6952
Year * Timing	3	0.2751	0.9842	0.3061	0.5736	0.2678	0.5343	0.3676	0.6120	0.7704
Nitrapyrin	1	0.5065	0.0258	0.3230	0.0607	0.5888	0.3453	0.1785	0.1161	0.0195
Year * Nitrapyrin	3	0.0834	0.0341	0.2288	0.0231	0.5213	0.0822	0.7954	0.1341	0.0038
UAN rate	1	0.4195	0.6107	0.5152	0.6818	0.9318	0.5630	0.4773	0.9201	0.4199
Year * UAN rate	3	0.8410	0.9671	0.6636	0.1578	0.1505	0.9861	0.7983	0.8892	0.6126
Timing * Nitrapyrin	1	0.8236	0.5852	0.8374	0.4041	0.5881	0.7327	0.3351	0.2187	0.8473
Year * Timing * Nitrapyrin	3	0.4786	0.4751	0.8920	0.9243	0.4357	0.9231	0.9886	0.9271	0.8657
Timing * UAN rate	1	0.4087	0.1929	0.5088	0.9395	0.4779	0.9268	0.0321	0.3612	0.1857
Year * Timing * UAN rate	3	0.1682	0.3082	0.1779	0.9839	0.8970	0.8822	0.6407	0.7871	0.2233
Nitrapyrin * UAN rate	1	0.4482	0.8594	0.3145	0.1565	0.6214	0.9456	0.2789	0.4594	0.6059
Year * Nitrapyrin * UAN rate	3	0.9339	0.1946	0.2853	0.1971	0.2190	0.7399	0.3948	0.5821	0.6709
Timing * Nitrapyrin * UAN rate	1	0.2947	0.2331	0.1917	0.0530	0.0465	0.9427	0.6659	0.7599	0.0898
Year * Timing * Nitrapyrin * UAN rate	3	0.2155	0.1143	0.9858	0.1400	0.1887	0.9734	0.3268	0.8488	0.1585

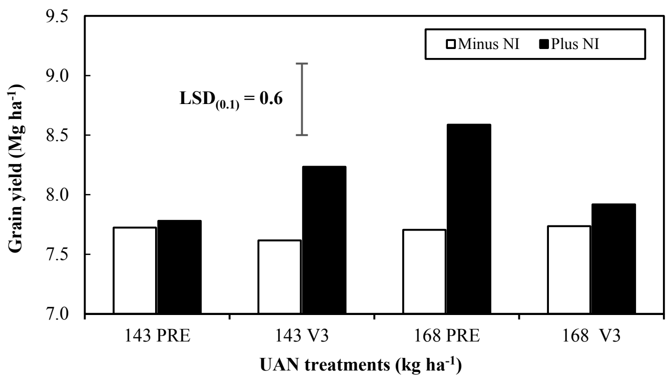

Figure 3. Corn grain yields were affected by UAN application timing (PRE or V3), application rate (143 or 168 kg ha^{-1}) and presence (Plus) or absence (Minus) of nitrification inhibitor (NI) (nitrapyrin). Data were combined over years (2012 to 2015). LSD, least significant difference at $P \leq 0.10$

There was no significant interaction between UAN application rates, timings and nitrapyrin for soil NO_3-N concentration at the 0 to 22 cm depth at V7 (Table 3). However, UAN application timings and experimental years did have a significant interaction for soil NH_4-N at the 0 to 22 cm depth at the V7 growth stage (Table 3). Soil NH_4-N concentration in 2013 for V3 plants was greater (39.3 mg kg^{-1}) than the PRE treatments at this growth stage (Table 5). At V7, soil NH_4-N concentration in 2013 for PRE applied treatments was significantly greater (27.9 mg kg^{-1}) than all the treatments applied PRE (Table 5). The V3 treatments generally resulted in greater soil NH_4-N concentrations over PRE applied treatments (Table 5). An interaction between year and nitrapyrin was noted for soil NO_3-N at 23 to 46 cm depth (Table 3). In 2014, V3 treatments in the absence of nitrapyrin had the greatest soil NO_3-N (13.7 mg kg^{-1}) (Table 6). It was 54% (7.4 mg kg^{-1}) greater than the equivalent treatment with nitrapyrin.

Significant main effects and interactions for soil N concentration in the harvest soil sample were assessed using a three-factor ANOVA at $P \leq 0.1$ (Table 3). UAN at 168 kg ha^{-1} (16 mg kg^{-1}) increased soil NO_3-N by 15% (3 mg kg^{-1}) compared to UAN at 143 kg ha^{-1} at the 0 to 22 cm soil depth (Table 7). There was a year by UAN application timing interaction for soil NH_4-N at the 0 to 22 cm depth. In 2012, soil NH_4-N (13 mg kg^{-1})

concentration was the greatest at 0-20 cm depth with V3 applied UAN, and was greater than all PRE applied N treatments (Table 8). However, V3 applied treatments in 2015 had the lowest soil NH_4-N concentration (4 mg kg^{-1}). Except for 2015, V3 treatments generally resulted in higher soil NH_4-N concentrations compared to PRE treatments. Soil NO_3-N at a 23 to 46 cm depth, had a year × UAN application rate × timing × nitrapyrin interaction. In 2012, V3 UAN at 143 kg ha^{-1} with nitrapyrin (24 mg kg^{-1}) had a 50% (12 mg kg^{-1}) greater soil NO_3-N concentration over the equivalent amount of UAN without nitrapyrin (Table 9). In contrast, V3 applied UAN at 168 kg ha^{-1} in 2013 with nitrapyrin (19 mg kg^{-1}) had a 13% (3 mg kg^{-1}) lower soil NO_3-N concentration compared to UAN without nitrapyrin.

Table 4. Soil NO_3-N and NH_4-N concentrations from 0 to 22 cm depth at V3 growth stage for N application timing of pre-emergence (PRE) and V3, absence (Minus) or presence (Plus) of nitrification inhibitor (NI, nitrapyrin), and UAN rate (143 and 168 kg ha^{-1}). Data were averaged over years (2012-2015)

UAN	PRE†			
	NO_3-N		NH_4-N	
	Minus NI	Plus NI	Minus NI	Plus NI
-------------- kg ha^{-1} -------------	-- mg kg^{-1} --			
-------------------- 0 to 22 cm --------------------				
Non-treated	------------------ 14.2 -----------------		------------------- 5.1 -------------------	
143	35.9	37.1	17.4	17.7
168	45.2	34.3	25.5	19.3
$LSD_{(0.01)}$ ‡	------------------ 19.4 -----------------		------------------ 17.2 -------------------	
-------------------- 23 to 46 cm --------------------				
Non-treated	------------------- 6.2 ------------------		------------------- 5.8 --------------------	
143	10.2	9.3	7.0	5.4
168	10.3	9.4	5.6	6.1
$LSD_{(0.01)}$	------------------- 4.1 ------------------		------------------- NS§ ------------------	

Note. †Only one soil sample from V3 application timing plots at the V3 growth stage was taken because V3 application timing treatments were applied after soil sampling. This one sample was assumed to represent all the V3 application timing plots. Soil NO_3-N and NH_4-N from 0 to 22 cm depth for V3 application timing plots were 13.9 and 6.0 mg kg^{-1}, respectively. Soil NO_3-N and NH_4-N from 23 to 46 cm depth for V3 application timing plots were 4.6 and 5.6 mg kg^{-1}, respectively; ‡Least significant difference at $P \leq 0.01$; § Non-significant.

Table 5. Soil NH_4-N concentrations from 0 to 22 cm depth at V7 corn growth stage for application timing (PRE and V3) and years (2012-2015)

Year	PRE	V3
	-------------------------- mg kg^{-1} --------------------------	
2012	15.8	31.6
2013	27.9	39.3
2014	19.0	19.0
2015	12.3	14.3
$LSD_{(0.1)}$ †	----------------------------- 7.1 ----------------------------	

Note. †Least significant difference at $P \leq 0.10$.

UAN 143 at kg ha^{-1} without nitrapyrin at V3 had soil NO_3-N that was 22% greater compared to the equivalent treatment with nitrapyrin. This was in contrast to what was observed in the equivalent treatment in 2012. This result indicates a possible difference in plant N uptake between years. During a drought year (2012), the highest soil NO_3-N concentrations were observed. All the treatments in 2012 resulted in significantly greater soil NO_3-N concentrations compared to their respective treatments in 2013, 2014 and 2015. The lowest soil NO_3-N concentrations were observed in 2015, and were similar to 2014.

Table 6. Soil NO_3-N from 23 to 46 cm depth at V7 for application timing (PRE and V3), absence (Minus) or presence (Plus) of nitrification inhibitor (NI, nitrapyrin) and years (2012-2015)

Year	PRE		V3	
	Minus NI	Plus NI	Minus NI	Plus NI
	mg kg^{-1}			
2012	2.3	3.7	1.9	2.3
2013	9.5	9.8	7.6	8.7
2014	8.5	10.0	13.7	6.3
2015	6.6	5.8	3.1	4.2
$LSD_{(0.10)}$ †	2.5			

Note. †Least significant difference at $P \leq 0.10$.

Table 7. Soil NO_3-N from 0 to 22 cm depth at harvest for UAN application rates (143 and 168 kg ha^{-1}). Data were averaged across the years (2012-2015)

UAN	NO_3-N
kg ha^{-1}	mg kg^{-1}
143	13.7
168	16.2
$LSD_{(0.10)}$ †	2.4

Note. †Least significant difference at $P \leq 0.10$.

Table 8. Soil NH_4-N from 0 to 22 cm depth at harvest for application timing (PRE and V3) and years (2012-2015)

Year	NH_4-N	
	PRE	V3
	mg kg^{-1}	
2012	4.2	13.3
2013	4.9	5.9
2014	4.6	6.4
2015	4.3	3.8
$LSD_{(0.10)}$ †	2.2	

Note. †Least significant difference at $P \leq 0.10$.

Table 9. Soil NO_3-N from 23 to 46 cm depth at harvest for application rates (UAN 143 kg ha^{-1} and UAN 168 kg ha^{-1}), application timing (PRE and V3), absence (Minus) or presence (Plus) of nitrification inhibitor (NI, nitrapyrin) and years (2012-2015)

Year	UAN 143 kg ha^{-1}				UAN 168 kg ha^{-1}			
	PRE		V3		PRE		V3	
	Minus NI	Plus NI	Minus NI	Plus NI	Minus NI	Plus NI	Minus NI	Plus NI
	mg kg^{-1}							
2012	12.3	11.6	11.9	23.9	12.5	11.2	22.3	19.4
2013	4.7	4.7	9.1	7.1	5.7	5.2	7.3	7.3
2014	1.7	3.3	1.8	1.9	1.7	2.0	2.1	3.7
2015	1.5	1.3	1.6	1.2	1.8	1.2	1.4	1.7
$LSD_{(0.10)}$ †	1.8							

Note. †Least significant difference at $P \leq 0.10$.

3.5 Leaf SPAD Meter Readings

An interaction for leaf SPAD meter readings between year and nitrapyrin at V8 and VT growth stages occurred (Table 3). SPAD readings at V8 and VT in 2015 were the lowest (33 to 43 SPAD units) among all years regardless of the presence or absence of nitrapyrin (Table 10). The highest SPAD reading was noted at VT in 2014 with or without nitrapyrin (58 SPAD units). Nitrapyrin in 2015 at V8 (43 SPAD units) and VT (38 SPAD units) had higher SPAD readings (4 and 5 SPAD units) compared to the absence of nitrapyrin. In 2012 and 2014, SPAD readings at VT increased over SPAD readings at V8, while in 2013 and 2015 SPAD readings decreased at VT compared to V8 regardless of presence or absence of nitrapyrin.

Table 10. SPAD meter readings in the absence (minus) or presence (plus) of nitrification inhibitor (NI, nitrapyrin) measured at the V8 and VT growth stages for the years of 2012 to 2015

Year	V8		VT	
	Minus NI	Plus NI	Minus NI	Plus NI
	SPAD Units			
2012	45.2	45.0	47.4	47.7
2013	56.4	55.2	44.3	44.8
2014	51.6	51.4	58.0	58.3
2015	39.4	42.8	33.1	38.1
$LSD_{(0.10)}$ †	2.2		2.2	

Note. †Least significant difference at $P \leq 0.10$.

All these differences in SPAD readings may have been affected by differences in daily and total precipitation amounts and distribution of precipitation events over the growing seasons. For example, 2012 was relatively a dry year with low daily precipitation events that may have reduced SPAD readings potentially due to low moisture content of soil which limits NO_3-N uptake by plants. Furthermore, 2015 was the wettest year of study and it received high precipitation events over the course of the season which may have increased N losses due to leaching and denitrification mechanisms. Nitrapyrin in 2015 at both V8 and VT stages had a significant effect compared to when nitrapyrin was not applied because nitrification inhibitors are often more effective where risk of N losses due to excessive wet soil conditions is high (Randall et al., 2003).

3.6 Grain Moisture, Test Weight, and Protein Content

Grain moisture had an interaction for UAN application rates, application timings and nitrapyrin at $P \leq 0.1$ (Table 3). UAN at 143 kg ha^{-1} without nitrapyrin at V3 (197 g kg^{-1}) had the highest grain moisture content, and was greater than all the other treatments (Table 11). The UAN at 168 kg ha^{-1} with nitrapyrin applied PRE (180 g kg^{-1}) resulted in the lowest grain moisture content, and was significantly lower (9 g kg^{-1}) than an equivalent treatment at V3. Grain moisture content was generally lower in treatments with nitrapyrin compared to treatment without nitrapyrin.

Table 11. Grain moisture and test weight in the absence (minus) and or presence (plus) of nitrification inhibitor (NI, nitrapyrin) for application timings (PRE and V3) and UAN rates (143 and 168 kg ha^{-1}), and grain protein concentration for application timings and UAN rates. Data were combined over years (2012-2015)

UAN	Moisture		Test Weight		Protein
	Minus NI	Plus NI	Minus NI	Plus NI	
kg ha^{-1}	g kg^{-1}		kg m^{-3}		g kg^{-1}
143 PRE	184	183	747	733	83
143 V3	197	182	728	735	85
168 PRE	184	180	735	740	85
168 V3	188	189	737	731	84
$LSD_{(0.1)}$ †	8		14		2

Note. †Least significant difference at $P \leq 0.10$.

There was an interaction between UAN application rates, application timings and nitrapyrin at $P \leq 0.1$ for grain test weight (Table 3). UAN at 143 kg ha^{-1} without nitrapyrin at the PRE timing increased grain test weight 2% (14 kg m^{-3}) compared to the equivalent treatment with nitrapyrin (Table 11). It was also 3% greater (19 kg m^{-3}) than its equivalent treatment applied at V3 (728 kg m^{-3}). Corn hybrids used in this study each year were not the same. This may be one possible reason the grain test weight was different among treatments.

There was an interaction between UAN application rates and application timings for grain protein (Table 3). PRE UAN at 143 kg ha^{-1} had the lowest grain protein concentration (83 g kg^{-1}) (Table 11). UAN at 143 kg ha^{-1} applied at PRE and UAN at 168 kg ha^{-1} applied at V3 both resulted in similar grain protein concentrations (85 g kg^{-1}), and were greater than V3 applied UAN at 143 kg ha^{-1}. Grain protein results are more likely a representation of leaf SPAD meter readings because both SPAD meter readings and grain protein concentration had similar trends. There was no difference among treatments for grain oil concentration, starch concentration or plant population (data not presented).

Since these treatments were assessed over four growing seasons, climatic variability within growing seasons affects the observed results especially the extreme weather events that occurred during this research. For example, 2015 was relatively the wettest season with a majority of precipitation of the season occurring in a short interval of time, while 2012 was one of the driest seasons on record (USDM, 2015). Hence, the impact of applying nitrapyrin with UAN may have been more positive due to the excess soil moisture.

4. Conclusions

An application of UAN at 143 kg ha^{-1} with nitrapyrin at V3 had the highest grain yield (8.6 Mg ha^{-1}), followed by 7.7 Mg ha^{-1} yield with UAN at 168 kg ha^{-1} with nitrapyrin applied PRE. Soil NO_3-N and NH_4-N concentrations were generally affected by UAN application rates and timings, and relatively less by the application of nitrapyrin. In the wettest year (2015), nitrapyrin increased leaf SPAD meter readings which were likely related to grain protein concentrations. Overall, the presence of nitrapyrin had lower overall grain moisture levels. Plant population, grain oil and starch concentrations were not affected by any of the treatments in the experiment. The highest corn yields were obtained with UAN at 143 kg ha^{-1} with nitrapyrin at V3 and UAN at 168 kg ha^{-1} with nitrapyrin applied at PRE. Based on these findings, a side-dress application of a lower rate of UAN with nitrapyrin at V3 may be effective when the risk of N losses during the growing season due to unfavorable precipitation events and other environmental variables are high. A PRE application of UAN with nitrapyrin was beneficial, but not as effective as applying the nitrapyrin with the side-dress application. However, further research into investigating the cost-benefit ratio of using nitrapyrin with UAN on corn production would be important to assist growers in making decisions on the best timings and rates of UAN in combination with nitrapyrin to utilize in poorly-drained soils.

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