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Pronitridine and Nitrapyrin With Anhydrous Ammonia for Corn

Gurbir Singh[1] & Kelly A. Nelson[1]

[1] Division of Plant Sciences, University of Missouri, Novelty, MO, USA

Correspondence: Gurbir Singh, Lee Greenley Jr. Memorial Research Center, Division of Plant Sciences, University of Missouri, Novelty, MO 63460, USA. Tel: 1-660-739-4410. E-mail: singhgu@missouri.edu

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Abstract

Nitrogen (N) losses due to leaching, denitrification and/or ammonia volatilization are of utmost concerns since they reduce farm profitability and adversely affect environmental quality. To combat these N losses, a new nitrification inhibitor (NI), pronitridine, can be used to slow down the nitrification process. A two-year (2014, 2015) field experiment was conducted to evaluate the effectiveness of pronitridine at different rates (9.4, 18.7, and 28.1 L ha^{-1}) with anhydrous ammonia (AA) at 112 kg N ha^{-1} when applied in the fall or pre-plant on claypan soils in northeast Missouri. Using pronitridine at 9.4 L ha^{-1} with AA in the fall during a low yielding year (2015) increased corn grain yield 7% compared to AA + nitrapyrin (2.3 L ha^{-1}). Agronomic efficiency and yields were greatest with pronitridine at 9.4 L ha^{-1} than AA + nitrapyrin. Grain N removal was highest for AA + pronitridine at 18.7 L ha^{-1} compared to AA + nitrapyrin. Pre-plant application of AA + pronitridine at 9.4 L ha^{-1} increased grain starch content compared to AA without NI, but it was not significantly different from AA + nitrapyrin. Results indicated that pronitridine was effective in increasing yields when applied in the fall and was similar to other NI's when applied pre-plant in the spring.

Keywords: corn, enhanced efficiency fertilizer, nitrification inhibitor, nitrapyrin, Instinct, N-serve, N management, in-season best management practice

1. Introduction

A best management practice (BMP) for reducing the environmental impact of N loss through denitrification, volatilization and leaching while sustaining or increasing corn yields includes the use of a nitrification inhibitor (NI) (Abalos, Jeffery, Sanz-Cobena, Guardia, & Vallejo, 2014; Malhi, Grant, Johnston, & Gill, 2001). Nitrification inhibitor slows down the conversion of ammonium to nitrate, thereby potentially reducing nitrate-N (NO_3-N) losses via leaching or denitrification (Decock, 2014; Wolt, 2004). Nitrapyrin [2-Chloro-6-(trichloromethyl)pyridine] is the most commonly used NI sold as N-Serve® (NS) and Instinct® (I), by Dow AgroSciences, Indianapolis, IN. Nitrapyrin (NS) is generally added to AA for fall N applications to prevent subsurface N leaching losses (Randall, Vetsch, & Huffman, 2003), whereas a micro-encapsulated liquid version of nitrapyrin (I) is used with urea and urea ammonium nitrate (UAN) application.

Extensive research has been conducted on NI's with several reviews on the effects of NI's on the reduction of N loss and the impact on crop production (Aulakh, Rennie, & Paul, 1984; Burzaco, Ciampitti, & Vyn, 2014; Cerrato & Blackmer, 1990; Cook et al., 2015; Fisk, Maccarone, Barton, & Murphy, 2015; Frame, 2017; Prasad, 1995; Wolt, 2004). Research on NI's has shown positive, neutral, or negative effects on grain yield (Quemada, Baranski, Nobel-de Lange, Vallejo, & Cooper, 2013; Wolt, 2004). Randall and Vetsch (2005) reported that the addition of nitrapyrin to fall-applied AA decreased average flow-normalized NO_3-N leaching losses by 10% compared to fall-applied AA without nitrapyrin. Similarly, N_2O emissions decreased 19-27% when nitrapyrin was used with UAN in Indiana (Omonode & Vyn, 2013). The addition of nitrapyrin to AA increased corn grain yield 0.94 Mg ha^{-1} compared to treatments without nitrapyrin (Vetsch & Randall, 2004). In contrast, Stehouwer and Johnson (1990) found no beneficial effect of nitrapyrin on corn grain yield for spring-applied N in Ohio. In Minnesota, grain yield increased with AA + nitrapyrin in one year out of six (Randall & Vetsch, 2005). In contrast, Blackmer and Sanchez (1988) reported corn grain yields were reduced when nitrapyrin was added and they reported that nitrapyrin increased the susceptibility of plants to moisture stress and induced ammonia toxicity to the plants.

Nitrification inhibitors play important role for managing N losses in claypan soils of north central and eastern Missouri (Habibullah, Nelson, & Motavalli, 2018; Nelson, 2018). In Missouri, NI's have been evaluated with N fertilizers for their effect on placement, timing, rates, tillage, N losses in water, N_2O emissions, corn grain quality and yield (Habibullah, Nelson, & Motavalli, 2017; Hanson, Maledy, & Jentes, 1987; Nash, Nelson, & Motavalli, 2013a, 2013b; Nash, Motavalli, & Nelson, 2012; Nelson, 2018; Nelson, Paniagua, & Motavalli, 2009). A new NI, pronitridine (CAS RN 1373256-33-7, Centuro™, Koch Agronomic Services, Wichita, KS), was recently developed to enhance the efficiency of applied N fertilizer by inhibiting the nitrification process and reducing N losses through leaching or denitrification (Gabrielson & Epling, 2016; Nelson, 2018; Vetsch & Schwab, 2014). In Minnesota, UAN was supplemented with three NI's treatments (two rates of pronitridine and one of nitrapyrin) to evaluate the effects on corn grain yield (Vetsch & Schwab, 2014). During a wet year, the lowest corn yields were observed with UAN in the absence of a NI. Nelson (2018) reported that UAN plus pronitridine applied pre-emergence had corn grain yields similar to UAN plus nitrapyrin in Missouri. In California, a significant reduction in N_2O emissions from UAN plus pronitridine treatments was found when compared to other treatments including calcium nitrate and AA with or without NI's (Waterhouse, Wade, Horwath, & Burger, 2017); however, no improvement in corn yields or N use efficiency was observed. There is lack of research on the use of pronitridine with AA application on corn in the Midwest US. Therefore, the objective of this research was to evaluate the effectiveness of pronitridine and nitrapyrin applied in the fall or spring (pre-plant) on corn grain yield, N uptake, and quality.

2. Materials and Methods

2.1 Study Location and Experimental Design

The experiment was conducted at the University of Missouri Greenley Research Center near Novelty, MO (40°1′41.4″ N, 92°11′18.6″ W) in 2014 and 2015. The soil was a Putnam silt loam (fine, smectitic, mesic, Vertic Albaqualfs). These soils typically have a clay layer approximately 45-50 cm below the soil surface which causes poor internal drainage (Nelson & Smoot, 2012), and subsequently results in gaseous N loss (Nash et al., 2012). Precipitation was collected on-site (Table 1) throughout the growing season using an automated weather station (Campbell Scientific, Inc., Logan, UT).

Table 1. Monthly precipitation average (10-year) and precipitation during the growing season at Novelty, Missouri in 2014 and 2015

Month	**Precipitation**		
	10-year average†	2014	2015
	-------------------------------- mm --------------------------------		
April	139	106	71
May	153	26	119
June	94	225	322
July	59	51	257
August	62	164	106
September	86	175	35
Total	612	747	911

Note.† Averaged from 2002 to 2013.

The study was arranged in a randomized complete block design with six replications in plots 3 by 15 m. This research was arranged as two-factor experiment with NI treatments [pronitridine (Centuro™, Koch Agronomic Services, Wichita, KS) at 9.4 L ha^{-1}, 18.8 L ha^{-1}, and 28.1 L ha^{-1}, nitrapyrin (NS) and nitrapyrin (I) at 0.5 kg a.i. ha^{-1}] which were either fall or preplant spring applied with 112 kg N ha^{-1} as AA. In addition to the above NI treatments, AA with UAN (32%) at a rate equivalent to the N present in 9.4 L ha^{-1} of pronitridine and a non-treated control treatment were also included in the experiment.

2.2 Field and Crop Management

Anhydrous ammonia was applied using a John Deere 2510 (Moline, IL) applicator. The pronitridine was injected using a separate tube on each applicator unit. The outlet was as close to the anhydrous stream as possible, *i.e.* 1 cm or less. Pronitridine contains a NI mixed into a 30% N fertilizer (0.38 kg N L^{-1}) that includes slow release N compound. To offset N in the pronitridine, a treatment not receiving pronitridine received 7.9 L ha^{-1} of UAN that was similar to the average N contribution from pronitridine. Nitrapyrin (NS) was applied using a Sidekick^{TM} injection system (Sioux Falls, SD) while nitrapyrin (I) was applied using the same application system as pronitridine. Fall treatments were applied after the soil temperature was below 10 ^{o}C at a depth of 15 cm. Spring pre-plant applications were done at a time consistent with local farming practices. Selected management practices are reported in Table 2.

Table 2. Selected management practices and application information in 2014 and 2015

Field information	**2014**	**2015**
Previous crop	Soybean	Soybean
Tillage	No-till	No-till
Planting date	9 Apr.	18 Apr.
Hybrid	DKC 62-97VT3	DKC 62-97VT3
Seeding rate (seeds ha^{-1})	81,500	81,500
Fertilizer application dates		
Fall	28 Oct. 2013	7 Nov. 2014
Preplant	26 Mar.	23 Mar.
Maintenance	11 Apr.	3 Apr.
N-P_2O_5-K_2O (kg ha^{-1})	22-90-135-22S-2Zn	18-90-90
Crop protection chemicals		
Fungicide	10 July, Azoxystrobin† (0.12 kg a.i. ha^{-1}) + propiconazole (0.10 kg a.i. ha^{-1})	NA‡
Herbicide		
POST	6 May, Acetochlor (2.65 kg a.i. ha^{-1}) + atrazine (1.88 kg a.i. ha^{-1}) + glyphosate (1.06 kg a.i. ha^{-1}) + DAS (0.02 kg L^{-1})	23 Apr. saflufenacil at 0.02 kg ha^{-1} + glyphosate (1.06 kg a.i. ha^{-1}) + UAN at 2.34 L ha^{-1} + MSO at 1% v/v
Late POST	11 June, Glyphosate (1.06 kg a.i. ha^{-1}) + topramezone (0.02 kg a.i. ha^{-1}) + atrazine (0.25 kg a.i. ha^{-1}) + DAS (0.02 kg L^{-1})	10 June, Glyphosate (1.55 kg a.i. ha^{-1}) + mesotrione (0.09 kg a.i. ha^{-1}) + DAS (0.02 kg L^{-1})
Harvest date	6 Oct.	15 Sept.

Note. † Acetochlor (2-chloro-2'-methyl-6'ethyl-N-ethoxymethylacetanilide); atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine); azoxystrobin, methyl (E)-2-(2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl)-3-methoxyacrylate; diammonium sulfate ($(NH4)_2SO_4$); glyphosate, (N-(phosphonomethyl)glycine); MSO, alcohol ethoxylate, phosphatidylcholine; mesotrione (2-[4-(Methylsulfonyl)-2-nitrobenzoyl]cyclohexane-1,3-dione); propiconazole, 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]Methyl]-1H-1,2,4-triazole; and saflufenacil, N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide.

‡ Abbreviations: DAS, Diammonium sulfate; NA, none applied; MSO, Methylated seed oil; POST, post emergence; UAN, urea ammonium nitrate.

2.3 Data Collection

Prior to fall N application, composite soil samples were taken from the plot area of each replication using an Uhland probe from four depths (0-15, 16-30, 31-60, and 61-90 cm). Collected soil samples were analyzed for soil properties given in Table 3 using standard soil testing analytical procedures for Missouri (Nathan, Stecker, & Sun, 2012). The 0-15 cm deep soil samples were analyzed for pH (0.01 M $CaCl_2$), organic matter content, cation exchange capacity, available P, K, Ca, Mg, and gravimetric soil moisture content. Soil samples from all depths were analyzed for nitrate and exchangeable ammonium concentrations.

Table 3. Soil properties at 0 to 15 cm and soil moisture content, soil nitrate (NO_3-N), and ammonium (NH_4-N) concentration at four depths in 2014 and 2015 at the study location

Soil test information	Soil Depth	2014	2015
	cm		
pH_s (0.02 M $CaCl_2$)	0-15	6.0±0.2[†]	6.6±0.2
Bray I-P (kg ha^{-1})	0-15	47.1±7.4	45±25
Exchangeable (1 M NH_4Cl)			
K (kg ha^{-1})	0-15	355±39	253±56
Ca (kg ha^{-1})	0-15	4483±493	5201±473
Mg (kg ha^{-1})	0-15	562±98	527±67
CEC ($cmol_c$ kg^{-1})[‡]	0-15	14.4±1.9	14.3±0.9
OM (g kg^{-1})	0-15	24.1±2.3	29.2±4.2
Soil moisture content (%)	0-15	20.3±0.6	20.5±0.6
	16-30	23.0±2.1	23.8±1.9
	31-60	26.8±3.4	28.0±3.6
	61-90	22.7±2.8	23.7±3.1
NO_3-N (mg kg^{-1})	0-15	4.9±0.9	8.1±2.4
	16-30	5.0±0.7	2.0±0.6
	31-60	2.5±0.7	0.6±0.3
	61-90	3.1±2.2	0.4±0.1
NH_4-N (mg kg^{-1})	0-15	5.7±1.1	3.9±1.6
	16-30	6.0±2.1	3.2±0.3
	31-60	6.9±2.3	5.1±0.9
	61-90	7.0±1.2	2.8±0.5

Note. [†] Standard deviation.

[‡] Abbreviations: CEC, cation exchange capacity; OM, organic matter.

A Minolta SPAD-502 meter (Konica Minolta Optics, Inc., Aurora, IL) was used to collect chlorophyll meter readings from 10 ear leaves $plot^{-1}$ at the VT growth stage of corn (Abendroth, Elmore, Boyer, & Marlay, 2011). The SPAD chlorophyll meter readings have been shown to be strongly related to chlorophyll concentration or greenness of plant leaves (Markwell et al., 1995; Scharf et al., 2006). To determine ear leaf N concentration, 10 ear leaves $plot^{-1}$ were collected at R1 growth stage, dried, and analyzed for total N concentration by combustion using a total C:N analyzer (LECO, TruSPEC CN Analyzer, St. Joseph, MI).

Plant populations were determined before harvest from one row extending the entire length of the plot. Grain yield, moisture, and test weight were determined using a Wintersteiger Delta (Salt Lake City, UT) equipped with a HarvestMaster GrainGage (SBDS800, Juniper Systems Inc., Logan, UT). Grain yields were adjusted to 150 g kg^{-1} prior to statistical analysis. Collected grain samples were analyzed for N concentration by combustion using a total C:N analyzer (LECO, TruSPEC CN Analyzer, St. Joseph, MI). Grain samples were also analyzed for protein, oil, and starch concentration with a Foss Infratec 1241 Grain Analyzer (Eden Prairie, MN). Agronomic efficiency was calculated as $(Y - Y_0)/F$ where Y = grain yield of the harvested portion of corn with nutrient applied, Y_0 = grain yield of corn with no nutrient applied, and F = amount of nutrient applied to determine the short-term impact of N on productivity (Fixen et al., 2015). Additionally, the yield increase for NI treatments was calculated by comparing NI treatments with that of AA with UAN.

2.4 Statistical Analysis

Prior to data analysis, all variables were subjected to normality analysis using the Univariate procedure in SAS (SAS, 2014). The Glimmix procedure in SAS was used for analysis. The fixed factor in the statistical model was NI treatments, timing of N applications and year whereas replications were considered as random factors. The three-way interaction between fixed factors was not statistically significant for any of the dependent variables and there was no significant difference in the timing of the application of the treatments. Therefore, timing of N application was dropped as a fixed factor and data were analyzed separately for fall and spring applied treatments. The T-grouping for least square means ($P = 0.1$) was used to determine differences among NI treatments and years.

3. Results and Discussion

3.1 Growing Season Precipitation and Its Effects on Corn Yield

The total precipitation for 2014 and 2015 was 135 and 299 mm higher, respectively, than the 10-year average precipitation received during the growing season from April to September (Table 1). June was the wettest month during the two growing seasons. June and July of 2015 received 228- and 198-mm higher rainfall compared to the 10-year average monthly precipitation. In 2015, wet conditions during the corn growing season probably contributed to lower overall yields compared to 2014 (Figure 1). Claypan soils, if not drained during wet years, can result in waterlogged conditions causing N loss (runoff or denitrification), and reduced N availability to the cash crop (Kaur, Zurweller, Nelson, Motavalli, & Dudenhoeffer, 2017). A reduction in N availability during wet years can reduce corn grain yield (Figure 1).

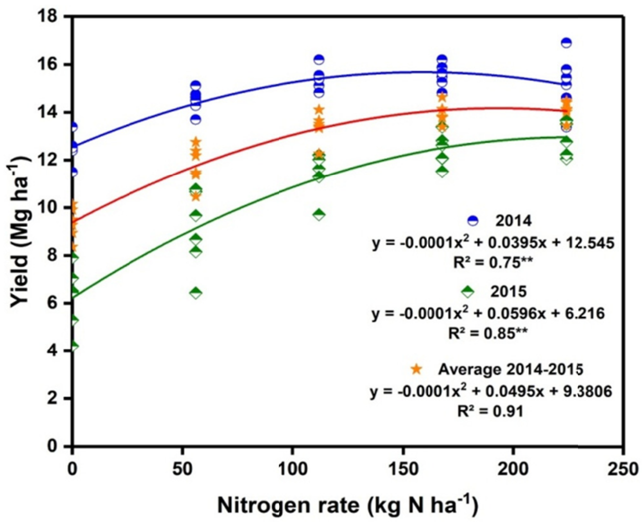

Figure 1. Corn yield response to N rates applied in the spring in the absence of a nitrification inhibitor in 2014 (blue line with circles), 2015 (green line with diamonds) and the average from 2014-2015 (orange line with stars). Individual points at each nitrogen rate represents six replicates in each year

3.2 Fall N Application

3.2.1 Chlorophyll Meter Readings

The chlorophyll meter readings for fall N applications were affected by an interaction between year and NI (Tables 4 and 6). Many studies have shown a positive correlation between chlorophyll meter readings and N fertilizer application rates (Schepers et al., 1992; Costa et al., 2001). The chlorophyll meter reading in the nontreated control in 2014 was 15.53 SPAD unit's greater than 2015. The 2015 growing season was comparatively wetter than 2014 (Table 1). The excessive soil moisture due to wet growing season conditions may have adversely affected plant growth and consequently, reduced N uptake due to restricted root growth and lowered N availability due to N denitrification loss (Kisaakye et al., 2015; Nash et al., 2015; Kaur et al., 2018). Reduction in N uptake under wet soil conditions might have resulted in lower chlorophyll meter readings and earleaf N concentration in our study (Table 6).

Table 4. Probability values (p-values) and numerator degrees of freedom (df) associated with the sources of variation in the statistical analysis of nitrification inhibitor treatments when N was applied in fall at 112 kg N ha^{-1}

Source of variation	df	Chlorophyll meter	Ear leaf N	Population	Moisture	Test weight	Yield	Yield increase[†]	Grain N	Grain N removal	Agronomic efficiency	Protein	Oil	Starch
		---------- p-values ----------												
Nitrification inhibitor	6	<0.0001	<0.0001	0.0923	0.0863	<0.0001	<0.0001	0.0990	<0.0001	<0.0001	0.0985	<0.0001	0.9469	0.2919
Year	1	0.0003	<0.0001	0.3628	<0.0001	<0.0001	<0.0001	0.8068	<0.0001	<0.0001	<0.0001	<0.0001	0.0751	<0.0001
Nitrification inhibitor*Year	6	<0.0001	0.0005	0.4566	0.7609	0.5947	<0.0001	0.2056	0.0880	0.8925	0.2074	0.0340	0.0327	0.0176

Note. [†] Yield increase for NI treatments was calculated by comparing NI treatments with that of AA with UAN.

Table 5. Probability values (p-values) and numerator degrees of freedom (df) associated with the sources of variation in the statistical analysis of nitrification inhibitor treatments when N was applied in spring (pre-plant) at 112 kg N ha^{-1}

Source of variation	df	Chlorophyll meter	Ear leaf N	Population	Moisture	Test weight	Yield	Yield increase†	Grain N	Grain N removal	Agronomic efficiency	Protein	Oil	Starch
		p-values												
Nitrification inhibitor	6	<0.0001	<0.0001	0.3290	0.0054	0.3670	<0.0001	0.3063	<0.0001	<0.0001	0.3369	<0.0001	0.4464	0.0040
Year	1	<0.0001	<0.0001	0.1388	<0.0001	0.0002	<0.0001	0.9803	<0.0001	<0.0001	0.0035	<0.0001	0.1047	<0.0001
Nitrification inhibitor*Year	6	0.0084	0.0549	0.6270	0.6933	0.4751	0.1184	0.8768	0.2255	0.7040	0.9529	0.0014	0.4672	0.6703

Note. † Yield increase for NI treatments was calculated by comparing NI treatments with that of AA with UAN.

Table 6. Corn response to nitrification inhibitor with anhydrous ammonia when N amount was 112 kg N ha^{-1} (α = 0.1). Results of significant interaction between nitrification inhibitor treatment and year

Nitrification Inhibitor	Application Rates	Year	Fall Application							Spring Application		
			Chlorophyll meter	Ear leaf N	Corn Grain Parameters					Chlorophyll meter	Ear leaf N	Grain Protein
					Yield	Grain N	Protein	Oil	Starch			
	L ha^{-1}		SPAD units	g kg^{-1}	Mg ha^{-1}	------------------ g kg^{-1} ------------------				SPAD units	-------- g kg^{-1} -------	
Pronitridine	9.4	2014	57.68abc§	32.43a	15.77a	13.02a	83a	38a	728f	60.03a	31.16a	82b
	9.4	2015	54.67cde	25.87cd	11.52cd	10.00bcd	68de	32c	748a	54.77d	25.52bcd	70de
Pronitridine	18.7	2014	58.82ab	32.59a	15.52a	13.40a	83a	37ab	731ef	58.55ab	32.43a	84a
	18.7	2015	55.93bcde	26.99bc	11.11cde	10.58bc	73b	35b	740bc	51.60e	25.25bcd	72cd
Pronitridine	28.1	2014	58.40abc	32.80a	15.43a	12.79a	82a	36ab	734de	58.03abc	32.44a	83ab
	28.1	2015	53.23ef	26.8bc	10.37e	10.09bcd	70bcd	35b	741b	53.00de	23.76d	70de
Nitrapyrin (NS)†	2.3	2014	59.88a	32.97a	15.25a	12.79a	81a	35b	735cde	58.48ab	32.71a	81b
	2.3	2015	51.25f	24.54d	10.78e	9.90cde	71bcd	35b	740bc	54.12de	25.88bc	72cd
Nitrapyrin (I)	2.6	2014	59.90a	32.88a	15.21a	13.03a	82a	35b	734de	59.95a	31.82a	80b
	2.6	2015	56.28abcd	28.32b	11.84bc	9.63de	72b	36ab	739bcd	55.82bcd	26.77b	74c
AA with UAN‡	7.9	2014	58.82ab	32.59a	15.55a	12.81a	80a	35b	735cde	59.92a	31.63a	85a
	7.9	2015	54.87cde	27.37bc	11.48cd	10.46bc	71bc	35b	743ab	55.52bcd	24.63cd	74c
Non-treated Control		2014	54.55def	28.53b	12.52b	10.73b	68cd	36ab	740bc	55.28cd	27.12b	68ef
		2015	39.02g	15.45e	5.08f	9.26e	64e	36ab	743ab	41.63f	17.14e	65f

Note. † Nitrapyrin was applied as N-serve (NS) or Instinct (I).

‡ UAN (32%) is equivalent to 9.4 L ha^{-1} Pronitridine.

§ Within a column and a given factor, means followed by the same letter are not statistically different (α = 0.1).

All NI treatments had significantly higher chlorophyll meter readings than the non-treated control in both years due to higher N availability and N uptake by corn plants with NI treatments than the non-treated control which received no N fertilizer application. No significant differences were observed between pronitridine applied at 9.4, 18.7 or 28.1 L ha^{-1}, nitrapyrin (NS or I), and AA with UAN (32%) in 2014. In 2015, use of nitrapyrin (I) resulted in chlorophyll meter readings that were 3.05 and 5.03 SPAD unit's higher than pronitridine at 28.1 L ha^{-1} and nitrapyrin (NS), respectively (Table 6). Similarly, Nelson (2018) did not find any differences between the pronitridine and nitrapyrin when used with UAN at 67 and 135 kg N ha^{-1}. However, increasing the N application rate to 202 kg N ha^{-1} resulted into higher chlorophyll meter readings with nitrapyrin (I) than pronitridine at 9.4 L ha^{-1}. Increasing the application rate of pronitridine in 2015 did not result in any differences in chlorophyll meter readings. Use of AA with UAN had chlorophyll meter reading that were 3.62 SPAD unit's higher than nitrapyrin (NS) applied in 2015. Use of nitrapyrin (I) and pronitridine at 9.4 or 18.7 L ha^{-1} showed no significant differences between years for chlorophyll meter readings. The nitrapyrin (NS), AA with UAN, and pronitridine at 28.1 L ha^{-1} treatments had chlorophyll meter reading that were 8.63, 3.95, and 5.17 SPAD unit's higher in 2014 compared to 2015, respectively.

3.2.2 Ear Leaf N Concentration

The ear leaf N concentration due to fall N applications was affected by the NI, year, and their interaction (Tables 4 and 6). Like chlorophyll meter readings, all NI treatments had significantly higher ear leaf N concentration than the non-treated control in each year, which was possibly due to higher N availability for uptake by plants in these treatments. No significant differences were observed between pronitridine rates (9.4, 18.7 or 28.1 L ha^{-1}), nitrapyrin (NS or I), and AA with UAN in 2014. Ear leaf N concentration was similar among different rates of pronitridine in both years. Nitrapyrin (I) had 2.45 g kg^{-1} greater ear leaf N concentration than pronitridine at 9.4 L ha^{-1} in 2015. Nitrapyrin (NS) had significantly lower ear leaf N concentration than all other NI except pronitridine at 9.4 L ha^{-1} and the non-treated control in 2015. In general, ear leaf N concentrations of all treatments were significantly higher in 2014 than 2015. The non-treated control in 2014 had 13.8 g kg^{-1} higher ear leaf N concentration than 2015. Multiple studies have reported positive or no effect of NI applied in fall on the ear leaf N concentration (Blackmer & Sanchez, 1988; Boswell, 1977; Nash et al., 2013b; Touchton, Hoeft, & Welch, 1979). Nitrapyrin added in fall with 67 kg N ha^{-1} as urea increased N concentration by 15% in the ear leaf when compared to urea without nitrapyrin (Touchton et al., 1979).

3.2.3 Grain Moisture, Test Weight, and Yield

Corn grain moisture of the fall N application was affected by the main effects of NI and year; however, the interaction between these factors was not significant (Table 4). Similar to chlorophyll meter readings and earleaf N concentration, grain moisture was not affected by the differences in application rates of pronitridine. Pronitridine at 18.7 L ha^{-1} increased grain moisture 6.41 and 6.08 g kg^{-1} compared to nitrapyrin (NS) and AA with UAN, respectively. An increase in grain moisture with pronitridine might delay corn harvesting slightly and affect grain quality. No differences in grain moisture were observed between pronitridine at any rate and nitrapyrin (I). All NI treatments had greater grain moisture than the non-treated control except AA with UAN or nitrapyrin (NS). Grain test weight was affected by NI's and year, but no interaction was present (Table 4). All of the NI treatments and AA with UAN had 1.27 to 1.55 kg hL^{-1} greater test weights than the non-treatment control treatment.

Corn grain yield was affected by the interaction between NI's and year for the fall N applications (Tables 4 and 6). Corn grain yields were greater in 2014 than 2015 for all treatments. The non-treated control had 7.44 Mg ha^{-1} greater yield in 2014 than 2015. Corn grain yield with all NI treatments was significantly higher than the non-treated control in both years. No significant differences were observed between NI treatments in 2014 which indicated that all NI's were equally efficient based on corn grain yield. An increase in the application rate of pronitridine from 9.4 to 28.1 L ha^{-1} reduced corn grain yield 1.15 Mg ha^{-1} in 2015 which could be due to greater N remaining in the ammonium form. Pronitridine at 28.1 L ha^{-1} reduced grain yield by 1.47 and 1.11 Mg ha^{-1} compared to nitrapyrin (I) and AA with UAN, respectively, in 2015. Use of nitrapyrin (I) increased corn grain yield 1.06 Mg ha^{-1} greater than nitrapyrin (NS) in 2015. Using pronitridine at 9.4 L ha^{-1} with AA in fall during the low yielding year (2015) increased corn yield 0.74 Mg ha^{-1} compared to AA plus nitrapyrin (NS) (Table 6).

During a low yielding year like 2015, the anoxic conditions due to higher water availability early in the season may have caused abiotic stress to corn plants. A supplemental application of N is recommended when a plant suffers abiotic stress during a wet growing season. Adding higher rates of NI's during a wet year can result in reduced availability of N in soil to the corn plants (Hendrickson, Walsh, & Keeney, 1978), which might result in a yield reduction. This can be the possible reason for lower corn grain yields with pronitridine when applied at higher rates compared to treatments that received pronitridine at lower rates in our study. In a study conducted by Blackmer and Sanchez (1988) in Iowa, a significant reduction in corn grain yield was observed when nitrapyrin was added to AA. The authors reported that nitrapyrin increased the susceptibility of plants to moisture stress and induced ammonia toxicity. To limit the yield losses due to NI's, an optimum combination of NI's application rate with N fertilizer is needed. In our study, pronitridine at 9.4 L ha^{-1} with AA during 2015 increased corn yield compared to AA plus nitrapyrin (NS). The yield increase with pronitridine at 9.4 L ha^{-1} could have also been due to a reduction in N loss via denitrification and timely availability of N to corn during the 2015 growing season.

3.2.4 Corn Grain N Content, Removal, and Agronomic Efficiency

Corn grain N content was significantly affected by the interaction between NI and year (Tables 4 and 6). All NI treatments had higher grain N content than the non-treated control in both years except nitrapyrin (NS or I) in 2015. No differences in grain N content were observed between NI treatments in 2014. In 2015, nitrapyrin (I) had 0.83 and 0.95 g kg^{-1} lower N content in the grain than AA with UAN and pronitridine at 18.7 L ha^{-1}, respectively. Grain N content in 2014 was greater than 2015 for all treatments. The non-treated control had 1.47 g kg^{-1} more grain N content in 2014 than 2015. Grain N removal was greater with the use of all NI treatments compared to non-treated control (Table 7). Pronitridine at 18.7 L ha^{-1} had 11.79 and 11.62 g kg^{-1} greater grain N removal than pronitridine at 28.1 L ha^{-1} and nitrapyrin (NS), respectively (Table 7). Nitrapyrin (I) and AA with UAN were similar to pronitridine at any rate for grain N removal. Pronitridine at 28.1 L ha^{-1} had 6.62 and 5.53 kg kg^{-1} lower agronomic efficiency than pronitridine at 9.4 L ha^{-1} and nitrapyrin (I), respectively. Nitrapyrin (NS) had 5.64 kg kg^{-1} lower agronomic efficiency than pronitridine at 9.4 L ha^{-1}.

Table 7. Corn response to nitrification inhibitor with anhydrous ammonia when N amount was 112 kg N ha^{-1} (α = 0.1). Results of significant nitrification inhibitor treatments

Nitrification Inhibitor	Application Rates	Fall Application					Spring Application				
		Grain Moisture	Test Weight	Grain N removal	Agronomic efficiency	Yield increase[¶]	Corn Grain Parameters				
							Moisture	Yield	N	N removal	Starch
	L ha^{-1}	g kg^{-1}	kg hL^{-1}	g kg^{-1}	kg kg^{-1}	%	g kg^{-1}	Mg h^{-1}	g kg^{-1}	kg ha^{-1}	g kg^{-1}
Pronitridine	9.4	173.42ab[§]	71.88a	160.30ab	43.22a	1.66a	174.75ab	13.53ab	11.06b	153.61a	738b
Pronitridine	18.7	178.33a	72.19a	162.87a	40.29abc	-1.13abc	181.75a	13.30ab	11.38b	155.11a	735cd
Pronitridine	28.1	174.25ab	72.10a	151.08b	36.60c	-4.66c	169.67cb	13.41ab	11.36b	156.55a	736bcd
Nitrapyrin (NS)[†]	2.3	171.92bc	71.95a	151.25b	37.58bc	-3.63bc	175.83ab	13.26b	11.6ab	156.46a	738bc
Nitrapyrin (I)	2.6	174.75ab	72.23a	155.97ab	42.13ab	1.17ab	173.08b	13.91a	11.46ab	161.90a	737bcd
AA with UAN[‡]		172.25bc	72.19a	160.21ab	-	-	177.25ab	13.12b	11.95a	160.00a	734d
Non-treated Control		167.50c	70.68b	90.72c	-	-	163.00c	9.36c	9.63c	93.83b	742a

Note. [†] Nitrapyrin was applied as N-serve (NS) or Instinct (I).

[‡] UAN (32%) is equivalent to 9.4 L ha^{-1} Pronitridine.

[§] Within a column and a given factor, means followed by the same letter are not statistically different (α = 0.1).

[¶] Yield increase for NI treatments was calculated by comparing NI treatments with that of AA with UAN.

Grain N content was reported to decrease when using nitrapyrin with AA by Hendrickson et al. (1978) whereas Boswell (1977) found no differences in grain N content for AA plus nitrapyrin when compared to non-treated AA in a three-year study. Tissue N content was lower in one out of three years of study for the AA only treatment compared to AA plus nitrapyrin (Boswell, 1977). Pronitridine and nitrapyrin used with UAN increased grain N removal and agronomic efficiency of corn in Missouri (Nelson, 2018).

3.2.5 Corn Grain Quality

The grain protein content was significantly higher in 2014 than 2015 for all treatments which was possibly due to wet conditions during the 2015 growing season. Grain protein content was greater with the use of all NI treatments compared to the non-treated control in 2014 and 2015, except for pronitridine at 9.4 L ha^{-1} in 2015. Greater protein concentration in grain could be due to higher grain N concentration since N is a primary component of protein (Hay et al., 1953; Tsai et al., 1992). No significant differences in protein content between NI treatments were observed in 2014. In 2015, pronitridine at 9.4 L ha^{-1} had 5 g kg^{-1} lower protein content than the pronitridine at 18.7 L ha^{-1}. Pronitridine at 9.4 L ha^{-1} had 5 and 4 g kg^{-1} lower protein content than nitrapyrin (I) and AA with UAN in 2015.

In 2014, nitrapyrin (NS and I) and AA with UAN had 3 g kg^{-1} higher oil content in grain than pronitridine at 9.4 L ha^{-1}. No significant differences were found for oil content between the non-treated control and other NI treatments in 2014. In 2015, pronitridine at 9.4 L ha^{-1} had 3 to 4 g kg^{-1} lower oil content than all other treatments including the non-treated control. Pronitridine at 9.4 L ha^{-1} had 5.56 g kg^{-1} more oil content in grain in 2014 than 2015.

The non-treated control had higher grain starch content than all treatments except nitrapyrin (NS) and AA with UAN in 2014. Similarly, pronitridine at 9.4 L ha^{-1} had a lower starch content than all treatments except pronitridine at 18.7 L ha^{-1} in 2014. However, pronitridine at 9.4 L ha^{-1} had higher starch content than all treatments except AA with UAN and non-treated control in 2015.

The grain starch concentration for pronitridine at 9.4, 18.7, and 28.1 L ha^{-1} was 20, 9, and 7 g kg^{-1} less in 2014 than 2015, respectively. Similarly, AA with UAN had 8 g kg^{-1} more starch content in 2015 compared to 2014. No such differences between years were obtained for starch content for nitrapyrin (NS or I) and non-treated control treatments. Higher starch content in corn grain under waterlogged conditions compared to non-waterlogged soil conditions was also observed by Kaur et al. (2017) in claypan soils of Missouri.

3.3 Spring N Application

3.3.1 Chlorophyll Meter Readings

Chlorophyll meter readings were greater in 2014 compared to 2015 for all treatments. The non-treated control had 13.65 SPAD unit's higher chlorophyll meter reading in 2014 than 2015. Chlorophyll meter readings for spring N application was significantly affected by the interaction between NI and year. All NI treatments had higher chlorophyll meter readings than the non-treated control each year. No differences were found between the

NI treatments in 2014. However, AA with UAN, nitrapyrin (I), and pronitridine at 9.4 L ha^{-1} had 3.92, 4.22, and 3.17 SPAD unit's higher chlorophyll meter readings in 2015 than pronitridine at 18.7 L ha^{-1}, respectively.

3.3.2 Ear Leaf N Concentration

Ear leaf N concentrations were greater in 2014 compared to 2015 for all treatments. The non-treated control had chlorophyll meter reading that was 13.65 SPAD units higher in 2014 than 2015. All treatments had higher ear leaf N concentrations compared to the non-treated control each year. Touchton et al. (1979) also reported 14% higher ear leaf N concentration with spring N applications at 134 kg ha^{-1} than the non-treated control. No differences were found between NI treatments in 2014. Nitrapyrin (I) and (NS) had 3.01 and 2.12 g kg^{-1} higher ear leaf N concentration than pronitridine at 28.1 L ha^{-1} in 2015. Increasing the application rate of pronitridine did not increased ear leaf N concentration. In 2015, nitrapyrin (I) increased ear leaf N concentration 2.14 g kg^{-1} greater than AA with UAN. Nitrapyrin (I) inhibits the nitrification process and may have allowed prolonged availability of N to corn plants throughout the growing season compared to single pre-plant application of AA with UAN.

3.3.3 Corn Grain Moisture and Yield

Pronitridine at 18.7 L ha^{-1} had 8.67 to 18.75 g kg^{-1} higher grain moisture than the pronitridine at 28.1 L ha^{-1}, nitrapyrin (I), and non-treated control. All NI treatments had 3.77 to 4.55 Mg ha^{-1} higher grain yield than the nontreated control. A consistent increase in corn yields with the use of NI's was presumably due to reduced nitrification rates and subsequently lower denitrification losses with the inhibitor (Nash et al., 2013b). Use of pronitridine at different rates provided similar yields as with nitrapyrin (NS) for spring N applications. However, no differences were obtained between the different rates of pronitridine when spring applied in this study. Nitrapyrin (I) had 0.65 to 4.55 Mg ha^{-1} greater yields than nitrapyrin (NS), AA with UAN, and the non-treated control, respectively.

3.3.4 Corn Grain N Content and Removal

Use of AA with UAN in spring had 2.32 g kg^{-1} higher grain N content than the non-treated control. Pronitridine at 9.4, 18.7, and 28.1 L ha^{-1} had 0.57 to 0.89 g kg^{-1} lower grain N content than AA with UAN. Higher corn grain N content might have resulted from availability of more N for uptake by plants than in non-treated control that received no N applications or pronitridine treatments. All NI treatments resulted in 59.78 to 68.07 kg ha^{-1} greater N removal than the non-treated control. In contrast, there was an 11% reduction in grain N concentration with nitrapyrin (NS) (Touchton et al., 1979). No significant differences were found between NI treatments in our research.

3.3.5 Corn Grain Quality

Grain protein content was significantly higher in 2014 than 2015 for all NI treatments except the non-treated control. A study conducted by Kaur et al. (2017) in the claypan soils reported that corn grain protein content was reduced with an increase in duration of waterlogged soil conditions. The same study reported that protein content of corn grain was reduced by 1.50 g kg^{-1} with each day of flooding (Kaur et al., 2017). Pronitridine applied at 18.7 L ha^{-1} had 4 to 17 g kg^{-1} greater grain protein concentration than the nitrapyrin (NS and I) and non-treated control in 2014.The greater protein concentration could be due greater assimilation of N in the grain (Tsai et al., 1992). In 2015, both nitrapyrin (I) and AA with UAN increased protein content 5 to 10 g kg^{-1} compared to the non-treated control and pronitridine applied at 28.1 L ha^{-1}. The non-treated control had greater starch content than all other NI treatments. Pronitridine at 9.4 L ha^{-1} had 3 to 4 g kg^{-1} greater starch content than pronitridine at 18.7 L ha^{-1} and AA with UAN. Nitrapyrin (NS) had 3 g kg^{-1} higher grain starch concentration than AA with UAN. Yearly differences in corn grain quality in response to different NI's used in this study might have occurred through variation in soil moisture and temperature conditions which affects the decomposition or dissolution of the NI's (Bronson, Mosier, & Bishnoi, 1992; McCarty & Bremner, 1989).

4. Conclusion

Our study indicates that the new NI pronitridine was effective in increasing corn grain yield in a low yielding year when rainfall received was 49% greater than the 10-yr average. During a wet year, denitrification loss of N is higher and when supplemented with a NI like pronitridine may limit the denitrification loss and make N more available during the corn growing season. Additionally, pronitridine at 9.4 L ha^{-1} + AA applied in fall increased the agronomic efficiency 5.64 kg kg^{-1} compared to nitrapyrin (NS) + AA indicating that fall applied pronitridine + AA on claypan soil was a better option for farmer to add to AA for corn. In addition to the observed increased yield and agronomic efficiency, fall application of N generally saves money and time for farmers and increases efficiency of planting during spring. In this study, spring applied pronitridine + AA was as effective in increasing yield as compared to other NI's + AA. The greater benefit of using pronitridine as NI is the handling of the pronitridine compared to nitrapyrin. Pronitridine does not corrode the mixing tank and exposure to pronitridine is not classified as hazardous.

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