

MANAGEMENT OF NITROGEN AND NITRIFICATION INHIBITORS FOR CORN  
AND WHEAT PRODUCTION ON CLAYPAN SOILS

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A Thesis  
presented to  
the Faculty of the Graduate School  
at the University of Missouri-Columbia

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In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science

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MAY 2017

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AND WHEAT PRODUCTION ON CLAYPAN SOILS

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and hereby certify that, in their opinion, it is worthy of acceptance.

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## **DEDICATION**

I would like to dedicate this work to my family for always supporting me.

## **ACKNOWLEDGMENTS**

I would first like to express gratitude to the United States Agency for International Development (USAID) for providing funding for this scholarship. I would then like to take the opportunity to express gratitude to my co-advisor, Dr. Peter Motavalli, for accepting me into the Soils Program at the University of Missouri. Dr. Motavalli was always willing to take time out of his busy schedule to share his wisdom and give advice on issues related to research and planning for the future. Next, I would like to express gratitude to my co-advisor, Dr. Kelly Nelson, for the guidance and experience he has granted me. The opportunity to work with Dr. Nelson at Greenley Memorial Research Center has provided invaluable experience in numerous aspects of agronomic research. I would also like to thank Dr. Muhammad Imtiaz and Shamim Akhtar from CIMMYT-Pakistan, and Dr. Thomas Rost and Luara Lovgren from UC-Davis for their hard work and commitment to make the initial selection, immigration process and our stay in the U.S. smoother. I would also like to acknowledge Chris Dudenhoffer, and the Greenley Memorial Research Center staff, in particular, Lynn Bradley, for all the time and assistance they provided during my research. I would like to sincerely thank Christy Copeland, Linda Journey and Judy Prevo from the International Programs office at the College of Food Agriculture and Natural Resources. They did everything during my entire stay to make my life easier and more enjoyable. I would additionally like to thank Dr. Robert Kremer for accepting my request of sitting on my thesis committee. His knowledge of soils has been critical during my research and will continue to benefit me in future. I would like to thank Anna Carrillo Arnal and Kristen Kalz for making my stay at Mizzou pleasant through their friendly support and love.

Lastly, I would like to thank my fellow graduate students for their help and support over the duration of my degree; in particular, I would like to acknowledge Gurpreet Kaur, Theodore Blumenschein, and Rafid Al Ubori, for their help in collecting data for this research project.

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# **CHAPTER 1**

## **LITERATURE REVIEW**

### **INTRODUCTION**

#### **Food Demand**

The global human population, which is currently 7.3 billion, is expected to reach 9.2 billion by 2050 (Zilberman et al., 2013). This population rise will increase the demand for food production and the potential for food insecurity. The productivity of existing arable land will have to be improved as opposed to solely employing more land for the production of food because the amount of arable land is finite (Driever et al., 2014). According to Brundtland et al. (1987), sustainable economic growth is a combination of economics and ecology, and can be described by the term sustainable development. Therefore, strategies for achieving future increases in food production need to focus on practices that are not only effective in increasing production but also are based on conserving the natural resource base and on minimizing potential environmental contamination. The question of how to increase food, feed and fiber while sustaining better environmental quality has been a vital issue for researchers and policy makers. One way of achieving this goal would be to enhance sustainable food productivity by improving plant nutrient use efficiency since under- or over-use of plant nutrients is a major constraint for sustainable cropping systems around the world and has led to environmental degradation of soil, water bodies and the atmosphere (Motavalli et al., 2008a).

#### **Corn Production**

According to (Grace et al., 2011a), global demand for corn as food, feed and bioenergy continues to rise. The North Central Region (NCR) of the U.S., which

comprises 12 Midwestern states (North Dakota, South Dakota, Kansas, Michigan, Iowa, Missouri, Nebraska, Wisconsin, Illinois, Indiana, Minnesota and Ohio), is a major producer of corn. Throughout the world, the U.S. produces and exports the largest amount of corn. The Midwestern U.S. region provides 80% of this production (Özdoğan et al., 2012). More than 25% of the global trade in wheat, maize, soybeans and, cotton credits to the United States (Adams et al., 1999). In China, wheat-maize system alone provides more than 50% of the total food produced (Yearbook, 1999).

### **Wheat Production**

Wheat is one of the most important crops in the world. Globally, it provides more than 20% of the calories, and 2.5 billion people consume a similar proportion of protein from wheat (Braun et al., 2010a). The North Central Region of the U.S. fulfills half of the nation's wheat requirements (Grace et al., 2011a). The current increase in global wheat productivity is only 1.1% per annum or even stagnant in some parts (Brisson et al., 2010a). In contrast, the global demand is predicted to increase to 1.7% per annum by 2050 (Rosegrant and Agcaoili, 2010a), which indicates the growing demand and current wheat yield gain per annum needed to be met. This deficit in wheat production illustrates the need for new strategies to achieve increased productivity in order to avoid shortfalls (Hawkesford et al., 2013a).

### **Nitrogen**

Nitrogen (N) is a substantial part of all living organisms and is essential for life. It has been estimated that approximately one-third of the people on earth are alive because of the use of manufactured N fertilizers to provide protein from plant and animal-based foods (Smil, 1997a). Biological growth in most land and water environments on earth are

N-limited. The overall sequence of biochemical changes of N in the environment is called the N cycle (Scharf, 2015). The N cycle in the last 2.5 billion years has been affected by humans more than any other phenomenon (Canfield et al., 2010).

### **Nitrification**

Nitrification has been defined as a microbially mediated conversion of reduced forms of N (e.g., ammonium ( $\text{NH}_4^+$ )) into oxidized forms (e. g. nitrate ( $\text{NO}_3^-$ )) (O'Sullivan et al., 2013; Gao et al., 2015). Ammonia oxidation i.e., oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , is the first step and rate-determinant step in the nitrification process. Nitrification is an essential to the global N cycle. It plays a vital role in agricultural production systems due to its various N-loss pathways.

Soil and environmental conditions influence the nitrification rate (Williamson et al., 1998; Di and Cameron, 2002). After rewetting of dry soil in incubation studies, a time lag of 4 to 10-day has been reported between addition of  $\text{NH}_4^+$  and its active nitrification (MacLean and McRae, 1987; Mulvaney et al., 1997; Williams et al., 1998). Adding up to  $200 \text{ mg kg}^{-1}$  of  $\text{NH}_4^+$  can increase nitrification (Malhi and McGill, 1982). Low soil moisture has adverse effects on activity of nitrifying bacteria because it reduces substrate ( $\text{NH}_4^+$ ) diffusion and intracellular water potential (Agehara and Warncke, 2005). Substrate diffusion is a problem at a water pressure greater than  $-0.6 \text{ MPa}$  while potential for cell dehydration increases at water potential less than  $-0.6 \text{ MPa}$  (Stark and Firestone, 1995). Soil pH in addition to soil moisture has also been reported to affect the nitrification process (Agehara and Warncke, 2005).

Nitrification has been reported to occur over a wide range of 4.5 to 10 pH, however, its optimum level is at 8.5 and it decreases with lower levels of pH (Montagnini

et al., 1989; Paavolainen and Smolander, 1998; Ste-Marie and Paré, 1999; Havlin et al., 2005). Urea temporarily increases soil pH soon after its application but ultimately brings down below the original pH level (Martikainen, 1985; Mulvaney et al., 1997).

Nitrification increases with increasing temperatures. However, it reaches its peak between 25 to 35 °C temperatures (Justice and Smith, 1962; Kowalenko and Cameron, 1976; Malhi and McGill, 1982; Breuer et al., 2002). Increased soil N mineralization has been associated with an increase in temperature (Campbell and Biederbeck, 1972; Stanford et al., 1973; Sierra, 1997; Kätterer et al., 1998). Agehara and Warncke (2005) reported that higher temperatures increased activity of nitrifying bacteria, and that soil  $\text{NO}_3\text{-N}$  were positively correlated to soil temperature. This increase in soil  $\text{NO}_3\text{-N}$  was associated with an increase in  $\text{NH}_4^+$  production during mineralization.

The nitrifying bacteria in soil have been reported to be inactive in cold soils and this inactivity prevails until the temperatures reach 4 to 5 °C (Schmidt, 1982). However, nitrification has also been observed in frozen soils (Nyborg and Malhi, 1979). The importance of the cumulative effect of relatively slow nitrification in cold soils from the period of fall-to-spring has been well documented (Frederick, 1956; Anderson and Boswell, 1964; Frederick and Broadbent, 1966; Sabey, 1969; Campbell et al., 1973; Gomes and Loynachan, 1984; Haynes, 1986).

In the case of corn fields, heavy rainfalls in the period of March-through-May is a major factor affecting soil N losses (Balkcom et al., 2003). A corn study conducted in Missouri compared fall application of anhydrous ammonia (AA) plus nitrapyrin, anhydrous ammonia alone with preplant application of these fertilizers (Nash et al., 2013a). They showed a 9 to 11% increase in corn grain yield when these fertilizers were

applied as preplant applications compared to a fall application. In three wet years, Nash et al. (2013b) reported a 2 Mg ha<sup>-1</sup> increase in corn grain yield when anhydrous ammonia with nitrapyrin was injected in no-till as a preplant application compared to a fall application.

### **Nitrification Inhibitors**

Nitrification inhibitors (NI) are chemical compounds which restrict, delay and/or slow down the nitrification process in order to reduce loss of nitrate before plants can utilize N (Motavalli et al., 2008a). In the late 1950s, extensive research was initiated to identify effective chemical NIs (Zerulla et al., 2001). However, little scientific knowledge is available about the effects of NIs on physiological aspects of corn grain yield and N use efficiency parameters, such as varying N rates and timing (Burzaco et al., 2014). Nitrification inhibitors behavior and persistence in soil are determined by diffusion into the atmosphere, differential movement in soils, sorption on clay or organic matter, and breakdown mechanisms (Slangen and Kerckhoff, 1984). Environmental and edaphic factors, such as moisture, temperature, and soil texture, are also determinant factors in understanding their behavior and persistence in soils (Prasad and Power, 1995). The crop species, management factors and soil climate greatly affect the efficacy of NIs (Chen et al., 2008).

Ammonium is a well-retained N form in soils due to the positive charge of the ion (Halvorson et al., 2014). Extending the time period that ammonium persists in soils will help prevent N losses due to leaching and gaseous N loss mechanisms (Chen et al., 2008; Halvorson et al., 2014). Use of a NI may also be advantageous in field operations in either one of the two or both ways: 1) allowing lower rates of applied N fertilizer or 2)

eliminating the need for a split application of N fertilizer during the growing season (Pasda et al., 2001). Although adding a NI does not always increase crop yields, it does help manage  $\text{NO}_3^-$  leaching and  $\text{NO}_x$  production (Edmeades, 2004). However, the nitrification process could be delayed from 4 to 10 weeks by these NIs when they are combined with urea or  $\text{NH}_4^+$ -containing N fertilizers and managed properly (Nelson and Huber, 2001; Bronson et al., 2008; Franzen, 2011).

A need for a new generation of NIs was reported long ago (Subbarao et al., 2006), and it was suggested that NIs should be less expensive, more efficient and suitable in both tropical and temperate environments (Watson, 2005). Christensen (2002) reported after surveys from farmers in the U.S. that NIs had been used on about 9% of the national corn area and that that proportion had not changed. Despite the fact that many chemicals have been tested as NIs, few chemicals have shown agronomic and economic viability, and are used in commercial agriculture as NIs (Slangen and Kerkhoff, 1984; Prasad and Power, 1995; McCarty, 1999; Frye, 2005). Nitrapyrin (2-chloro-6-trichloromethylpyridine, trade name N-Serve or Instinct), DCD (dicyandiamide) and DMPP (3, 4-dimethylpyrazole phosphate, trade name ENTEC) are some of the most studied NIs (Goos and Johnson, 1999; Dittert et al., 2001; Nelson and Huber, 2001; Pasda et al., 2001; Weiske et al., 2001a; b; Zerulla et al., 2001; Calderón et al., 2005; Xu et al., 2005; Islam et al., 2007a; b; Franzen, 2011). A new NI, KAS-771G77, was recently developed for use with AA (Vetsch and Schwab, 2014; Gabrielson and Epling, 2016).

### ***Nitrapyrin***

Nitrapyrin has been reported to be ineffective due to its sorption on soil colloids, hydrolysis, and loss by volatilization (Hoefl, 1984a; Liu et al., 1984a); however, it has

resulted in reduced N losses and increased plant N uptake (Chen et al., 1998a; 1998b). Research trials on nitrapyrin in diverse environments over many years in the Midwestern USA has shown increased corn yield by 7%, increased soil retention of N by 28%, decreased N leaching by 16%, and decreased NO<sub>x</sub> emissions by 51% (Wolt, 2004). It has been reported that a fall application of AA with nitrapyrin in tile drainage system had reduced drainage loss of nitrate by 10% as opposed to fall application of AA without nitrapyrin (Randall and Vetsch, 2005). Nitrification inhibitors with reduced rates of fertilizers have usually shown increased yields (Frye, 2005). After two years, Awale et al. (2015) reported that N availability was similar in sugar beet when 146 kg N ha<sup>-1</sup> with nitrapyrin compared to 180 kg N ha<sup>-1</sup> without nitrapyrin.

#### ***KAS-771G77***

KAS-771G77 was recently developed for use with AA (Vetsch and Schwab, 2014; Gabrielson and Epling, 2016). Limited research has reported on the effects of nitrification inhibitors for wheat production using AA (Kidwaro and Kephart, 1998), while no research has integrated NI technology with new AA applicator technology for use with wheat. A three-year (2012 to 2014) corn study in Minnesota on poorly-drained glacial till soils under continuous corn and subsurface tile drained soil evaluated the effects of two rates of KAS-771G77 (9.4 and 18.7 L ha<sup>-1</sup>) and one rate of nitrapyrin (2.6 L ha<sup>-1</sup>, 0.57 kg ai ha<sup>-1</sup>) on corn yield using four rates (67, 134, 202 and 269 kg ha<sup>-1</sup>) of UAN (Vetsch and Schwab, 2014). It was noted that 2014, the wettest year among study years, had significantly low yield both when no NI was applied or when KSA-771G77 applied at 18.7 L ha<sup>-1</sup> compared to KSA-771G77 applied at 9.4 L ha<sup>-1</sup> and nitrapyrin application.

## **Nitrogen Management**

Nitrogen availability and losses are important factors affecting N management (Awale et al., 2015). In-depth understanding of the suitability of different management practices for different environmental conditions will allow for improved decisions as to when and where these N fertilizers will be economically and agronomically effective (Motavalli et al., 2008a). Nutrient losses can be reduced per unit of crop production when the right source of nutrient is applied at the right rate, time and place (Snyder et al., 2009). This fertilizer “rights” concept is often termed as 4R nutrient stewardship. The 4R nutrient stewardship is linked with the sustainable development goal of increasing economic, social and environmental benefits (Bruulsema et al., 2009).

Nitrate ( $\text{NO}_3^-$ ) leaching, runoff and erosion, and gaseous losses from denitrification and ammonia volatilization are the primary mechanisms of N loss from agricultural fields (Follett and Delgado, 2002; Cui et al., 2010). Despite the fact that synthetic N fertilizers are responsible to a large extent in enhancing agricultural crop and livestock production, and fulfilling nutritional requirements of a growing human population, increasing amounts of reactive N in the environment has also caused detrimental effects on environmental quality (Vitousek et al., 1997a; Howarth et al., 2002a; Howarth, 2004a). Eutrophication, which is caused by an excess of N (and phosphorus) in water bodies, such as fresh-water ecosystems, estuaries and coastal areas (Lepistö et al., 2006; Savage et al., 2010), is an environmental pollution challenge. Soil nitrous oxide ( $\text{N}_2\text{O}$ ) emissions are another environmental problem, for which agriculture has been identified as the major anthropogenic contributor worldwide (Gill et al., 2010). This excessive N in the environment has major detrimental effects. These effects include

acidification of soil and water resources, loss of biodiversity in aquatic and terrestrial ecosystems and invasion of N-loving weeds, increased greenhouse gas levels due to emissions of N<sub>2</sub>O, increased atmospheric haze and production of airborne particulate matter, depletion of stratospheric ozone, increased ozone-induced injury to crops, forests and other ecosystems (Galloway and Cowling, 2002a). It has been reported that 60% of coastal rivers and bays in the U.S. have been moderately or severely degraded by nutrient pollution, particularly by N (Howarth et al., 2002a). Several factors, such as variation in soil properties, climatic conditions, crop growth, and management practices (e.g., soil tillage method, selection of N source, timing and method of application), affect the relative magnitude of these N loss processes (Motavalli et al., 2008a). Research is needed to better understand management practices and environmental conditions to optimize the use of these N fertilizers, reduce N losses to the environment and enhance agricultural productivity.

### **Crop Nitrogen Use Efficiency**

One definition of N use efficiency (NUE) of a cropping system is the proportion of N fertilizer that is removed from harvest crop biomass during the growing season to the ratio of crop yield per unit of applied N fertilizer (Novoa and Loomis, 1981; Dobermann, 2005; Ladha et al., 2005). Cereal species' NUE has been generally reported from 30 to 50 kg grain kg<sup>-1</sup> N (Raun and Johnson, 1999; Balasubramanian et al., 2004; Dobermann, 2005; Ciampitti and Vyn, 2012a). Under practical farming conditions, lower NUE is usually associated with excessive N application amount (Ladha et al., 2005; Ciampitti and Vyn, 2011, 2012b), greater spatial variability of factors controlling NUE, and poor land management practices (Cui et al., 2010). Crop grain yield usually increases

with increase in plant N uptake. This is in accordance with the law of diminishing returns that increased plant N uptake is associated with increased N supply (Cassman et al., 2002; Ciampitti and Vyn, 2012b, 2013); however, beyond a threshold incremental gains in N supply are accompanied by less than proportional grain yield (Burzaco et al., 2014).

Due to lack of information, farmers are unable to devise management strategies to improve their N practices on large scale and estimate the amounts of N fertilizer needed to achieve maximum NUE in fields (Ladha et al., 2005). It is important to understand the contribution of farmers' behavior to current N application practical problems. Lower NUE is one of those existing problems; and therefore, it is imperative to improve N management techniques to achieve maximum potential NUE in cereal production systems (Dobermann, 2005). Globally, recovery efficiency of N ( $RE_N$ ) within research trials for wheat and corn is 54 and 63%, respectively (Cui et al., 2010). According to Cassman et al. (2002), the average U.S. Corn Belt on-farm corn  $RE_N$  was 37%.

Crops only uptake a portion of applied N fertilizer during a growing season. Pan (2001) conducted a study using  $N^{15}$  as a tracer in an intensive wheat-corn system, and the results showed that only 25% of fertilizer N applied was utilized by crops, 30-50% lost to the environment and 25-45% accumulated in the soil. The poor efficiency of applied fertilizer N is often explained by its losses (up to 92%) from the plant-soil system (Awale et al., 2015). Experimental plots generally do not represent the efficiencies achieved in farmers' fields (Cassman et al., 2002). It has been suggested that in order to meet the 2025 cereal demand, the global partial factor productivity of N (PFPN) for cereals should be increased at a rate of 0.1 to 0.4  $kg\ kg^{-1}year^{-1}$  (Dobermann and Cassman, 2005). Synchrony between crop N demand and N supply from all sources throughout growing

season is important in improving the  $RE_N$  in crop production (Tilman et al., 2002; Cassman et al., 2002; Dobermann, 2005). In-season application of N may be beneficial in site-specific N supply and crop demand, and may result in higher NUE (Flowers et al., 2004; Shanahan et al., 2008).

### **Common Nitrogen Fertilizer Sources**

Anhydrous ammonia (AA), urea, urea ammonium nitrate (UAN), ammonium nitrate (AN) and ammonium sulfate are readily soluble N fertilizers, and are common synthetic fertilizers used in row-crop agriculture (Millar et al., 2010a). According to (Bouwman et al., 2002c), urea-based fertilizers account for 43% of global N fertilizer sales. Urea, due to its highly soluble nature, poses a large potential for N loss through ammonia volatilization, sub-surface drainage in poorly drained soils (Drury et al., 2009a) or leaching in sandy soils (Wilson et al., 2009a). Some of the most common N fertilizer sources used in the U.S. Corn-Belt include UAN, AA and ammonium nitrate (AN). A recently developed polymer-coated urea (PCU) is sparsely used in the region as well.

### ***Urea Ammonium Nitrate***

The UAN form of N fertilizer comes in liquid form and is considered a useful source of fertilizer N in mixing with other nutrients or chemicals. Half of the N in UAN is in the urea form while the other half consists of  $NH_4^+$  and  $NO_3^-$  forms at 25% each. Research is limited on use of UAN as an N fertilizer source. One study, conducted over two years in Indiana, compared UAN application rates (0, 90 and 180 kg N ha<sup>-1</sup>), application timing (preemergence and sidedress at the V6 stage of corn growth) and use of nitrapyrin on corn yield, soil N<sub>2</sub>O emissions and yield-scaled N<sub>2</sub>O emissions (Burzaco et al., 2013a). There was a 3 Mg ha<sup>-1</sup> increase in yield when UAN was applied as a

sidedress application with nitrapyrin and 180 kg N ha<sup>-1</sup> compared to a preemergence application without nitrapyrin at 90 kg N ha<sup>-1</sup>. Although nitrapyrin significantly reduced both daily and cumulative N<sub>2</sub>O emissions when averaged across both years, UAN rate was the primary factor influencing corn yield, yield-scaled N<sub>2</sub>O emissions, soil N<sub>2</sub>O fluxes and cumulative soil N<sub>2</sub>O emissions followed by N application timing and nitrapyrin. The increments in N application rates may have overshadowed the effects of nitrapyrin on yield and N<sub>2</sub>O emissions. A three-year (2012 to 2014) corn study in Minnesota on poorly-drained glacial till soils under continuous corn and subsurface tile drained soil evaluated the effects of two rates of KAS-771G77 (9.4 and 18.7 L ha<sup>-1</sup>) and one rate of nitrapyrin (2.6 L ha<sup>-1</sup>, 0.57 kg ai ha<sup>-1</sup>) on corn yield using four rates (67, 134, 202 and 269 kg ha<sup>-1</sup>) of UAN (Vetsch and Schwab, 2014).

### ***Anhydrous Ammonia***

Anhydrous ammonia (AA) is a common source of N for corn production in Missouri (Nash et al., 2013a). Anhydrous ammonia application in the fall is a common N management practice in the Corn Belt of the Midwestern United States (Kyveryga et al., 2004; Parkin and Hatfield, 2010; Nash et al., 2012). The time-period between November and April is usually not suitable for AA application due to excessively wet or frozen soil conditions (Kyveryga et al., 2004). Recommendations for fall application of AA suggest waiting until the soil temperature drops to 10 °C at a depth of 10 to 15 cm to reduce possible N loss (Nelson and Hansen, 1968; Follett et al., 1981).

It has been reported that fertilizers injected in soil result in higher soil N<sub>2</sub>O emissions compared to surface broadcasted N fertilizers (Bouwman et al., 2002). Since temperatures in the period after November and before April are usually low and the rate

of nitrification is believed to be low in cooler temperatures, N losses because of  $\text{NO}_3^-$  leaching and denitrification are reduced in this period providing the option of AA application in late fall instead of early spring (Kyveryga et al., 2004). The nitrifying bacteria in soil have been reported to be inactive in cold soils and this inactivity prevails until the temperatures reach 4 to 5 °C (Schmidt, 1982). However, nitrification has also been observed in frozen soils (Nyborg and Malhi, 1979).

The importance of the cumulative effect of relatively slow nitrification in cold soils from the period of fall-to-spring has been well documented (Frederick, 1956; Anderson and Boswell, 1964; Frederick and Broadbent, 1966; Sabey, 1969; Campbell et al., 1973; Gomes and Loynachan, 1984; Haynes, 1986).

In the case of corn fields, heavy rainfall in the period of March through May is a major factor affecting N losses (Balkcom et al., 2003). A corn study conducted in Missouri compared fall application of anhydrous ammonia plus nitrapyrin, anhydrous ammonia alone with preplant application of these fertilizers (Nash et al., 2013a). They showed a 9 to 11% increase in corn grain yield when these fertilizers were applied as preplant applications compared to a fall application. In three wet years, Nash et al. (2013b) reported a 2 Mg ha<sup>-1</sup> increase in corn grain yield when AA with nitrapyrin was injected in no-till as a preplant application compared to a fall application.

### ***Polymer-coated Urea***

Polymer-coated urea (PCU) is slow or controlled-release N fertilizer, and its application is a newly developed N management practice (Blaylock et al., 2004, 2005; Motavalli et al., 2008; Nelson et al., 2009b). Nash et al. (2013) reported that PCU increased corn grain yields 12 to 14% compared to non-coated urea (NCU) when it was

applied in fall and preplant strip-till. A corn study conducted in Missouri compared fall application of strip-till placement of PCU and strip-till placement of NCU with preplant application of these fertilizers (Nash et al., 2013a). They showed a 9 to 11% increase in corn grain yield when these fertilizers were applied as preplant applications compared to a fall application.

### **Common Nitrogen Management Practices**

Management strategies that have been examined to control and/or reduce soil N loss include improved timing of N fertilizer and manure applications, better use and development of soil, plant and manure testing procedures to determine in-season N availability, better N fertilizer and manure recommendations, switching to use of variable-rate of N fertilizer applications and other more effective N fertilizer application methods, increased adoption of nutrient management planning, application of urease or nitrification inhibitors (NI), and use of N fertilizer sources that are suitable for local environmental conditions (Dinnes et al., 2002; Motavalli et al., 2008a; Cui et al., 2010). In addition, improved local weather forecasting and modeling may also assist farmers and agricultural professionals to devise well-informed plans and make rational decisions for N use (Bruulsema, 2007).

### ***Sources of N Fertilizer***

Since N fertilizer may have a variable fate under different climatic and management conditions, accurately quantifying its losses to the environment is difficult (Snyder et al., 2009). Reaching a broad general conclusion regarding N source effects is difficult because fertilizer source, tillage, soil temperature and soil moisture interact with each other differently depending on environmental conditions. This variability makes it

difficult to draw general conclusions on the effects of different N fertilizer sources (Harrison and Webb, 2001; Bouwman et al., 2002a; Venterea et al., 2005). Carefully selecting the type of fertilizer in accordance with soil environmental conditions can substantially reduce N loss (McTaggart et al., 1994). A research study conducted in claypan soil found that urea-derived N either leached or was transported laterally in runoff when there was no tile drainage (Blevins et al., 1996). However, they estimate approximately 35% of urea N fertilizer loss occurred through gaseous emissions. This suggests that extensive research is needed to determine the effects of different N fertilizer sources under diverse soil and climatic conditions.

#### ***Application Rate of N Fertilizer***

Each agronomic crop has a potential critical level for each essential nutrient element. When N rates exceed a certain agronomic threshold, the probability of N loss to the environment from agricultural soils increases (Snyder et al., 2009). In addition, excess soil N during the early stages of crop development sometimes results in excessive crop growth which may expose the crop to be more susceptible to disease infection and lodging, especially in the case of wheat and other small grains (Cui et al., 2010). Since there are variations across cropping systems, environments, and within fields, N rates cannot be generalized and yet there are N fertilizer recommendations. However, quantifying the surplus between N-applied and the crop's total plant N-uptake during the growing season can help to set approximate N rates for a particular crop under specific soil and climatic conditions (Van Groenigen et al., 2010). After harvest, the recommended residual amount of soil  $\text{NO}_3^-$ -N content in 90 cm soil layer is to maintain  $90 \text{ kg N ha}^{-1}$  (Hofman, 1999; Cui et al., 2008c; d). No crop response to added N was

reported in intensive wheat-maize production system in China when soil inorganic N exceeded  $190 \text{ kg NO}_3^- \text{-N ha}^{-1}$  in the top 90 cm before planting (Cui et al., 2008c, 2008d). Variability within the soil profile N content and a crop's N requirements make it difficult to set up general recommendations under varied temporal and spatial conditions. Therefore, it is important to test different N fertilizer rates under diverse conditions to make recommendations more accurate for specific growing conditions.

### ***Application Timing of N Fertilizer***

The goal of N management is to make the best use of N by increasing crop productivity while not causing N pollution. Best N management practices improve soil N supply to the root zone within a reasonable range and quantity while considering temporal and spatial synchrony between crop N requirements and soil N supply simultaneously (Cui et al., 2010). By doing so, potential environmental loss of N from leaching, nitrification, denitrification, and other processes could subsequently be reduced (Burzaco et al., 2013a) and greater yields could be achieved. Therefore, 60 to 70% of N should be available to the crop during the fastest growing stages of the crop in order to obtain synchrony between crop demand and N supply (Cui et al., 2010). A study conducted in Spain showed that wheat NUE was 14% when it was applied at sowing but increased to 55% when applied at the beginning of stem elongation as a topdress (López-Bellido et al., 2005). Another study conducted in Iowa, reported that 50 to 64% of the N applied in a fall-application was lost from the upper 1.5 m of soil (Sanchez and Blackmer, 1988). Timing is a critical part of the 4R framework to enhance NUE. Although spring applications of N fertilizer have shown greater N synchrony between soil mineral N supply and crop N demand (Stehouwer and Johnson, 1990; Randall et al.,

2003), the combined effects of a nitrification inhibitor (NI) and spring applied N (e.g., pre-emergence and sidedress) has not been studied in depth (Burzaco et al., 2014).

### **North Central Missouri and Claypan Region**

Missouri has many regions, which are vulnerable to conditions of temporary soil saturation. North central Missouri, southern Illinois and southeast Kansas comprise the central claypan region which accounts for 4 million ha comprising predominantly of these soils. This claypan region has a subsoil layer at 20 to 40 cm from the surface which is 100% higher in clay content compared to the above horizon (Anderson et al., 1990a; Jung et al., 2006a; Myers et al., 2007a). This claypan poses a major challenge to agricultural practices due to its poor drainage characteristics. Poorly-drained soils and areas in low-lying landscape positions have a higher potential for saturated soil conditions which may cause N losses and negatively affect crop yields (Nelson et al., 2009; Nash et al., 2012, 2013a). A better understanding of the effects and interactions of N sources, application-rates and application-timings with and without a NI on crop production and N losses could help maintain or increase grain yield. Little research has been conducted in poorly-drained claypan soils to determine accurate research-based recommendations on when these N fertilizers would be effective, how they behave in different environments and cropping systems, and how they should be managed in those environments and cropping systems (Motavalli et al., 2008a). In addition, there is a lack of research studies which have compared the simultaneous impact of multiple management factors together (Burzaco et al., 2013a) on corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) in the Midwestern, United States.

## **RESEARCH OBJECTIVES**

The overall objective of this research was to evaluate whether NIs and/or N management can increase or maintain corn and wheat production in poorly drained claypan soils in Northeast Missouri.

### **Specific Research Objectives**

- 1) To determine the effects of application of nitrapyrin with UAN application rates and application timings on corn production in claypan soils in Northeast Missouri.
- 2) To evaluate the efficacy of a new NI (KAS-771G77) in comparison with nitrapyrin on claypan soils for winter wheat production using split N applications.
- 3) To assess winter wheat production under poorly drained claypan soils using KAS-771G77 and nitrapyrin with AA, PCU and AN. These fertilizers were separated into fall vs spring applications.
- 4) To measure soil N status, and selected crop response variables during the growing season and at harvest among the applied treatments.

## **HYPOTHESES**

### **Hypothesis 1**

Nitrification inhibitors (i.e., nitrapyrin and KAS-771G77) will suppress the nitrification process in soil, which will increase NUE, reduce N loss and increase corn and wheat yields.

### **Hypothesis 2**

Nitrification inhibitors presence or absence and their application timings will interact differently with N fertilizer sources application rates and application timings under different field conditions.

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**CHAPTER 2**  
**MANAGEMENT OF NITRAPYRIN WITH UREA AMMONIUM NITRATE ON**  
**CORN YIELD AND SOIL NITROGEN**

**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. Field research was conducted on corn (*Zea mays* L.) from 2012 to 2015 in Northeast Missouri. 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<sup>-1</sup>), with and without a NI (nitrapyrin), and a non-treated control which were arranged in randomized complete block design. UAN applied at a rate of 143 kg ha<sup>-1</sup> with nitrapyrin at the V3 growth resulted in the highest yield (8.6 Mg ha<sup>-1</sup>). Similarly, pre-emergence application of UAN 168 kg ha<sup>-1</sup> with nitrapyrin resulted in greater yields (7.7 Mg ha<sup>-1</sup>). UAN application rates and timings affected soil NO<sub>3</sub>-N and NH<sub>4</sub>-N concentration more than nitrapyrin presence or absence during the growing season. A side-dress application of a lower rate of UAN with nitrapyrin at V3 corn growth stage may be useful when risk of N losses during the growing season due to unfavorable precipitation events and other environmental variables is high. A pre-emergence application of UAN with nitrapyrin was useful and it may eliminate the need for split-application of N fertilizer later in the season. Workload on growers soon before planting or during growing season, excessive wet field conditions in early spring, reduced N fertilizer use efficiencies due to uncertain climatic conditions during growing season, and environmental concerns of pollution from

N escaping from agriculture production systems may give an incentive to growers and policy makers to increase the use of nitrapyrin in the future.

## **INTRODUCTION**

Corn (*Zea mays* L.) is a major source of feed and food world-wide (Lobell et al., 2013). Global production of corn was reported to be over 1 billion MT, of which the U.S. accounts for 35% followed by China and Brazil with 21% and 8%, respectively (FAOSTAT, 2016). Despite the continued increase in overall production, emphasis has been given to increase yields in the face of climate change (Lobell et al., 2013) and rapidly increasing human population (Zilberman et al., 2013). Corn is a nitrogen (N) intensive crop which means large additional N inputs are added to the soil to maintain productivity (Millar et al., 2010a). The Midwest region of the U.S is known for growing corn on extended areas and is referred to as the Corn-Belt of the United States. Various soil biogeochemical processes such as nitrification, denitrification and leaching sometimes decrease N use efficiency of applied fertilizer due to unpredictable heavy precipitation in early spring.

Careful selection of N fertilizer sources, application rates, and application timing 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 region (Randall et al., 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 retard or slow down the conversion of ammonium to nitrate ( $\text{NO}_3^-$ ) 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  $\text{NO}_2\text{-N}$  loss when UAN was combined with a urease and NI (Halvorson et al., 2010a; Halvorson and Grosso, 2012). However, none of the studies reported significant increases in grain yields. The NI might not have profound effects on yield in those studies since it was an irrigated system typically and NI typically works best in excessive water conditions.

In row-crop agriculture, synthetic fertilizers such as urea, UAN, ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), AA and ammonium sulfate ( $(\text{NH}_4)_2\text{SO}_4$ ) are commonly used (Millar et al., 2010a). The UAN form of N fertilizer comes in liquid form and is considered a useful source of fertilizer N in mixing with other nutrients or chemicals. Half of the N in UAN is in the urea form while the other half consists of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  forms at 25% each. Research is limited on use of UAN as an N fertilizer source. One study, conducted over two years in Indiana, compared UAN application rates (0, 90 and 180 kg N ha<sup>-1</sup>), application timing (preemergence and sidedress at the V6 stage of corn growth) and use of nitrapyrin on corn yield, soil  $\text{N}_2\text{O}$  emissions and yield-scaled  $\text{N}_2\text{O}$  emissions (Burzaco et al., 2013a). There was a 3 Mg ha<sup>-1</sup> increase in yield when UAN was applied as a sidedress application with nitrapyrin and 180 kg N ha<sup>-1</sup> compared to a preemergence application without nitrapyrin at 90 kg N ha<sup>-1</sup>. Although nitrapyrin significantly reduced both daily and cumulative  $\text{N}_2\text{O}$  emissions when averaged across both years, UAN rate was the primary factor influencing corn yield, yield-scaled  $\text{N}_2\text{O}$  emissions, soil  $\text{N}_2\text{O}$  fluxes and cumulative soil  $\text{N}_2\text{O}$  emissions followed by N application timing and

nitrapyrin. The increments in N application rates may have overshadowed the effects of nitrapyrin on yield and N<sub>2</sub>O emissions.

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

## **MATERIALS AND METHODS**

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. The soil was 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., 1990a; Jung et al., 2006a; Myers et al., 2007a). This claypan layer has a 100% higher clay content than the above horizon. The depth to claypan at this particular location ranges from 46 to 60 cm (data not presented). Each year soil sampling occurred prior to planting at each site using a stainless steel push probe from depth increments of 0 to 22 and 23 to 46 cm to characterize selected initial soil properties (Table 2.1). Standard soil test analytical methods were used by the University of Missouri Soil and Plant Testing Lab to analyze these samples (Nathan et al., 2006a). Additional soil samples were collected from the 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<sub>4</sub>-N and NO<sub>3</sub>-N concentrations and were analyzed for soil NH<sub>4</sub><sup>+</sup> and soil NO<sub>3</sub><sup>-</sup> using a 2M KCl extraction and analysis with a Lachat QuickChem (Hach Corp., Loveland, CO) automated ion analyzer.

The field site for each growing season was different from the previous year and all sites had been in corn-soybean (*Glycine max.* L) rotations. Soybean residues on the surface of the soil were not disturbed and field sites in all years were maintained as 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<sup>-1</sup>), and the presence or absence of nitrapyrin. A non-treated control was included. Both the PRE and V3 applications were surface dribble-banded. Nitrapyrin (Instinct, Dow AgroSciences, Indianapolis, IN) was applied at 0.513 kg ai ha<sup>-1</sup>.

The corn hybrids planted each year were DKC62-77VT3P in 2012, DK62-97RIB in 2013 and 2014, and DK62-08 in 2015 in 76 cm wide row using a John Deere 7000 (Deere and Co., Moline, IL). Seeding rate was 79,000 seeds ha<sup>-1</sup> in 2012 and 82,000 seeds ha<sup>-1</sup> in 2013, 2014 and 2015. Field operation timeline and maintenance fertilizer details are reported in Table 2.1. Crop protection chemical applications are listed in Table 2.2. Chlorophyll (SPAD) meter readings (Minolta SPAD-502, Konica Minolta Optics, Inc., Tokyo, Japan) were recorded for 10 plant per plot at V8 and VT growth stages (Ritchie and Hanway, 1989). Corn grain yields were determined with a small-plot two row combine (Wintersteiger Inc., Salt Lake City, UT) and adjusted to 155 g kg<sup>-1</sup> moisture content before statistical analysis. Additional corn response measurements included grain protein concentration (Foss Intratec 1241, Eden Prairie, MN), harvested plant population, oil concentration, starch concentration, test weight and moisture content. The duration of

the growing season in 2012, 2013, 2014 and 2015 was 144, 136, 182 and 148 days, respectively.

All statistical analyses were conducted using the SAS statistical program (SAS 9.4 Software, Cary, NC). 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  $P \leq 0.1$  were used for mean separation. ANOVA tables for the main effects and interactions for soil  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  at V7 and harvest, yield and other crop response variables are reported in Tables 2.3 and 2.4, respectively.

## **RESULTS AND DISCUSSION**

### **Precipitation**

Precipitation data for 2001 to 2015 were obtained from the Missouri Historical Agricultural Weather Database website (MU Extension, 2016). These data were analyzed for daily and cumulative precipitation for the study year (April 1 to November 1), and compared with the average cumulative precipitation from 2001 to 2011 (Figure 2.1). Precipitation in 2012 was 35% (277 mm) less compared to average cumulative precipitation (784 mm). 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 October 31. A 13-day difference was noted between harvest and harvest soil sample, and this period received a 34% (135 mm) of the total precipitation of the season. Daily cumulative precipitation in 2013 (795 mm) did not differ from the average cumulative precipitation (784 mm). Harvest and harvest soil sampling occurred on same day. Cumulative daily

precipitation in 2014 was 9% (74 mm) high than 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 this period received 47 mm precipitation. In 2015, daily cumulative precipitation amounted for 962 mm and it was 23% (179 mm) higher than average daily cumulative of the period of 2001 to 2011. The time-period between harvest and harvest soil sample in 2015 was 43 days, and there was 36 mm of rainfall during that time.

### **Average Daily Air temperature**

Average daily air temperature from January 1 to December 31 for 2012, 2013, 2014 and 2015 are reported in Figure 2.2. In all four years, temperature was generally below the freezing point (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  $\pm 10$  °C more than it was noted in 2014 and 2015. In 2014 and 2015, the air temperature remained below 0 °C for longer intervals during that period. However, the time-period for which corn crop was in the field, temperature across years was similar. Temperature 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. It was followed by a decline in early-November which again ended up in several daily average temperatures below the freezing point in December.

## **Grain Yield**

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  $P \leq 0.1$  among UAN application timing, rate, and nitrapyrin was noted (Table 2.4). The PRE UAN at 168 kg N ha<sup>-1</sup> with nitrapyrin resulted in the highest grain yield (8.6 Mg ha<sup>-1</sup>) and was 11% greater compared to pre-emergence application of UAN at 168 kg N ha<sup>-1</sup> without nitrapyrin (7.7 Mg ha<sup>-1</sup>) (Figure 2.3). The UAN at 143 kg N ha<sup>-1</sup> with nitrapyrin at V3 (8.2 Mg ha<sup>-1</sup>) resulted in a 7% increase over UAN at 143 kg N ha<sup>-1</sup> without nitrapyrin at V3 (7.6 Mg ha<sup>-1</sup>). No significant difference was noted between yields of PRE UAN at 143 kg N ha<sup>-1</sup> with or without nitrapyrin. Similarly, no significant yield difference was observed between V3 UAN at 168 kg N ha<sup>-1</sup> with or without nitrapyrin.

## **Soil Nitrogen at V3, V7 and Harvest**

### ***V3 Stage***

Results for soil NO<sub>3</sub>-N and soil NH<sub>4</sub>-N at 0 to 22 and 23 to 46 cm depths for the V3 growth stage were analyzed using a single-factor one-way ANOVA at  $P \leq 0.01$  because side-dress treatment applications had not occurred at that time (Table 2.5). 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 were greater than the non-treated control (14.2 mg kg<sup>-1</sup>) for soil NO<sub>3</sub>-N from 0 to 22 cm. Other than that, all the treatments resulted in similar soil NO<sub>3</sub>-N concentrations from 0 to 22 cm at the V3

growth stage. At V3, only PRE applied UAN at 168 kg N ha<sup>-1</sup> without nitrapyrin (25.5 mg kg<sup>-1</sup>) resulted in significantly higher soil NH<sub>4</sub>-N concentration in the 0 to 22 cm depth compared to the non-treated control (5.1 mg kg<sup>-1</sup>). At the V3 growth stage in the 23 to 46 cm depth, PRE applied UAN at 168 kg N ha<sup>-1</sup> with nitrapyrin (10.3 mg kg<sup>-1</sup>) had higher soil NO<sub>3</sub>-N concentration compared to the non-treated control (6.2 mg kg<sup>-1</sup>).

### ***V7 Stage***

Soil N concentrations at V7 were analyzed using a three-factor ANOVA at  $P \leq 0.1$ . There was no significant interaction between UAN application rates, timings and nitrapyrin for soil NO<sub>3</sub>-N concentration in the the 0 to 22 cm depth at V7 (Table 2.3). However, UAN application timings and experimental years did have a significant interaction for soil NH<sub>4</sub>-N in the 0 to 22 cm depth at the V7 growth stage (Table 2.3). Soil NH<sub>4</sub>-N concentration in 2013 for V3 plants was greater (39.3 mg kg<sup>-1</sup>) than all the treatments at this growth stage (Table 2.6). At V7, soil NH<sub>4</sub>-N concentration in 2013 for PRE applied treatments was significantly greater (27.9 mg kg<sup>-1</sup>) than all the treatments applied PRE. The V3 treatments generally resulted in greater soil NH<sub>4</sub>-N concentrations over PRE applied treatments. An interaction between year and nitrapyrin was noted for soil NO<sub>3</sub>-N at 23 to 46 cm depth (Table 2.3). In 2014, V3 treatments in the absence of nitrapyrin had the greatest soil NO<sub>3</sub>-N (13.7 mg kg<sup>-1</sup>) (Table 2.7). It was a 54% (7.4 mg kg<sup>-1</sup>) greater than the equivalent treatment without nitrapyrin.

### ***Harvest***

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 2.3). The UAN at 168 kg ha<sup>-1</sup> (16 mg kg<sup>-1</sup>) increased soil NO<sub>3</sub>-N by 15% (3 mg kg<sup>-1</sup>) compared to the

UAN at 143 kg ha<sup>-1</sup> for the 0 to 22 cm soil depth (Table 2.8). There was a year by UAN application timing interaction for soil NH<sub>4</sub>-N in the 0 to 22 cm depth. In 2012, V3 applied UAN at 0 to 22 cm depth soil NH<sub>4</sub>-N (13 mg kg<sup>-1</sup>) concentration was the greatest, and was greater than all PRE applied N treatments (Table 2.9). However, V3 applied treatments in 2015 had the lowest soil NH<sub>4</sub>-N concentration (4 mg kg<sup>-1</sup>). Except for 2015, V3 treatments generally resulted in higher soil NH<sub>4</sub>-N concentrations compared to PRE treatments. Soil NO<sub>3</sub>-N at a 23 to 46 cm depth, had a year x UAN application rate x timing x nitrapyrin interaction. In 2012, V3 UAN at 143 kg ha<sup>-1</sup> with nitrapyrin (24 mg kg<sup>-1</sup>) had a 50% (12 mg kg<sup>-1</sup>) greater soil NO<sub>3</sub>-N concentration over the equivalent amount of UAN without nitrapyrin (Table 2.10). To the contrary, V3 applied UAN at 168 kg ha<sup>-1</sup> in 2013 with nitrapyrin (19 mg kg<sup>-1</sup>) had a 13% (3 mg kg<sup>-1</sup>) lower soil NO<sub>3</sub>-N concentration compared to UAN without nitrapyrin. UAN 143 at kg ha<sup>-1</sup> without nitrapyrin at V3 increased soil NO<sub>3</sub>-N 22% compared to the equivalent treatment with nitrapyrin. This was in contrast to what was observed in the equivalent treatment in 2012. This indicates a possible difference in N uptake between years. During a drought year (2012), the highest soil NO<sub>3</sub>-N concentrations were observed. All the treatments in 2012 resulted in significantly greater soil NO<sub>3</sub>-N concentrations compared to their respective treatment in 2013, 2014 and 2015. The lowest soil NO<sub>3</sub>-N concentrations were observed in 2015, and were similar to 2014.

### **Leaf SPAD Meter Readings at V8 and VT growth stages**

An interaction for leaf SPAD meter readings between year and nitrapyrin at V8 and VT growth stages was found (Table 2.4). 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 2.11). 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.

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  $\text{NO}_3\text{-N}$  uptake by plants. Furthermore, 2015 was relatively the wettest year of study and it received high precipitation events over the course of 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 NIs typically work best where risk of N losses due to excessive wet soil conditions is high.

## **Grain Quality and Plant Population**

### ***Grain Moisture***

Grain moisture interacted for UAN application rates, application timings and nitrapyrin at  $P \leq 0.1$  (Table 2.4). UAN at  $143 \text{ kg ha}^{-1}$  without nitrapyrin at V3 ( $197 \text{ g kg}^{-1}$ ) had the highest grain moisture content, and was greater than all the other treatments (Table 2.11). The UAN at  $168 \text{ kg ha}^{-1}$  with nitrapyrin applied PRE ( $180 \text{ g kg}^{-1}$ ) resulted in the lowest grain moisture content, and was significantly lower ( $9 \text{ g kg}^{-1}$ ) than an

equivalent treatment at V3. Grain moisture content was generally lower in treatments with nitrapyrin compared to treatment without nitrapyrin.

### ***Grain Test Weight***

There was an interaction between UAN application rates, application timings and nitrapyrin at  $P \leq 0.1$  for grain test weight (Table 2.4). UAN at  $143 \text{ kg ha}^{-1}$  without nitrapyrin at the PRE timing increased grain test weight 2% ( $14 \text{ kg m}^{-3}$ ) compared to the equivalent treatment with nitrapyrin (Table 2.12). It was also greater by a significant 3% ( $19 \text{ kg m}^{-3}$ ) amount over its equivalent treatment applied at V3 stage ( $728 \text{ kg m}^{-3}$ ). Corn hybrids used in this study were not the same. This may be one possible reason the grain test weight is different among treatments.

### ***Grain Protein***

There was an interaction between UAN application rates and application timings for grain protein at  $P \leq 0.1$  (Table 2.4). UAN at  $143 \text{ kg ha}^{-1}$  applied at PRE ( $83 \text{ g kg}^{-1}$ ) had lower grain protein concentration than all treatments (Table 2.11). UAN at  $143 \text{ kg ha}^{-1}$  applied at PRE and UAN at  $168 \text{ kg ha}^{-1}$  applied at V3 both resulted in the same amount of grain protein concentration of  $85 \text{ g kg}^{-1}$ , and were greater than V3 applied UAN at  $168 \text{ kg ha}^{-1}$  ( $84 \text{ g kg}^{-1}$ ). Grain protein results are more likely a representation of leaf SPAD meter readings because both SPAD meter readings and grain protein concentration have similar patterns.

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 greatly altered these results. For example, 2015 was

relatively the wettest season with a majority of precipitation of the season occurring in a short interval of time. Hence, the impact of nitrapyrin that may have been more positive. Workload on growers soon before planting or during the growing season, excessive wet field conditions in early spring, reduced N fertilizer use efficiencies due to uncertain climatic conditions during the growing season, and the environmental pressure of potential N pollution may give an incentive to growers, researchers and policy makers to promote the use of NIs in the future.

## **CONCLUSIONS**

The UAN at 143 kg ha<sup>-1</sup> with nitrapyrin applied at V3 had the highest grain yield (8.6 Mg ha<sup>-1</sup>), followed by 7.7 Mg ha<sup>-1</sup> yield of UAN at 168 kg ha<sup>-1</sup> with nitrapyrin applied PRE. Soil NO<sub>3</sub>-N and NH<sub>4</sub>-N concentrations were generally affected by UAN application rates and timings compared to nitrapyrin. In the wettest year (2015), nitrapyrin increased leaf SPAD meter readings. SPAD meter readings were more likely related to grain protein concentrations. The presence of nitrapyrin decreased grain moisture content. Plant population, grain oil and starch concentrations were not affected by any of the factors in the experiment. Based on this research, the highest yields were obtained with UAN at 143 kg ha<sup>-1</sup> with nitrapyrin at V3 and UAN at 168 kg ha<sup>-1</sup> with nitrapyrin applied at PRE. Based these findings, a side-dress application of a lower rate of UAN with nitrapyrin at V3 may be useful when the risk of N losses during the growing season due to unfavorable precipitation events and other environmental variables is high. A PRE application of UAN with nitrapyrin was useful but not as effective as the side-dress application. However, further research into investigating the cost-benefit ratio of using nitrapyrin with UAN on corn production system may best evaluate the consistency

of the crop response to this combination and the underlying reason behind observed interactions.

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Table 2.1. Experimental timeline, soil initial properties from 0 to 22 cm and 23 to 46 cm depth, and soil maintenance fertilizer details from 2012 to 2015.

Source	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 <sup>†</sup>	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.
Soil Initial Properties				
----- 0 to 22 cm -----				
pH	5.9	5.7	6.4	6.3
Neutralizable acidity (N.A.), cmol <sub>c</sub> kg <sup>-1</sup>	1.7	3.5	0.8	0.9
Organic matter (O.M.), %	3.3	2.1	2.4	2.3
Bray 1 phosphorus (P), kg ha <sup>-1</sup>	30	37	26	53
Calcium (Ca), kg ha <sup>-1</sup>	4,822	3,624	3,873	5,049
Magnesium (Mg), kg ha <sup>-1</sup>	584	305	361	580
Potassium (K), kg ha <sup>-1</sup>	228	182	155	293
Cation exchange capacity (CEC), cmol <sub>c</sub> kg <sup>-1</sup>	14.9	12.9	11.0	14.7
Nitrate-nitrogen (NO <sub>3</sub> -N), mg kg <sup>-1</sup>	6.6	13.1	9.2	8.0
Ammonium-nitrogen (NH <sub>4</sub> -N), mg kg <sup>-1</sup>	4.0	6.8	2.4	2.4
----- 23 to 46 cm -----				
pH	NA <sup>‡</sup>	5.1	5.5	5.2
Neutralizable acidity (N.A.), cmol <sub>c</sub> kg <sup>-1</sup>	NA	5.4	2.9	4.9
Organic matter (O.M.), %	NA	2.0	1.7	2.2
Bray 1 phosphorus (P), kg ha <sup>-1</sup>	NA	20	13	13
Calcium (Ca), kg ha <sup>-1</sup>	NA	3,283	3,578	4,941
Magnesium (Mg), kg ha <sup>-1</sup>	NA	293	407	874
Potassium (K), kg ha <sup>-1</sup>	NA	114	103	137
Cation exchange capacity (CEC), cmol <sub>c</sub> kg <sup>-1</sup>	NA	15.0	13.7	21.1
Nitrate-nitrogen (NO <sub>3</sub> -N), mg kg <sup>-1</sup>	4.3	4.9	6.4	3.4
Ammonium-nitrogen (NH <sub>4</sub> -N), mg kg <sup>-1</sup>	4.1	7.1	4.1	3.6
Soil Maintenance Fertilizer				
Fertilizer type	N-P-K	NA <sup>§</sup>	N-P-K-S-Zn	NA
Application rate, lb/acre	17-80-120	NA	20-80-140- 20-2	NA
Application date	12 Apr.	NA	?	NA

<sup>†</sup> Corn growth development stages (Ritchie and Hanway, 1989)

<sup>‡</sup> Data were only collected from 23 to 46 cm depth for NO<sub>3</sub>-N and NH<sub>4</sub>-N for 2012.

<sup>§</sup> Maintenance fertilizer was not applied in 2013 and 2015.

Table 2.2. Plant protection chemical application timings, rates and date from 2012 to 2015.

Year	Herbicide Common Name	Timing	Rate (kg a.i. ha <sup>-1</sup> )	Date
2012	Simazine <sup>†</sup>	Fall Applied	1.12	3 Oct. 2011
	Glyphosate <sup>‡</sup>		0.43	
	Ace. + Flu. + Clo. <sup>§</sup>	Post Application 1	1.19	2 Apr
	Glyphosate		1.26	
	Glyphosate	Post Application 2	0.43	5 Jun
Mesotrione	0.11			
2013	Simazine	Fall Application	1.12	28 Nov .2012
	Acetolchlor <sup>¶</sup>	Post Application 1	1.61	14 May
	Atrazine <sup>#</sup>		2.25	
	Glyphosate	Post Application 2	0.43	22 May
	Mesotrione		0.11	
Lambda-cyhalothrin <sup>††</sup>	0.04			
2014	Acetochlor + Atrazine	Application 1	3.97	6 May
	Atrazine		0.56	
	Glyphosate		0.87	
	Glyphosate	Application 2	0.87	11 Jun
	Topramezone <sup>‡‡</sup>		0.012	
Atrazine	0.28			
2015	Saflufenacil <sup>§§</sup>	Before Emergence	0.025	23 Apr
	Glyphosate		1.42	
	Acetolchlor	After Emergence 1	2.53	28 Apr
	Atrazine		1.68	
	Topramezone	After Emergence 2	0.012	6 Jun
	Glyphosate		1.08	

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

Table 2.3. Three-factor ANOVA table for soil analyses at V7 and harvest corn growth stages.

Source	df	V7				Harvest			
		0-22 cm		23-46 cm		0-22 cm		23-46 cm	
		NO <sub>3</sub> -N	NH <sub>4</sub> -N						
----- Pr > F -----									
Year	3	0.1315	0.2340	0.2687	<.0001	<.0001	0.2599	<.0001	0.0002
Year (Rep)	4	0.6379	0.0111	0.5732	0.0000	0.8181	0.1766	0.9900	0.0002
Timing	1	0.2540	0.0025	0.2099	0.4094	<.0001	0.0003	<.0001	0.7683
Year * Timing	3	0.7964	0.0547	0.5184	0.9133	<.0001	<.0001	<.0001	0.8149
Nitrapyrin	1	0.4537	0.4117	0.7075	0.3387	0.1304	0.4601	0.4400	0.6845
Year * Nitrapyrin	3	0.7819	0.6897	0.2800	0.2425	0.6103	0.7984	0.4700	0.8976
UAN rate	1	0.2983	0.6423	0.4552	0.4816	0.0907	0.2893	0.4500	0.8026
Year * UAN rate	3	0.5936	0.1548	0.3363	0.9314	0.2949	0.6572	0.8000	0.7818
Timing * Nitrapyrin	1	0.4725	0.2390	0.2855	0.5880	0.8053	0.2031	0.2900	0.8814
Year * Timing * Nitrapyrin	3	0.4765	0.6307	0.0693	0.9863	0.7623	0.4793	0.2100	0.9203
Timing * UAN rate	1	0.2861	0.4974	0.6926	0.6970	0.3976	0.3590	0.5100	0.7882
Year * Timing * UAN rate	3	0.1804	0.6466	0.6275	0.5265	0.6335	0.3409	0.5600	0.9162
Nitrapyrin * UAN rate	1	0.1309	0.5343	0.8019	0.5718	0.3089	0.5671	0.1600	0.2562
Year * Nitrapyrin * UAN rate	3	0.8214	0.9732	0.8643	0.9628	0.7643	0.4539	0.0400	0.9683
Timing * Nitrapyrin * UAN rate	1	0.8032	0.7680	0.8539	0.6812	0.3266	0.4785	0.4000	0.8848
Year * Timing * Nitrapyrin * UAN rate	3	0.9330	0.7722	0.7133	0.4268	0.3245	0.2135	0.0329	0.6352

Table 2.4. Three-factor ANOVA table for selected corn production and quality variables.

Source	df	SPAD		Plant	Grain					
		V8	VT	Population	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	0.323	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

Table 2.5. Soil NO<sub>3</sub>-N and NH<sub>4</sub>-N concentration 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<sup>-1</sup>). Data were averaged over years (2012-2015).

UAN ---- kg ha <sup>-1</sup> ----	PRE <sup>†</sup>			
	NO <sub>3</sub> -N		NH <sub>4</sub> -N	
	Minus NI	Plus NI	Minus NI	Plus NI
	----- 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 <sub>(0.01)</sub> <sup>‡</sup>	----- 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 <sub>(0.01)</sub>	----- 4.1 -----		----- NS <sup>§</sup> -----	

<sup>†</sup>Only one soil sample from V3 application timing plots at the V3 growth stage was taken because V3 application timing treatment were applied after soil sampling. This one sample was assumed to represent all the V3 application timing plots. Soil NO<sub>3</sub>-N and NH<sub>4</sub>-N from 0 to 22 cm depth for V3 application timing plots were 13.9 and 6.0 mg kg<sup>-1</sup>, respectively. Soil NO<sub>3</sub>-N and NH<sub>4</sub>-N from 23 to 46 cm depth for V3 application timing plots were 4.6 and 5.6 mg kg<sup>-1</sup>, respectively.

<sup>‡</sup>Least significant difference at  $P \leq 0.01$

<sup>§</sup>Non-significant

Table 2.6. Soil NH<sub>4</sub>-N 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 <sup>-1</sup> -----	
2012	15.8	31.6
2013	27.9	39.3
2014	19.0	19.0
2015	12.3	14.3
LSD <sub>(0.1)</sub> <sup>†</sup>	----- 7.1 -----	

<sup>†</sup>Least significant difference at  $P \leq 0.1$

Table 2.7. Soil NO<sub>3</sub>-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 <sup>-1</sup> -----			
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 <sub>(0.1)</sub> <sup>†</sup>	----- 2.5 -----			

<sup>†</sup>Least significant difference at  $P \leq 0.1$

Table 2.8. Soil NO<sub>3</sub>-N from 0 to 22 cm depth at harvest for UAN application rates (143 and 168 kg ha<sup>-1</sup>). Data is averaged across the years (2012-2015).

UAN	NO <sub>3</sub> -N
----- kg ha <sup>-1</sup> -----	--- mg kg <sup>-1</sup> ---
143	13.7
168	16.2
LSD <sub>(0.1)</sub> <sup>†</sup>	----- 2.4 -----

<sup>†</sup>Least significant difference at  $P \leq 0.1$

Table 2.9. Soil NH<sub>4</sub>-N from 0 to 22 cm depth at harvest for application timing (PRE and V3) and years (2012-2015).

Year	NH <sub>4</sub> -N	
	PRE	V3
	----- mg kg <sup>-1</sup> -----	
2012	4.2	13.3
2013	4.9	5.9
2014	4.6	6.4
2015	4.3	3.8
LSD <sub>(0.1)</sub> <sup>†</sup>	----- 2.2 -----	

<sup>†</sup>Least significant difference at  $P \leq 0.1$

Table 2.10. Soil NO<sub>3</sub>-N from 23 to 46 cm depth at harvest for application rates (UAN 143 kg ha<sup>-1</sup> and UAN 168 kg ha<sup>-1</sup>), application timing (PRE and V3), absence (Minus) or presence (Plus) of nitrification inhibitor (NI, nitrapyrin) and years (2012-2015).

Year	UAN 143 kg ha <sup>-1</sup>				UAN 168 kg ha <sup>-1</sup>			
	PRE		V3		PRE		V3	
	Minus NI	Plus NI	Minus NI	Plus NI	Minus NI	Plus NI	Minus NI	Plus NI
	----- mg kg <sup>-1</sup> -----							
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 <sub>(0.1)</sub> <sup>†</sup>	----- 1.8 -----							

<sup>†</sup>Least significant difference at  $P \leq 0.1$

Table 2.11. 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 <sub>(0.1)</sub> <sup>†</sup>	----- 2.2 -----		----- 2.2 -----	

<sup>†</sup>Least significant difference at  $P \leq 0.1$

Table 2.12. Grain moisture and test weight in the absence (minus) and or presence (plus) of nitrification inhibitor (NI, nitrapyrin), application timing (PRE and V3) and UAN rate (143 and 168 kg ha<sup>-1</sup>), and grain protein for application timing and UAN rate. Data is combined over years (2012-2015).

UAN	Moisture		Test Weight		Protein
	Minus NI	Plus NI	Minus NI	Plus NI	
---- kg ha <sup>-1</sup> ----	----- g kg <sup>-1</sup> -----		----- kg m <sup>-3</sup> -----		--- g kg <sup>-1</sup> ---
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 <sub>(0.1)</sub> <sup>†</sup>	----- 8 -----		----- 14 -----		--- 0.2 ---

<sup>†</sup>Least significant difference at  $P \leq 0.1$

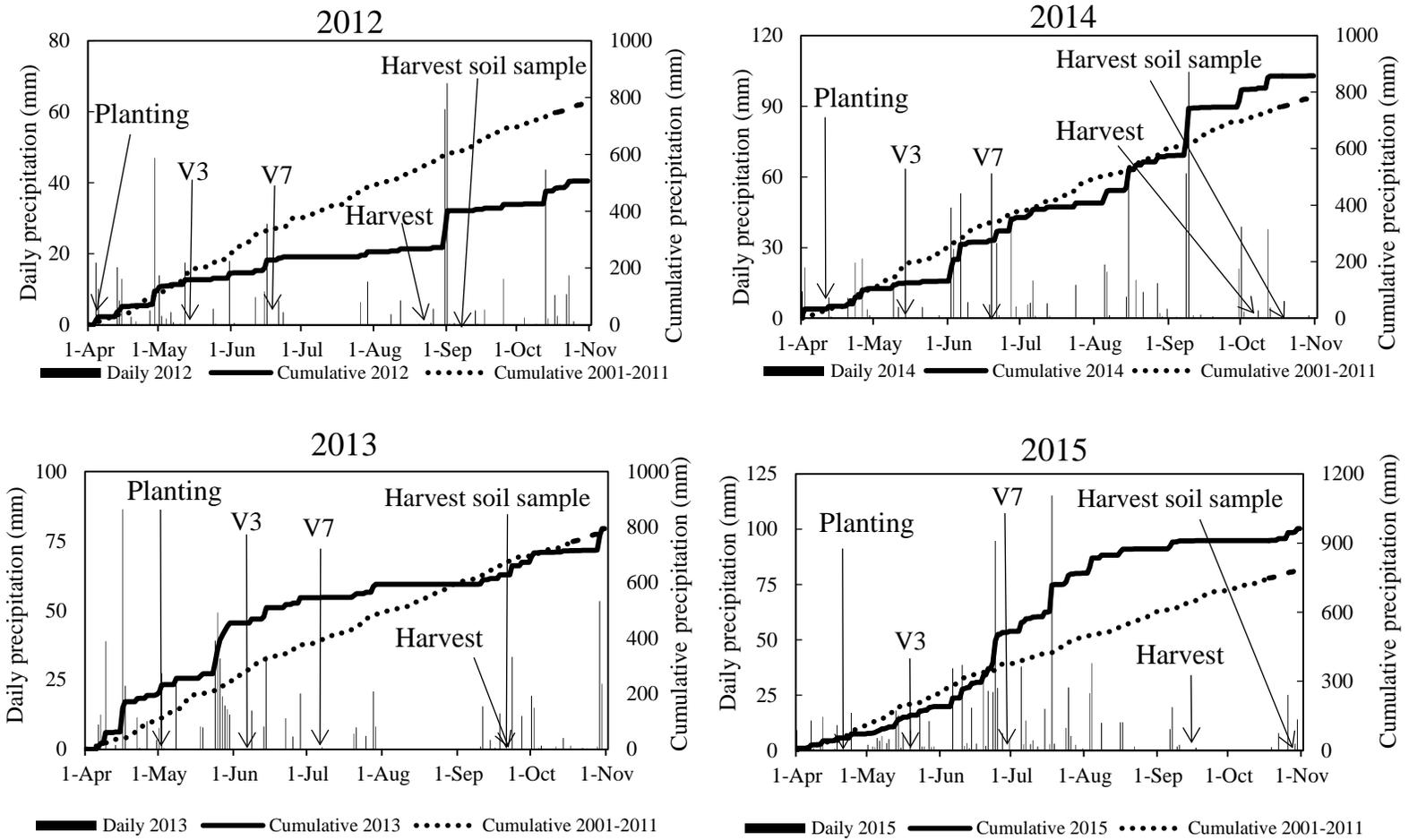


Figure 2.1. Precipitation history for the growing seasons of 2012, 2013, 2014 and 2015. Bars represent daily precipitation; solid line represents cumulative precipitation over the season; and dotted-line represents cumulative precipitation from 2001 to 2011. V3 and V7 are corn growth stages.

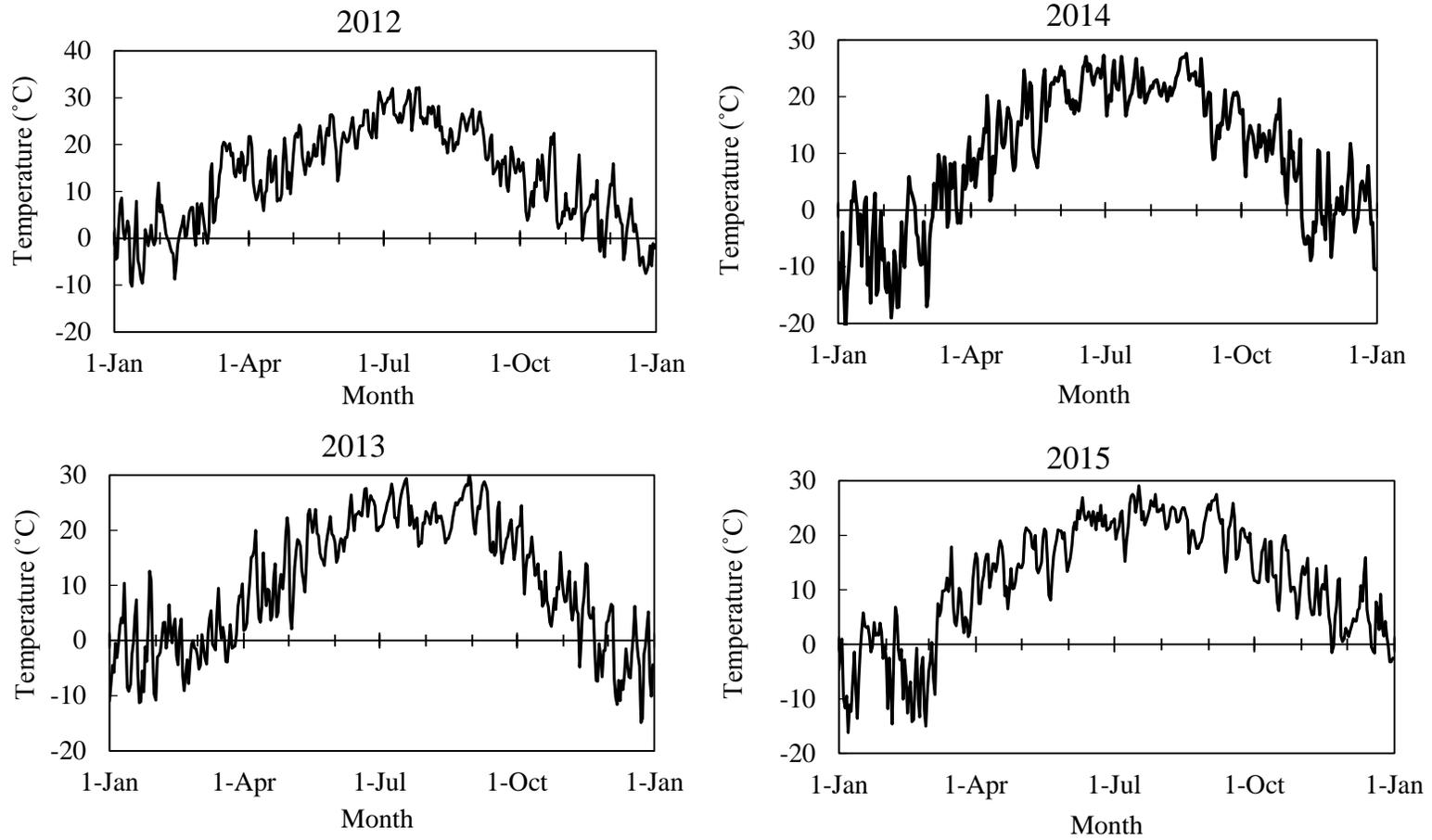


Figure 2.2. Daily average air temperature (°C) for the years of 2012, 2013, 2014 and 2015.

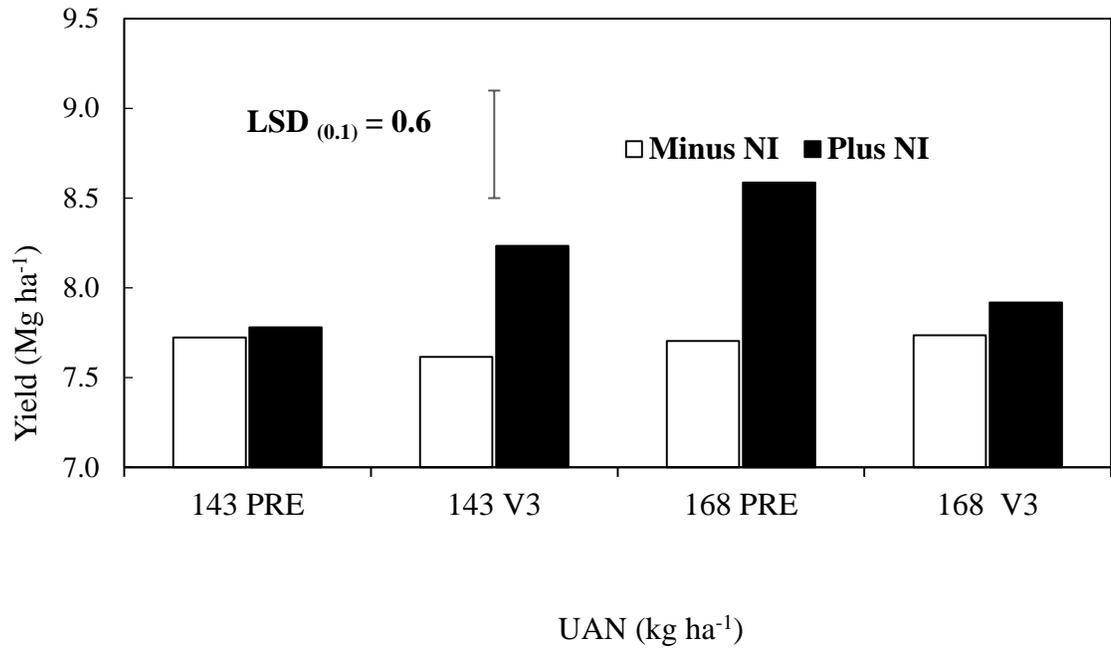


Figure 2.3. Corn grain yield as a function of interaction of UAN application timing (PRE or V3), application rate (143 or 168 kg ha<sup>-1</sup>) and presence (Plus) or absence (Minus) of nitrification inhibitor (NI) (nitrapyrin). Data were combined over years (2012 to 2015). LSD, least significant difference at  $P \leq 0.1$ .

**CHAPTER 3**  
**MANAGEMENT OF NITRAPYRIN AND KAS-771G77 WITH UREA**  
**AMMONIUM NITRATE ON WINTER WHEAT YIELD AND SOIL NITROGEN**

**ABSTRACT**

Synchrony between soil mineral nitrogen (N) supply and crop N demand is important for optimal plant growth. Excessively wet conditions expose poorly-drained soils to an increased potential of N loss and reducing N use efficiency of crops. A two-year experiment on wheat was initiated in 2014 and concluded in 2016 at the University of Missouri's Greenley Memorial Research Center (40°1'17"N, 92°11'24.9"W) near Novelty, Missouri. The objective of this experiment was to evaluate the effectiveness of nitrapyrin and KAS-771G77 applied with an early or late split application timing (40:60%) of 79 or 112 kg N ha<sup>-1</sup> on winter wheat (*Triticum aestivum* L.) soil and plant N status as well as grain yield. Year and UAN rate interacted for grain yield. The highest grain yields were achieved in 2016. Yields were similar (3,550 to 3,686 kg ha<sup>-1</sup>) in 2015 between UAN application rates. UAN at 112 kg N ha<sup>-1</sup> resulted in 551 kg ha<sup>-1</sup> yield over UAN at 79 kg N ha<sup>-1</sup> in 2016. Nitrapyrin and KAS-771G77 did not significantly affect soil ammonium or nitrate-N concentrations compared to the absence of a nitrification inhibitor. Nitrapyrin with the high UAN rate produced the highest grain test weight. In optimal growing conditions, split application of a high UAN application rate could be adjusted. However, use of nitrification inhibitors is a risk management strategy for unpredictable severe wet field conditions.

**INTRODUCTION**

Wheat is one of the most important crops in the world. Worldwide, 2.5 billion people obtain more than 20% of their calories and about 20% of the protein from it (Braun et al., 2010b). A growth gap between current global wheat production of 1.1% per

year (Dixon et al., 2009) and predicted global wheat production 1.7% per year by 2050 (Rosegrant and Agcaoili, 2010b) has already been reported. According to Brisson et al. (2010b), the growth in wheat production is stagnant in some parts of the world. It is critical to devise new and/or investigate existing strategies to achieve increased productivity to avoid shortfalls in production in the future (Hawkesford et al., 2013b). Midwestern states (North Dakota, South Dakota, Michigan, Iowa, Missouri, Indiana, Nebraska, Wisconsin, Illinois, Kansas, Minnesota and Ohio) are part of the North Central Region of the United States, and produce over half of the wheat in the U.S. (Grace et al., 2011b).

Nitrogen (N) is essential to all forms of life. Its availability largely affects the productivity of many ecosystems (Vitousek et al., 2002). Approximately one third of the world population would have not lived had the amount of protein attributed to the use of manufactured N fertilizers was not obtained (Smil, 1997b). Synthetic N fertilizers commonly used in row-crop agriculture include urea, anhydrous ammonia, ammonium nitrate, ammonium sulfate and urea ammonium nitrate (UAN) (Millar et al., 2010b). Urea-based fertilizers are the most widely sold (43%) N fertilizers in the world (Bouwman et al., 2002b; d). Due to its highly soluble nature, the potential risk of N losses in sub-surface drainage (Drury et al., 2009b) and denitrification (Nash et al., 2012b) in poorly-drained soils, and leaching in sandy soils (Wilson et al., 2009b) is frequently associated with urea based N fertilizers. These N losses from synthetic fertilizers decrease agricultural crop and livestock production, and the fulfillment of nutritional requirements of rapidly increasing human population (Vitousek et al., 1997b; Howarth et al., 2002b; Howarth, 2004b). Harmful environmental effects due to N loss mechanisms

include loss of biodiversity in aquatic and terrestrial ecosystems and invasion of N-loving weeds, acidification of soil and water resources, increased greenhouse gas levels due to emissions of N<sub>2</sub>O, depletion of stratospheric ozone, increased ozone-induced injury to crop, forest and other ecosystems increased atmospheric haze, and production of airborne particulate matter (Galloway and Cowling, 2002b). Factors such as variation in management practices (e.g., selection of N source, soil tillage method, timing and method of application), climatic conditions, soil properties, and crop growth affect the relative magnitude of N loss processes (Motavalli et al., 2008b).

Low-lying landscapes and poorly-drained soil conditions cause soils to be saturated during prolonged wet conditions from frequent rainfall events. These saturated conditions increase the risk of N loss and adversely affect crop yields (Nelson et al., 2009b; Nash et al., 2012b, 2013b). North central Missouri, southeast Kansas and southern Illinois are part of the central claypan MRLA (Anderson et al., 1990b; Jung et al., 2006b; Myers et al., 2007b). The claypan region includes 4 million hectares comprised predominantly of soils characterized by a subsoil layer of 100% more clay content at a depth of 20 to 40 cm compared to surface-layer making it a poorly-drained soil. Factors, such as fertilizer N sources, application timings, rates, and nitrification inhibitors (NI), interact differently in different conditions. Precise understanding of the effects of individual factors and their interactions on N losses as well as crop production may increase or maintain optimal crop grain yields. Lack of research studies on poorly-drained claypan soils to determine accurate research-based recommendations has been reported (Motavalli et al., 2008b). In addition, the simultaneous cumulative impact of

multiple management factors have not been studied extensively (Burzaco et al., 2013b) in winter wheat production in the Midwestern U.S.

Urea ammonium nitrate is available in solution form which allows it to be mixed easily with other chemicals such as NIs or other nutrients. Half of its N is in the urea form and the other half is in  $\text{NO}_3^-$  (25%) and  $\text{NH}_4^+$  (25%). Research is limited on the use of UAN as a N fertilizer source for winter wheat on claypan soils. A two-year Indiana study compared UAN application rates (0, 90 and 180 kg ha<sup>-1</sup>), application timings (preemergence and sidedress at V6 stage of corn (*Zea mays* L.) growth) and use of NI (nitrapyrin, N-Serve™) on yield, N<sub>2</sub>O emissions and yield-scaled N<sub>2</sub>O emissions (Burzaco et al., 2013). That study reported a 3 Mg ha<sup>-1</sup> increase in yield when UAN was applied as side-dress at 180 kg ha<sup>-1</sup> with nitrapyrin compared to a pre-emergence application at 90 kg N ha<sup>-1</sup> without nitrapyrin. Nitrapyrin significantly reduced both daily and cumulative N<sub>2</sub>O emissions when averaged across years. However, UAN rate influenced corn yield, yield-scaled N<sub>2</sub>O emissions, N<sub>2</sub>O fluxes and cumulative N<sub>2</sub>O emissions the most followed by application timing and nitrapyrin. The range in N application rates were large which may have overshadowed the effect of nitrapyrin on yield and N<sub>2</sub>O emissions suggesting further research is needed to investigate the effect of nitrapyrin with lower UAN rates.

Another corn study conducted in Minnesota over a three-year period under continuous corn and subsurface tile drainage on poorly-drained glacial till soils evaluated the effects of two rates (9.4 and 18.7 L ha<sup>-1</sup>) of a newly developed NI, KAS-771G77 (Gabrielson and Epling, 2016) and one rate of nitrapyrin (2.6 L ha<sup>-1</sup>, 0.57 kg ai ha<sup>-1</sup>) on corn yield (Vetsch and Schwab, 2014). These NIs were applied with four different rates

67, 134, 202 and 269 kg ha<sup>-1</sup> of UAN. A relatively wet year resulted in the lowest yield in the absence of a NI or 18.7 L ha<sup>-1</sup> of KAS-771G77 compared to at 9.4 L ha<sup>-1</sup> of KAS-771G77 and nitrapyrin application. No research has reported on the effects of KAS-771G77 on wheat response with UAN. The objective of this experiment was to evaluate the effectiveness of nitrapyrin and KAS-771G77 applied with an early or late split application timing (40:60%) of 79 or 112 kg N ha<sup>-1</sup> on winter wheat soil and plant N status and grain yield.

## **MATERIALS AND METHODS**

A two-year experiment on wheat was initiated in 2014 at the University of Missouri's Greenley Memorial Research Center (40°1'17"N, 92°11'24.9"W) near Novelty, Missouri. A single year was assigned based on the harvest date so the 2014-2015 season was reported as the 2015 experiment. The soil series was a Kilwinning silt loam (fine, smectitic, mesic Vertic Epiaqualfs). The experiment was a three-factor randomized complete block design (RCBD) with six and five replications in 2015 and 2016, respectively. Factors included two split-applied UAN rates (79 kg ha<sup>-1</sup> and 112 kg ha<sup>-1</sup>), three nitrification inhibitor timings (none, nitrification inhibitor with the first application timing, or nitrification inhibitor with the second application timing), and nitrification inhibitor [nitrapyrin (Instinct II, Dow AgroSciences, Indianapolis, IN) at 0.513 kg a.i. ha<sup>-1</sup> and KAS-771G77 (Koch Agronomic Services, Wichita, KS) at 9.3 L ha<sup>-1</sup>]. The split applications of UAN were applied as 40% at greenup and 60% one month afterwards. A non-treated control was included in the design. The location was different each year and wheat was planted following soybean harvest. Wheat was drilled (Great Plains Solid Stand 10, Assaria, KS) at 107 kg seeds ha<sup>-1</sup> in 19.1 cm wide rows. The crop

was no-till seeded for 2015 while vertical tillage (Case IH 330, Racine, WI) occurred for 2016 due to the presence of winter annual weeds and extensive residue. Plot size was 3 by 15 m. Wheat cultivars were 'MFA2525' planted on 21 October 2014 for 2015 and 'MFA2449' planted on 17 September 2015 for 2016. Cultivars changed from 2015 to 2016 due to the unavailability of MFA2525.

Initial soil samples were collected from the non-treated control on 19 March 2015 (2015 study-year) and 11 November 2015 (2016 study-year) to determine selected soil properties (Table 3.1). Soil samples were collected from 0 to 15 and 16 to 30 cm depths using a stainless-steel push probe. The soil samples were analyzed using standard soil test analytical methods at the University of Missouri Soil and Plant Testing Laboratory (Nathan et al., 2006). Additional analyses of soil  $\text{NH}_4^+$  -and  $\text{NO}_3^-$ -N were determined using a 2M KCl extraction and Lachat QuickChem automated ion analyzer (Hach Corp., Loveland, CO).

Field management, field characteristics, soil maintenance and crop protection chemicals are reported in Table 3.1. Chlorophyll meter readings for 10 plants per plot were recorded to determine chlorophyll content of the flag leaf using a SPAD meter (Minolta SPAD-502, Tokyo, Japan). At physiological maturity, whole plant samples were collected from each plot from a 30 by 76 cm quadrat to determine aboveground dry biomass. This sample was dried, weighed, ground to pass a 1 mm sieve (Wiley Mill, Swedesboro, NJ), and analyzed for TKN concentration (QuickChem, 1992) which was used to calculate tissue N uptake and tissue N recovery efficiency ( $\text{RE}_\text{N}$ ) (Fixen et al., 2014). Wheat grain yields were determined with a small-plot combine (Wintersteiger Delta, Salt Lake City, UT) and adjusted to  $130 \text{ g kg}^{-1}$  moisture content before analysis.

Grain samples were collected from each plot to determine test weight and moisture using a grain analysis computer (GAC 2100, DICKEY-john Corporation, Auburn, IL).

Precipitation data for the growing seasons were collected from the Missouri Historical Agricultural Weather Database website (MU Extension, 2017). Data were analyzed for each study year from October to July for 2015 and September to July for 2016. The precipitation data was assigned based on the harvest date such as the 2014-2015 season was reported as 2015. Daily and cumulative rainfall was reported in comparison with average cumulative rainfall for a 10-year period (2004 to 2014) in Figure 3.1.

Data were first subjected to single-factor analysis of variance (ANOVA) using the SAS statistical program (SAS Institute, 2016) to determine significant differences between N treatments and the non-treated control (NTC). This was followed by a three-factor ANOVA in the absence of the NTC since only one non-treated control was included in the design. Results were presented when significant differences among treatments were observed at  $P \leq 0.05$ . Means were then separated using Fisher's Protected Least Significant Difference (LSD).

## **RESULTS AND DISCUSSION**

### **Precipitation**

Cumulative precipitation in 2015 (1006 mm) was 20% (204 mm) greater than the 10-year average precipitation (Figure 3.1). Nearly 80% (792 mm) of the precipitation occurred after the first N application. Most of the rainfall during this period was from May through July with two unusually high rainfall events in June (95 mm) and July (115 mm). The month of June received an even distribution of precipitation in the range of 20

to 39 mm. From planting (21 October) until just prior to first N treatment application on 19 March, the 2015 daily cumulative rainfall was below the average cumulative rainfall. However, near the end of June precipitation exceeded the 10-year average.

The precipitation distribution pattern in 2016 was different from 2015. The cumulative daily amount was 19% (154 mm) less than the 10-year average (795 mm). The highest individual daily rainfall events occurred during mid-November (43 mm) and mid-December (45 mm), which was lower than 2015. The overall precipitation over the season, except from January to the end of March, was low but sufficient for high yields.

### **Average Daily Air Temperature**

Average daily air temperature from September 1 of the planting-year to August 31 of the harvest-year for the years of 2015 and 2016 is shown in Figure 3.2. In 2015, temperature between late-November and early-March was below the freezing point (0°C). In 2016, freezing temperatures were only observed in early-January to mid-February. Generally, temperatures in 2015 in the months of November through March were colder compared to 2016. In addition, temperature dropped below 10°C earlier and reached back to 10°C later in 2015 compared to 2016. This means temperature in 2015 remained colder for extended period of time compared to 2016.

### **Soil Nitrogen**

Soil NO<sub>3</sub>-N and NH<sub>4</sub>-N concentrations for the 0 to 15 and 16 to 30 cm depths of UAN at 79 (Figure 3.3A-D) and 112 (Figure 3.4A-D) kg N ha<sup>-1</sup> one-month (1 MAA), two-months (2 MAA), and three-months (3 MAA) after the first treatment application (MAA) were similar among treatments. Ammonium-N concentration at a 0-15 cm depth in the soil was 6-7 mg kg<sup>-1</sup> 1 MAA for the non-treated control and UAN treatments,

while the concentration was similar among treatments 2 MAA. There was 8-10 ppm ammonium-N in the soil profile (0-15 cm deep) for all treatments 3 MAA.

Soil  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations for both UAN applications from 16 to 30 cm depth were not affected by the NIs (Figure 3.3 A and C). This may be because of one of two reasons: 1) the N from UAN fertilizer may have not reached the soil depth of 16 to 30 cm at this point of time and/or 2) NIs did not move into soils as quickly as N to affect soil  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations even if the N leached into the soil because sorption/adsorption of NI to soil colloids, in particular nitrapyrin and DMPP, have been reported to reduce their effectiveness (Hoeft, 1984b; Liu et al., 1984b; Barth et al., 2001). Generally,  $\text{NH}_4\text{-N}$  concentration 3 MAA at both soil depths remained the highest followed by 2 MAA. This result probably supported the fact that  $\text{NO}_3^-$  is relatively more soluble and moves quickly in soils or there was greater plant uptake of  $\text{NO}_3^-$  compared to  $\text{NH}_4^+$ .

#### **Tissue Biomass, N Concentration and Uptake, and $\text{RE}_\text{N}$**

Nitrogen treatments increased tissue biomass, N concentration, and uptake compared to the non-treated control (data not presented). There was a four-way interaction between year, nitrification inhibitor timing, nitrification inhibitor, and UAN rate ( $P = 0.0038$ ) (Tables 3.2 and 3.3). Total biomass was generally greater in 2015 compared to 2016. KAS-771G77 with first application of UAN at  $112 \text{ kg ha}^{-1}$  in 2015 had the highest ( $29410 \text{ kg ha}^{-1}$ ) biomass that was greater than all the treatments. The presence of a NI with UAN at  $112 \text{ kg ha}^{-1}$  affected biomass more (17%) in the first application timing compared to with UAN at  $79 \text{ kg ha}^{-1}$  or compared to the second application timing. However, including a NI in the second application timing in 2015

resulted in a 25 to 33% greater biomass than in 2016. Biomass with UAN at 79 kg ha<sup>-1</sup> with or without a NI was similar when first and second applications of UAN in 2015 and 2016 were assessed separately except for KAS-771G77 in 2016 applied with the first or second application timing. When applied with the first application timing, KAS-771G77 plus UAN at 79 kg N ha<sup>-1</sup> had an 1850 to 2320 kg ha<sup>-1</sup> greater biomass compared to UAN or UAN plus nitrapyrin.

There was a significant main effect of UAN application rate on tissue N concentration ( $P = 0.0002$ ) (Table 3.2). Tissue N concentration of wheat treated with UAN at 112 kg N ha<sup>-1</sup> had a 1 g kg<sup>-1</sup> greater N concentration than UAN at 79 kg N ha<sup>-1</sup>. Tissue N concentration was similar between NIs ( $P = 0.8600$ ) and NI timings ( $P = 0.1024$ ) (Tables 3.2 and 3.4).

Tissue N uptake was affected by UAN rate ( $P < 0.0001$ ) (Table 3.2). A 25% (51 kg ha<sup>-1</sup>) increase in tissue N uptake of UAN at 112 kg N ha<sup>-1</sup> was observed compared to UAN at 79 kg N ha<sup>-1</sup> (data not presented). Years and nitrification inhibitor interacted ( $P=0.0062$ ) for tissue N uptake (Tables 3.2 and 3.4). Nitrification inhibitors (nitrapyrin and KAS-771G77) increased tissue N uptake 40 to 60% in 2015 compared to 2016. However, nitrapyrin and KAS-771G77 had similar tissue N uptake in individual years. The overall reduced tissue N uptake was probably affected by cumulative precipitation and its distribution frequency over the years which resulted in different tissue N uptake amounts as well as total biomass production (Table 3.3) which is used to calculate N uptake.

Crop RE<sub>N</sub> is an estimate of the proportion of N fertilizer that is removed from the harvested crop biomass during the growing season to the ratio of crop yield per unit of

applied N fertilizer (Novoa and Loomis, 1981; Dobermann, 2005; Ladha et al., 2005).

There was a significant interaction ( $P < 0.0001$ ) between years and nitrification inhibitor (Tables 3.2 and 3.4). Crop  $RE_N$  in 2016 for nitrapyrin was the largest (198%), and was greater than KAS-771G77 in 2015, but no difference between NIs was observed in 2016.

### **Plant Population, Grain Moisture, Test Weight, and Yield**

Plant population and grain moisture at harvest were similar for all treatments (Table 3.2). A significant interaction between year, UAN rate and NI was reported for grain test weight (Table 3.2). However, no significant difference among treatments within 2015 and 2016 was observed (Table 3.5). Test weights were generally greater in 2016 compared to 2015 which could be due to a change in the cultivar or weather conditions.

Grain yield was 1,260 to 3,120 kg ha<sup>-1</sup> greater than the non-treated control for all the treatments in 2015 ( $P < 0.0001$ ) and 2016 ( $P < 0.0001$ ) (data not presented) indicating a N yield response. The three-factor ANOVA indicated an interaction between year and UAN rates (Table 3.2). Grain yields were similar among N rates in 2015 (Table 3.5). In 2016, UAN at 112 kg ha<sup>-1</sup> had 531 kg ha<sup>-1</sup> greater yields compared to UAN at 90 kg N ha<sup>-1</sup> (4,871 kg ha<sup>-1</sup>). Yields in 2016 at both UAN application rates were significantly greater than 2015. No significant differences between nitrification inhibitors ( $P = 0.3917$ ) or nitrification inhibitor application timing ( $P = 0.5994$ ) was observed for yield.

The significant effect of UAN rate on wheat grain yield in 2015 contradicts the law of diminishing returns which states that an increase in plant N uptake is associated with N supply, and grain yield usually increases with increase in plant N uptake (Cassman et al., 2002a; Ciampitti and Vyn, 2012, 2013). Per this law, UAN 112 kg ha<sup>-1</sup> should have produced greater yields over UAN 79 kg ha<sup>-1</sup> in 2015. It could be due to the

overall high (20%) precipitation compared to 10-year average and lack of precipitation from planting to first treatment application on March 19 (20%) while 80% of the season's precipitation occurred afterwards from the month of May to July with two, 100 and 110 mm rainfall events in June and July, respectively. In comparison, 2016 received 20% lower precipitation than the 10-year average, but, the precipitation was evenly distributed over the season with relatively low daily rainfall events favored the overall high grain yields in 2016. Since beyond a certain threshold, incremental gains in N supply are accompanied by less than proportional grain yield (Burzaco et al., 2014). This suggests that in optimal growing conditions with a split application of UAN application that rates could be further adjusted. A split-application of N is typically recommended for wheat (Woolfolk et al., 2002); therefore, NI treatments may be better suited when a single application is used along with reduced rates of N.

## **CONCLUSIONS**

Nitrapyrin and KAS-771G77 did not affect soil N status compared to non-treated UAN in the two years of this research. Application timing of KAS-771G77 and application rate of UAN affected tissue biomass greatest. UAN application rate increased tissue N concentration and tissue total N uptake, but there was no effect of the NIs or timing of the NI. Nitrapyrin combined with UAN had the highest crop  $RE_N$  in 2015, but no effect was observed in 2016. Plant population and grain moisture were not affected by UAN rate, NI, or NI timing. Nitrification inhibitors had similar grain test weights although differences were observed between 2015 and 2016. Yields were similar (3,550 to 3686 kg ha<sup>-1</sup>) in 2015 between UAN application rates. However, UAN at 112 kg N ha<sup>-1</sup> increased yield 551 kg ha<sup>-1</sup> over UAN at 79 kg N ha<sup>-1</sup> in 2016. Application of nitrapyrin

or KAS-771G77 or their application timings with split applied UAN rate (low or high) did not affect winter wheat yields. However, use of NIs is a risk management strategy for unpredictable extreme wet field conditions. In optimal growing conditions, a split application of a high UAN application rate could be reduced.

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Table 3.1. Treatment and evaluation dates, selected soil initial properties, maintenance fertilizer and crop protection applications for 2015 and 2016.

----- Timeline -----				
Study-year	2015		2016	
Initial soil sampling	19 March		11 Nov. 15	
Planting	21 Oct. 14		17 Sep. 15	
First treatment application	19 March		10 March	
First in-season soil sampling (1 MAA)	20 April		15 April	
Second treatment application	22 April		15 April	
Plant population	20 April		6 May	
Second in-season soil sampling (2 MAA)	18 May		6 May	
Spad	27 May		23 May	
Dry aboveground biomass	3 June		31 May	
Third in-season soil sampling (3 MAA)	17 June		9 June	
Harvest	7 July		24 June	
----- Selected Soil Initial Properties -----				
Study-year	2015	2016	2015	2016
Soil depth, cm	0 to 15	0 to 15	16 to 30	16 to 30
pH	5.1	4.7	5.5	5.0
Neutralizable acidity (NA), cmol <sub>c</sub> kg <sup>-1</sup>	4.5	9.4	4.1	7.0
Cation exchange capacity (CEC), cmol <sub>c</sub> kg <sup>-1</sup>	16	22	17	23
Bray 1 phosphorus (P), kg ha <sup>-1</sup>	22	8	73	14
Calcium (CA), kg ha <sup>-1</sup>	3,998	4,048	4,281	4,679
Magnesium (Mg), kg ha <sup>-1</sup>	388	496	428	914
Potassium (K), kg ha <sup>-1</sup>	181	122	367	260
Organic matter (OM), %	2.8	2.2	3.2	2.6
Nitrate-nitrogen (NO <sub>3</sub> -N), mg kg <sup>-1</sup>	4.1	1.5	19.4	3.0
Ammonium-nitrogen (NH <sub>4</sub> -N), mg kg <sup>-1</sup>	4.4	4.1	9.7	6.2
----- Maintenance Fertilizer -----				
	Application rate		Application date	
Study-year, 2016				
N-P-K-S-Zn (kg ha <sup>-1</sup> )	16-52-0-13-2.6		23 Sep. 2015	
----- Plant Protection -----				
	Application rate		Application date	
Study-year, 2015				
Glyphosate <sup>†</sup> (kg a.i. ha <sup>-1</sup> )	1.17		21 Oct. 2014	
2,4-D <sup>‡</sup> (kg a.i. ha <sup>-1</sup> )	0.35		21 Oct. 2014	
Azoxystrobin <sup>§</sup> (kg a.i. ha <sup>-1</sup> )	0.15		13 May 2015	

<sup>†</sup>N-(phosphonomethyl) glycine.

<sup>‡</sup>Isooctyl (2-ethylhexyl) ester of 2,4-Dichlorophenoxyacetic acid.

<sup>§</sup>methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate.

One (1 MAA), two (2 MAA), and three (3 MAA) months after the first fertilizer application timing.

Table 3.2. Three-factor ANOVA of plant and grain parameters.

Source	DF	Tissue				Plant	Grain		
		Biomass	N	N uptake	RE <sub>N</sub> <sup>†</sup>	Population	Moisture	Test Wt <sup>‡</sup>	Yield
		Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
Year	1	<.0001	0.0003	<.0001	0.0001	0.0110	<.0001	<.0001	<.0001
Rep(Year)	2	0.7126	0.2343	0.5338	0.0691	0.0003	0.0001	0.0018	<.0001
NI timing	2	0.5278	0.1024	0.3054	0.4181	0.4416	0.6407	0.2781	0.5994
Year * NI <sup>§</sup> timing	2	0.7198	0.9978	0.6815	0.7419	0.1226	0.5637	0.5853	0.3556
NI	1	0.9028	0.8600	0.4483	0.2457	0.3130	0.8689	0.5176	0.3917
Year * NI	1	0.0020	0.0616	0.0062	<.0001	0.0808	0.5302	0.1764	0.6884
UAN <sup>††</sup> rate	1	<.0001	0.0002	<.0001	0.1661	0.1085	0.2563	0.0643	<.0001
Year * UAN rate	1	0.6790	0.0757	0.1791	0.8678	0.2713	0.4368	0.0182	0.0058
NI timing * NI	2	0.4812	0.2444	0.4568	0.4508	0.2906	0.9960	0.5673	0.5846
Year * NI timing * NI	2	0.0763	0.4556	0.1912	0.3542	0.2101	0.7555	0.3585	0.7127
NI timing * UAN rate	2	0.0756	0.7528	0.2246	0.3134	0.9162	0.7589	0.6314	0.7362
Year * NI timing * UAN rate	2	0.7857	0.2933	0.4945	0.5267	0.8620	0.8881	0.8794	0.6955
NI * UAN rate	1	0.4435	0.6838	0.4161	0.5321	0.1620	0.9568	0.2336	0.7505
Year * NI * UAN rate	1	0.0902	0.8215	0.1724	0.5767	0.2601	0.4095	0.0012	0.3899
NI timing * NI * UAN rate	2	0.1738	0.9136	0.8147	0.8021	0.8166	0.9182	0.5840	0.5834
Year * NI timing* NI * UAN rate	2	0.0038	0.5064	0.0517	0.0947	0.2731	0.8650	0.3288	0.5831

<sup>†</sup>REN, recovery efficiency of nitrogen; <sup>‡</sup>Wt, weight; <sup>§</sup>NI, nitrification inhibitor; <sup>††</sup>UAN, urea ammonium nitrate

Table 3.3. The effect of nitrification inhibitor (NI) and timing, year, and urea ammonium nitrate (UAN) rate on total tissue dry biomass.

Year	NI Application Timing					
	First Application			Second Application		
	Without NI	Nitrapyrin	KAS-771G77	Without NI	Nitrapyrin	KAS-771G77
	kg ha <sup>-1</sup>					
2015						
UAN <sup>†</sup> 79	20770	21560	21050	21760	20300	19680
UAN 112	22440	24320	29410	22090	21390	21870
2016						
UAN 79	11050	11520	13370	14740	14390	10830
UAN 112	16460	14240	13200	13520	17750	20160
LSD ( $P = 0.05$ ) <sup>‡</sup>	----- 1470 -----					

<sup>†</sup>UAN kg ha<sup>-1</sup>

<sup>‡</sup>Least significant difference at  $P \leq 0.05$

Table 3.4. The effect of nitrification inhibitor on tissue N concentration, tissue N uptake, and tissue RE<sub>N</sub>. Main effects were presented unless a significant interaction was observed.

Nitrification inhibitor	Tissue N g kg <sup>-1</sup>	Tissue N uptake		Tissue RE <sub>N</sub> <sup>†</sup>	
		2015	2016	2015	2016
		---- kg N ha <sup>-1</sup> ----		---- % ----	
Nitrapyrin	11	261	111	197	57
KAS-771G77	11	223	133	126	98
LSD ( $P=0.05$ ) <sup>‡</sup>	NS <sup>§</sup>	---- 61 ----		---- 65 ----	

<sup>†</sup>RE<sub>N</sub> was calculated as,

(Crop N uptake (+N source) - Crop N uptake (soil alone)) \* 100 / Total N added  
(Barraclough et al., 2010)

<sup>‡</sup>Least significant difference at  $P \leq 0.05$

<sup>§</sup>Non-significant

Table 3.5. The effect of nitrification inhibitor, UAN rate and year on grain test weight, and the effect of urea ammonium nitrate (UAN) rate and year on grain yield.

UAN kg ha <sup>-1</sup>	Test weight				Yield <sup>†</sup>	
	2015		2016		2015	2016
	Nitrapyrin	KAS-771G77	Nitrapyrin	KAS-771G77		
	----- kg m <sup>-3</sup> -----				--- kg ha <sup>-1</sup> ---	
79	740	740	751	754	3,550	4,871
112	738	742	759	754	3,686	5,402
LSD ( $P = 0.05$ ) <sup>‡</sup>	----- 16 -----				----- 532 -----	

<sup>†</sup>Non-treated control grain yield was 2140 kg ha<sup>-1</sup> in 2015 and 2420 kg ha<sup>-1</sup> in 2016

<sup>‡</sup>Least significant difference at  $P \leq 0.05$

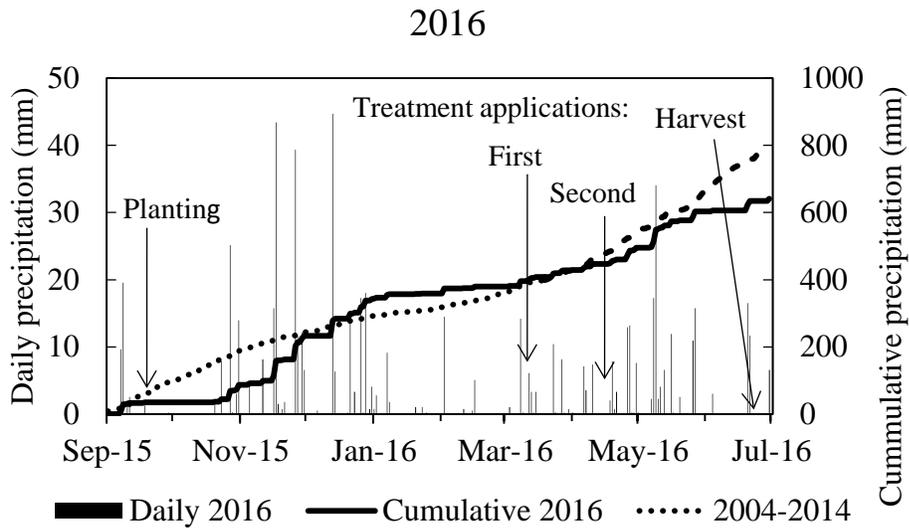
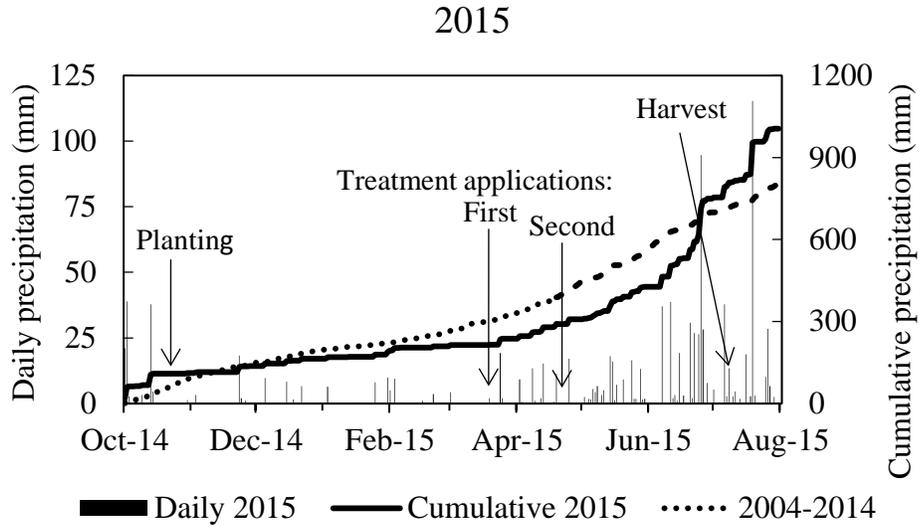


Figure 3.1. Precipitation history for the study years of 2015 and 2016. The bars represent daily precipitation of the study year, the solid-line represents the cumulative precipitation of the study year and the dotted-line represents the average cumulative precipitation from 2004 to 2014.

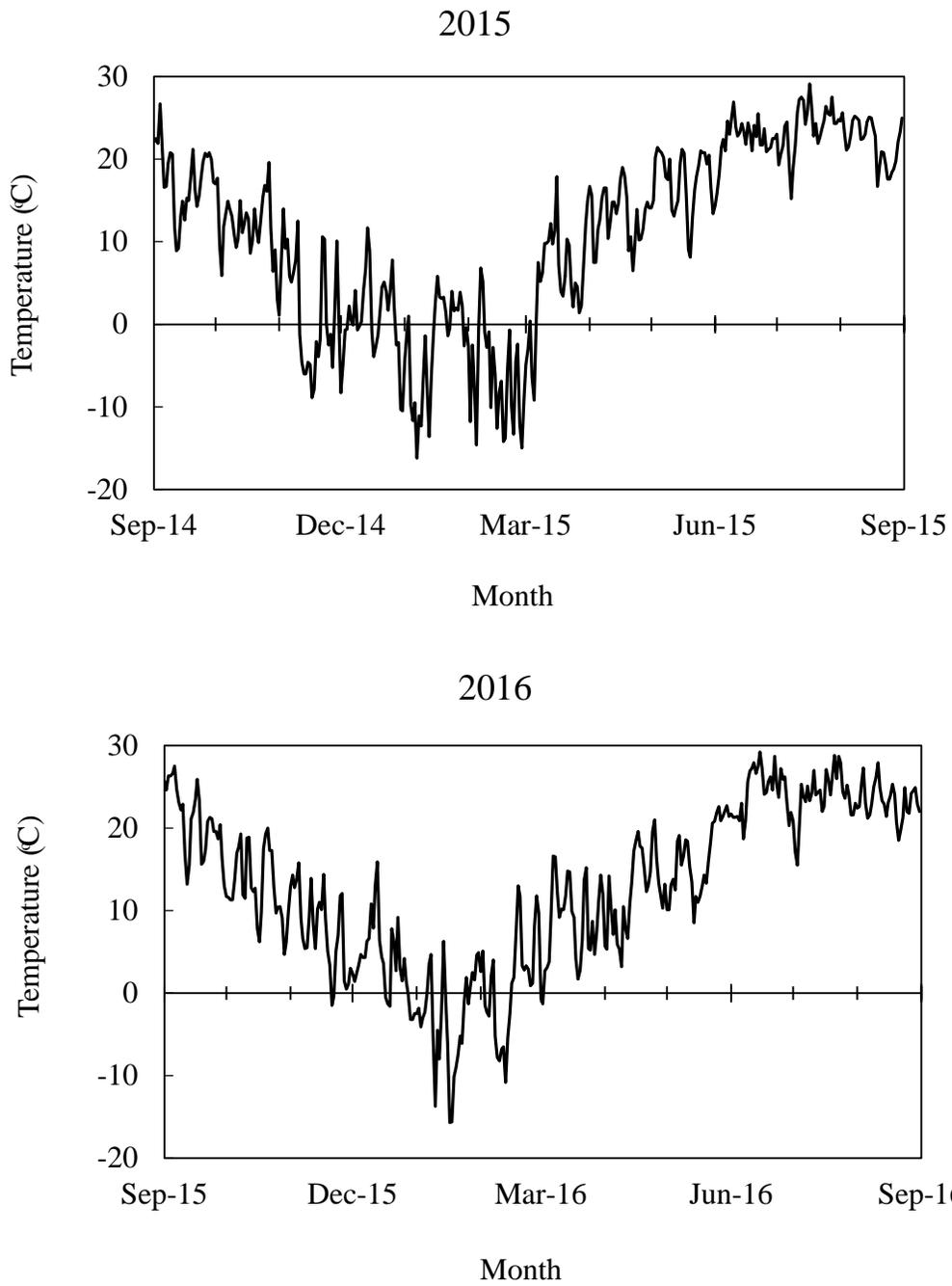


Figure 3.2. Daily average air temperature (°C) for the years of 2015 and 2016.

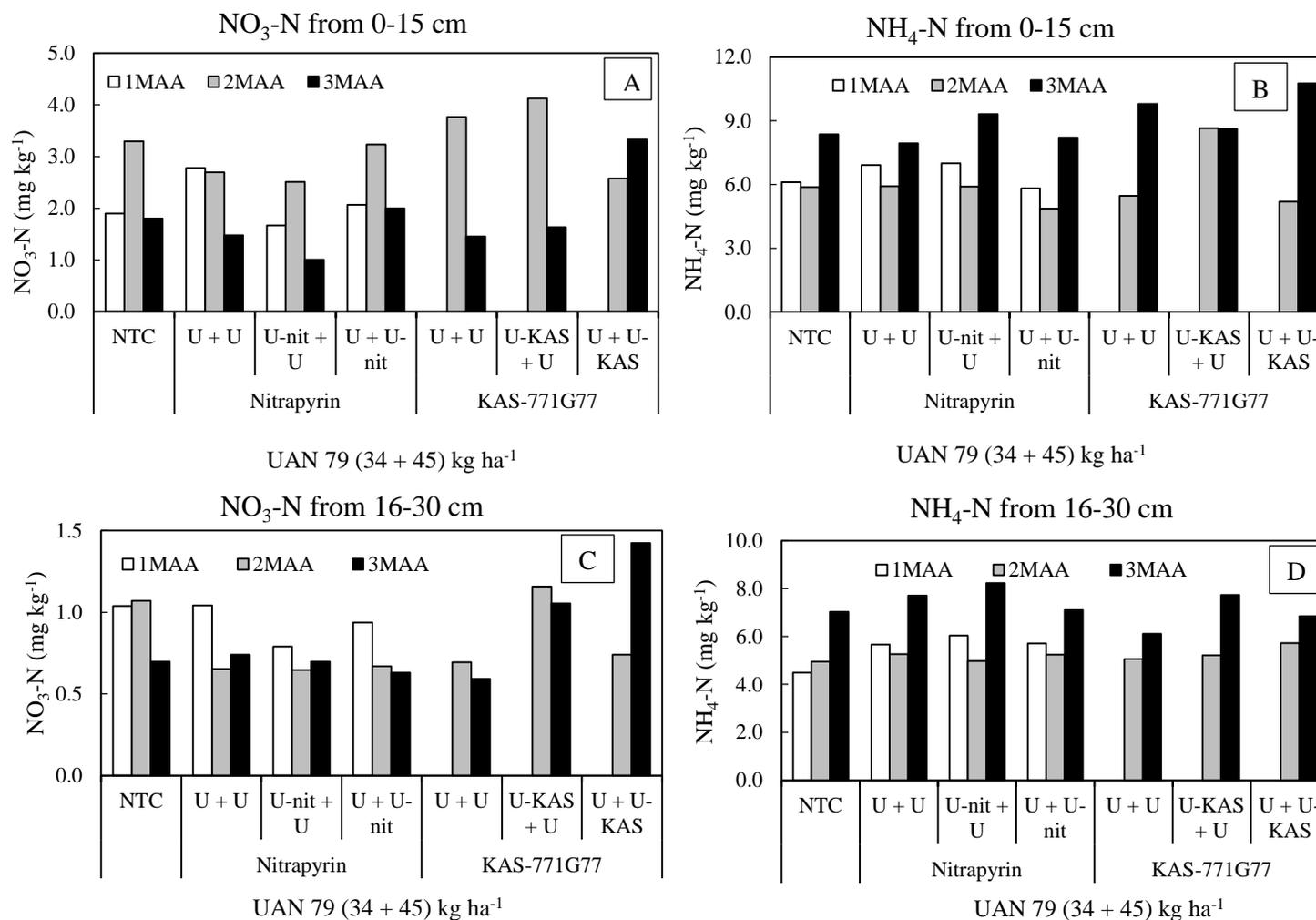


Figure 3.3. Soil test ammonium-nitrogen (NH<sub>4</sub>-N) concentration at 0-15 (A) and 16-30 (C) cm depths, and soil test nitrate-nitrogen (NO<sub>3</sub>-N) at a 0-15 (B) and 16-30 (D) cm depths 1-, 2-, and 3-months after application (MAA) of a 40:60% split application of urea ammonium nitrate (U) at totaling 79 kg N ha<sup>-1</sup>. Abbreviations: KAS, KAS-771G77; nitrapyrin; NTC, non-treated control; U, urea ammonium nitrate. One-month after application samples were only taken for the non-treated control and nitrapyrin treatments.

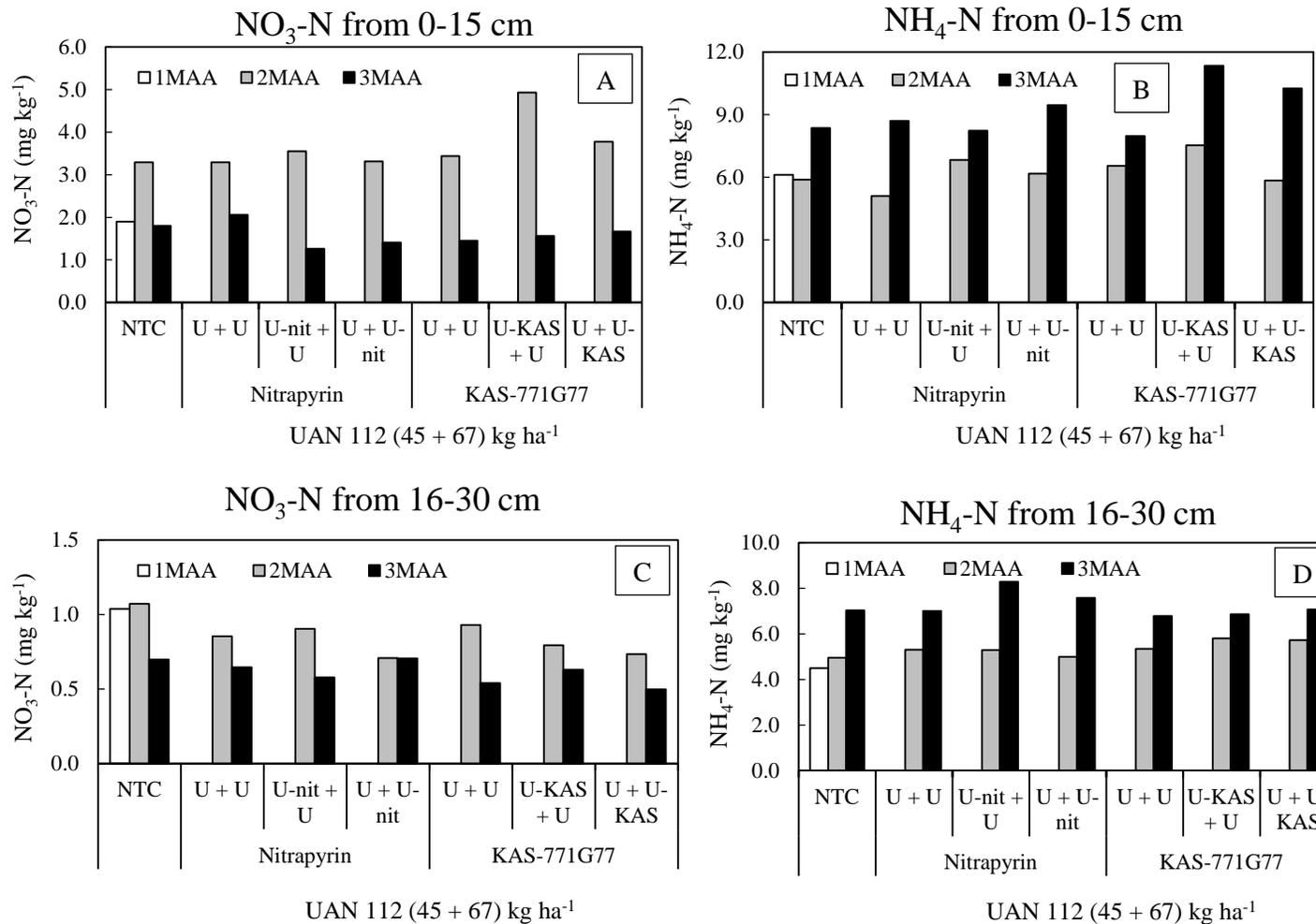


Figure 3.4. Soil test ammonium-nitrogen (NH<sub>4</sub>-N) concentration at 0-15 (A) and 16-30 (C) cm depths, and soil test nitrate-nitrogen (NO<sub>3</sub>-N) at a 0-15 (B) and 16-30 (D) cm depths 1-, 2-, and 3-months after application (MAA) of a 40:60% split application of urea ammonium nitrate (U) at totaling 112 kg N ha<sup>-1</sup>. Abbreviations: KAS, KAS-771G77; nitr, nitrapyrin; NTC, non-treated control; U, urea ammonium nitrate. One-month after application samples were not taken for UAN at 112 kg N ha<sup>-1</sup>.

**CHAPTER 4**  
**A COMPARISON OF NITRAPYRIN AND KAS-771G77 NITRIFICATION**  
**INHIBITORS TIMINGS WITH ANHYDROUS AMMONIA FOR WINTER**  
**WHEAT**

**ABSTRACT**

Poorly-drained soils and low-lying landscape positions have higher potential for saturated soil conditions causing nitrogen (N) losses and negatively affecting wheat (*Triticum aestivum* L.) crops grain yields. Two four-year experiments were conducted in northeastern Missouri to investigate the performance of a new nitrification inhibitor (NI), KAS-771G77, in winter wheat. Treatments were applied in two separate experiments: 1) in the fall after wheat emergence followed by spring topdress (early planted wheat) and 2) in the fall prior to wheat planting followed by spring topdress (late planted wheat). Nitrogen treatments included a non-treated control, three anhydrous ammonia (AA) treatments (with nitrapyrin, KAS-771G77, or urea ammonium nitrate) fall applied polymer coated urea (PCU), spring applied ammonium nitrate (AN); and fall-spring split-applied AN at a total of 112 kg N ha<sup>-1</sup>. For early planted wheat, spring applied AA + KAS-771G77 produced the highest yields (5,120 kg ha<sup>-1</sup>). For late-planted wheat, fall-applied AA + KAS-771G77 (5,030 kg ha<sup>-1</sup>) produced the highest yields with an 8% (413 kg ha<sup>-1</sup>) greater yield than fall-applied PCU. Based on these results spring application of AA + KAS-771G77 as topdress is recommended over its fall application after wheat emergence. While in contrast, preplant fall application of AA + KAS-771G77 is recommended over its spring application as topdress. SPAD meter readings and other crop response variables were affected differently by different N fertilizer sources, application timings and nitrification inhibitors.

## **INTRODUCTION**

Global human population is currently 7.3 billion and is expected to reach 9.2 billion by 2050 (Zilberman et al., 2013). Food production demand and food insecurity are often associated with an increase in population. Increased food demand and food insecurity should be addressed in the face of increasing population and climate change. Improving plant nutrient use efficiency is one way to enhance sustainable food productivity. Sustainable food production requires optimal use of plant nutrients. Under- or over-use of plant nutrients is a major problem in the world and has detrimental effects on water bodies, atmosphere and soil (Motavalli et al., 2008). Globally, wheat is one of the most important crops, and it provides more than 20% of the calories alone. Approximately 2.5 billion people consume similar proportion of protein from wheat (Grace et al., 2011). The current increase in wheat production is 1.1% per annum (Dixon et al., 2009) or even stagnant in some regions (Brisson et al., 2010), while its demand is predicted to increase to 1.7% per annum by 2050 (Rosegrant and Agcaoili, 2010). The gap between growing demand and current production necessitates the need for new strategies to achieve increased productivity (Hawkesford et al., 2013).

Nitrogen (N) is essential to life, but biological growth on Earth in most land and water environments is N-limited. Therefore, additional N is required to sustain life in these conditions. According to Snyder et al. (2009), nutrient losses can be reduced per unit of crop production when the right nutrient source is applied at the right place, time, and rate. The goal of N management is to make the best use of N and increase crop productivity. Soil N management focuses on plant availability and environmental N loss to increase N use efficiency (NUE) (Awale et al., 2015). To better synchronize crop

demand and N supply, 60 to 70% of N should be applied during the fastest growing stages of development (Cui et al., 2010). Improved NUE in the face of environmental pressure from agricultural-driven pollution and rapidly increasing food demand due to continuously increasing human population necessitates improved NUE (Subbarao et al., 2006).

Anhydrous ammonia, urea, urea ammonium nitrate, ammonium nitrate and ammonium sulfate are the most common synthetic N fertilizers used in row-crop agriculture (Millar et al., 2010). Typically, split applications of N are made to winter wheat (Kelley, 1995; Woolfolk et al., 2002; Kelley and Sweeney, 2005). A spring N application is usually difficult due to wet soil conditions which could increase gaseous N loss (Nash et al., 2012a, 2012b). Anhydrous ammonia is usually more cost-effective than other sources, but application into wheat was limited to a preplant application (Kidwaro and Kephart, 1998) due to soil disturbance of most injection knives until John Deere introduced the high speed low draft applicator (Woli et al., 2014). This applicator allows anhydrous ammonia to be injected below the soil surface using a single blade, boot, and closing wheels that cause minimal soil disturbance and may allow sidedress applications in wheat with limited injury to the wheat plants. Fall applications of AA are common for corn in this region (Parkin and Hatfield, 2010; Nash et al., 2013a). However, a wheat study in Spain showed a 14% N use efficiency when N was applied at the sowing stage, but this increased to 55% efficiency when applied at the beginning of stem elongation (López-Bellido et al., 2005).

Nitrification inhibitors are chemicals which restrict, delay or slow down the nitrification process (Motavalli et al., 2008). According to Subbarao et al. (2006), a few

efforts have been devoted to the development of new generation of NIs since the 1960s despite their potential agricultural and environmental benefits resulting from chemical regulation of nitrification. So far, nitrapyrin (2-chloro-6-trichloromethyl-pyridine), DMPP (3, 4-dimethylpyrazole phosphate) and DCD (dicyandiamide) are some of the most studied nitrification inhibitors (Goos and Johnson, 1999; Dittert et al., 2001; Nelson and Huber, 2001; Pasda et al., 2001; Weiske et al., 2001a, 2001b; Zerulla et al., 2001; Calderón et al., 2005; Xu et al., 2005; Islam et al., 2007a, 2007b; Franzen, 2011), while new NIs have been studied in research trials (Trenkel, 2010). A need for a new generation of NIs was previously reported (Subbarao et al., 2006), and it was suggested that NIs should be less expensive, more efficient and suitable in both tropical and temperate environments (Watson, 2005).

Nitrapyrin (N-Serve or Instinct, Dow AgroSciences, Indianapolis, IN) first appeared in the Midwestern United States in the 1960s and it was registered in 1974 (Huber et al., 1977). Nitrapyrin has been extensively studied in corn (Sanchez and Blackmer, 1998; Randall and Vetsch, 2005; Nash et al., 2013a, 2013b). An Iowa study reported a 50 to 64% N loss from the upper 1.5 m of soil in the absence of nitrapyrin when applied in the fall (Sanchez and Blackmer, 1988). Nash et al., (2013a) conducted a study on corn (*Zea mays* L.) in Missouri comparing fall- and preplant applications of anhydrous ammonia (AA) with and without nitrapyrin which showed a 9 to 11% increase in grain yield when N was applied in the spring, while another study reported a 2 Mg ha<sup>-1</sup> increase in corn grain yield when AA plus nitrapyrin was utilized compared to the absence of nitrapyrin (Nash et al., 2013c). Nitrapyrin, due to its sorption on soil colloids, hydrolysis and loss by volatilization, has been reported to be ineffective in some soil

types (Hoefl, 1984; Liu et al., 1984). However, reduced N losses, maintaining N in the ammonium form (Kidwaro and Kephart, 1998), have resulted in increased N uptake associated with its use (Chen et al., 1998a, 1998b).

Nitrapyrin has been evaluated under diverse environmental conditions over many years in the Midwestern USA, and has increased corn yield 7%, decreased N leaching 16%, increased soil retention of N 28%, and decreased NO<sub>x</sub> emission 51% (Wolt, 2004). Use of NIs with reduced rates of N fertilizers has usually increased yields in soils that are susceptible to N loss processes (Frye, 2005). A two-year study conducted on sugar beet (*Beta vulgaris* L.) reported no difference in N availability when 146 kg N ha<sup>-1</sup> with nitrapyrin was compared with 180 kg N ha<sup>-1</sup> without nitrapyrin, while soil N levels were either similar or higher with nitrapyrin application compared to a split N application (Awale et al., 2015). A 10% decrease in nitrate loss in a tile drainage system was reported when a fall application of AA with nitrapyrin was compared to the absence of nitrapyrin (Randall and Vetsch, 2005). Similarly, a grassland study conducted in New Zealand using DCD resulted in 61% reduction in NO<sub>3</sub><sup>-</sup> leaching (Clough et al., 2007). A two-year experiment in Indiana reported a yield increase of 3 Mg ha<sup>-1</sup> in corn when UAN at 90 kg N ha<sup>-1</sup> was applied as sidedress application with nitrapyrin compared a preemergence application of UAN without nitrapyrin (Burzaco et al., 2013a). In addition, nitrapyrin reduced both daily and cumulative N<sub>2</sub>O emissions.

A new NI, KAS-771G77, was recently developed for use with AA (Vetsch and Schwab, 2014; Gabrielson and Epling, 2016). Limited research has reported on the effects of NIs for wheat production using AA (Kidwaro and Kephart, 1998), while no research has integrated nitrification inhibitor technology with new AA applicator

technology for use with wheat. A three-year (2012 to 2014) corn study in Minnesota on poorly-drained glacial till soils under continuous corn and subsurface tile drained soil evaluated the effects of two rates of KAS-771G77 (9.4 and 18.7 L ha<sup>-1</sup>) and one rate of nitrapyrin (2.6 L ha<sup>-1</sup>, 0.57 kg ai ha<sup>-1</sup>) on corn yield using four rates (67, 134, 202 and 269 kg ha<sup>-1</sup>) of UAN (Vetsch and Schwab, 2014). It was noted that 2014, the wettest year among study years, had significantly low yield both when no NI was applied or when KSA-771G77 applied at 18.7 L ha<sup>-1</sup> compared to KSA-771G77 applied at 9.4 L ha<sup>-1</sup> and nitrapyrin application.

Poorly-drained soils and low-lying landscape positions have higher potential for saturated soils conditions which cause N losses from the root zone and negatively affects crop yields (Nelson et al., 2009a; Nash et al., 2012a, 2013b). Soil oxygen deficiency or low soil redox potential, which are usually characterized with waterlogged soil conditions, have been linked to reduced grain yields in these conditions (Tan et al., 2008; Milroy et al., 2009).

Northeastern Missouri, southern Illinois and southeast Kansas constitute the central claypan region which includes over 4 million ha land area. This claypan soil has a subsurface layer at 20 to 40 cm from the surface that is characterized by 100% higher clay content compared to the above horizon (Anderson et al., 1990; Jung et al., 2006; Myers et al., 2007). Enhanced efficiency fertilizers (EEF) such as polymer-coated urea (PCU) have been evaluated for fall applications for corn and wheat on claypan soils (Nash et al., 2012c; Motavalli et al., 2013; Nelson et al., 2014). A better understanding of the effects and interactions of N sources, application-rates and application-timing of anhydrous ammonia with and without NIs on crop production and N losses could help

maintain or increase grain yields. Little research has been conducted in poorly-drained claypan soils to determine accurate research-based recommendations on when these N fertilizers would be effective or how they behave in different environments and cropping systems, and how they should be managed in those environments and cropping systems (Motavalli et al., 2008). In addition, there is lack of research studies which have compared simultaneous impact of multiple management factors together (Burzaco et al., 2013b) on wheat in the Midwestern United States. Therefore, the objective of this research was to evaluate the effect of a new nitrification inhibitor, KAS-771G77, and nitrapyrin when fall and spring applied with anhydrous ammonia compared to standard N management strategies in the region on wheat greenness, biomass, tissue N, grain moisture, N concentration, and yield.

## **MATERIALS AND METHODS**

Field research utilized two wheat experiments from 2013 to 2016 at the University of Missouri's Greenley Memorial Research Center (40°1'17"N, 92°11'24.9"W) near Novelty. The soil series in both the experiments in 2013 and 2014 was a Kilwinning silt loam (fine, smectitic, mesic Vertic Epiaqualfs), while in 2015 and 2016 it was a Putnam silt loam (fine, smectitic, mesic Vertic Albaqualfs). Each year different field sites were selected for each experiment and were maintained as no-till cropping systems following soybean in the crop rotation. Each experiment was arranged as a randomized complete block design (RCBD) with four to six replications.

Wheat was no-till drilled (19.1 cm wide rows). 'MFA2525' was planted for the 2013, 2014, and 2015 crop, while 'MFA2449' was planted for the 2016 crop. In one experiment, wheat (early planted) was planted following soybean harvest and the fall N

treatments were applied after the soil temperature was 10°C. On the same day, fall N treatments were applied in a separate experiment (late planted) and wheat was planted shortly thereafter. Since these experiments were separated by space and were not randomized, they will be discussed separately and referred to as early planted and late planted wheat experiments. Nitrogen fertilizer treatments consisted of a non-treated control, anhydrous ammonia (AA) at 112 kg N ha<sup>-1</sup> applied in the fall and spring in the presence or absence of a nitrification inhibitor (Instinct<sup>®</sup>, nitrapyrin at 0.513 kg ai ha<sup>-1</sup> or KAS-771G77 at 9.3 L ha<sup>-1</sup>), fall applied polymer-coated urea at 112 kg N ha<sup>-1</sup>, and a split application of ammonium nitrate at 34 kg N ha<sup>-1</sup> in the fall and 78 kg N ha<sup>-1</sup> in the spring. Since KAS-771G77 included 1.4 kg N ha<sup>-1</sup>, UAN was applied at 1.4 kg N ha<sup>-1</sup> (8.4 L ha<sup>-1</sup>) as a control treatment with anhydrous ammonia. Nitrapyrin, KAS-771G77, and UAN were delivered in a separate tube and mixed at the point of anhydrous ammonia exiting from the applicator in the furrow. This prevented freezing of KAS-771G77 or UAN in the anhydrous ammonia lines. Nitrapyrin was applied in the same manner as KAS-771G77 so the application method was not a confounding factor.

Initial soil samples were collected from the non-treated control to determine selected initial soil characteristics (Table 4.1). Three soil samples were collected, which were composited afterwards, from the 0 to 15 cm depth using a stainless-steel push probe. The soil samples were analyzed using standard soil test analytical methods at the University of Missouri Soil and Plant testing Laboratory (Nathan et al., 2006b). Additional analyses of soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N were determined using a 2M KCl extraction and Lachat QuickChem automated ion analyzer (Hach Corp., Loveland, CO).

Additional field characteristics, management, application timings, maintenance fertilizer, and crop protection details are reported in Table 4.2 (early and late planted wheat experiments). Depending on the year, chlorophyll meter readings for 10 plants were recorded from late-May to early-June to determine chlorophyll content of the flag leaf using a SPAD meter (Minolta SPAD-502, Tokyo, Japan). At physiological maturity, whole-plant samples were collected from each plot from a 30 by 76 cm quadrat to determine aboveground dry biomass. This sample was dried, weighed, ground (Wiley Mill, Swedesboro, NJ), and analyzed for TKN concentration (QuickChem, 1992) which was used to calculate tissue N uptake and tissue N recovery efficiency ( $RE_N$ ) (Fixen et al., 2014). Wheat grain yields were determined with a small-plot combine (Wintersteiger Delta, Salt Lake City, UT) and adjusted to 130 g kg<sup>-1</sup> moisture content before analysis. Grain samples were collected from each plot to determine test weight and moisture using a grain analysis computer (GAC 2100, DICKEY-john Corporation, Auburn, IL). The grain samples were also analyzed for TKN (QuickChem, 1992) which was used to calculate grain N uptake and grain N recovery efficiency. Rainfall data for the growing seasons were collected from the Missouri Historical Agricultural Weather Database website (MU Extension, 2017). Data were analyzed for each study year from October to July for 2013, 2014 and 2015, and from September to July for 2016. The precipitation data was assigned based on the harvest date such as the 2012-2013 season was reported as 2013. Each study-year daily and cumulative rainfall was reported in comparison with the average cumulative rainfall from 2002 to 2012 in Figures 4.1 and 4.2.

Data were subjected to single-factor analysis of variance (ANOVA) using the SAS statistical program (SAS Institute, 2016). Data were subjected to an *F Max* test for

homogeneity (Kuehl, 1994) and combined over years when appropriate. Results were presented when significant differences among treatments were observed at  $P \leq 0.01$  and means were separated using Fisher's Protected Least Significant Difference.

## **RESULTS AND DISCUSSION**

### **Precipitation**

Rainfall varied each year (Figures 4.1 and 4.2). For example, 2013 and 2015 received 15% (140 mm) and 22% (220 mm) more than the cumulative average precipitation, while 2014 and 2016 received 19% (153 mm) and 17% (131 mm) less rainfall than the average cumulative of 2002 to 2012 (786 mm). It is worth noting that the time-period from planting to harvest also varied across years (Table 4.2).

### **Average Daily Air Temperature**

Average daily air temperature from September 1 of the planting-year to August 31 of the harvest-year for the years of 2013, 2014, 2015 and 2016 (Figure 4.3). Temperatures between 2013 and 2016, and 2014 and 2015 were similar. In 2015, temperature between late-November and early-March was below the freezing point (0°C). In 2016, freezing temperatures were only observed in early-January to mid-February. Generally, temperatures in 2015 in the months of November through March were colder compared to 2016. In addition, temperature dropped below 10°C earlier and reached back to 10°C later in 2015 compared to 2016. This means temperature in 2015 remained colder for extended period of time compared to 2016.

### **Early Planted Wheat**

All treatments resulted in higher chlorophyll (SPAD) meter readings than the non-treated control (Table 4.3). Spring-applied AA + nitrapyrin had the greenest flag leaf SPAD reading (49 SPAD units) which was greater than all N treatments except for that of

spring applied AA + KAS-771G77 and AA alone. Anhydrous ammonia (45 SPAD units) applied in the fall was 3 SPAD units lower than its equivalent spring application. In contrast, the SPAD reading for the AN treatment was not affected by its application timing.

Total aboveground biomass and tissue N concentration of all N treatments was greater than the non-treated control (Table 4.3). No other differences were noted among treatments for total above ground biomass or tissue N concentration. All treatments had similar grain moisture levels compared to the non-treated control. However, the AN treatment had grain moisture ( $142 \text{ g kg}^{-1}$ ) that was lower at both its application timings (split and spring applied) compared to fall applied AA + nitrapyrin ( $151 \text{ g kg}^{-1}$ ).

Grain N concentration of the non-treated control ( $15 \text{ g kg}^{-1}$ ) was lower than all other treatments (Table 4.3). As observed in tissue N concentration, grain N concentration in the spring applied treatments was generally higher compared to those of fall applied treatments. Crop N recovery efficiency ( $RE_N$ ) of spring applied AA + KAS-771G77 (40%) was the greatest, which was greater than fall applied AA + nitrapyrin (29%), spring applied AN (28%) and fall followed by spring applied AN (27%). Spring applied treatments with NIs (nitrapyrin or KAS-771G77) had greater  $RE_N$  compared to fall applied treatments.

All N treatments had higher grain yields compared to the non-treated control ( $3370 \text{ kg ha}^{-1}$ ) (Table 4.3). This suggests that the wheat crop responded to all N treatments in the experiment. Spring application of AA + KAS-771G77 produced the highest yields ( $5120 \text{ kg ha}^{-1}$ ) with over an 8% ( $410 \text{ kg ha}^{-1}$ ) yield increase compared to spring applied AA + nitrapyrin. Spring applied AA + KAS-771G77 increased yield 9 to

10% compared to the AN treatment. Generally, AA + NI applied in the spring resulted in higher overall grain yields compared to a fall application. At both application timings, AN resulted in the lowest yields. A split-application of AN has been a standard treatment in this region (Nash et al., 2012b). Among the fall applied treatments, PCU had the highest yields (4,920 kg ha<sup>-1</sup>); however, fall applied PCU yields were lower than spring applied AA treatments.

### **Late Planted Wheat**

Flag leaf SPAD readings for the non-treated control (37 SPAD units) were significantly lower than all other N treatments (Table 4.4). Spring applied AA + KAS-771G77 resulted in the greenest (48 SPAD units) flag leaf. Spring-applied AA + KAS-771G77 had higher (3 to 4) SPAD meter readings compared to fall-applied PCU, fall-applied AA and spring-applied AN. No significant differences were observed between treatments with NIs across different application timings. However, both fall and spring application timings of AA + KAS-771G77 resulted in 2 SPAD units higher compared to fall or spring applied AA + Nitrapyrin.

All N treatments increased aboveground biomass compared to the non-treated control (8,570 kg ha<sup>-1</sup>) (Table 4.4). No differences were observed among treatments with NIs among application timings. Spring-applied AN increased aboveground biomass 16% compared to spring applied AA. Nitrogen treatments increased tissue N concentration 2 to 5 g kg<sup>-1</sup> compared to the non-treated control. Fall followed by a spring application of AN resulted in the highest (15 g kg<sup>-1</sup>) tissue N concentration which was 13% greater than spring applied AA + UAN or AN. In addition, spring applied AA + KAS-771G77 and AA + Nitrapyrin had 2 g kg<sup>-1</sup> greater tissue N concentration compared to fall applications

of AA + KAS-771G77, AA + Nitrapyrin, or PCU. Although tissue N concentration for fall followed by spring application of AN was greater when it was spring applied AN, spring applied treatments with a NI generally resulted in higher tissue N concentration over fall applied treatments.

Total tissue N uptake was 48 to 93 kg ha<sup>-1</sup> lower in the non-treated control compared to all other treatments (Table 4.4). A split application of AN resulted in the highest amount (170 kg N ha<sup>-1</sup>) of total tissue N uptake, which was 27, 21, and 23% greater than fall applied AA + KAS-771G77, AA + UAN and PCU, respectively. Fall-applied AA + nitrapyrin resulted in slightly greater total tissue N uptake than rest of the treatments at this application timing. Spring-applied AA + KAS-771G77 had 21% greater total tissue N uptake compared to an equivalent fall application. All fall-applied treatments resulted in lower total tissue N uptake compared to their equivalent spring applied treatment.

Crop N recovery efficiency (RE<sub>N</sub>) of fall followed by spring application of AN resulted in the highest RE<sub>N</sub> (82%) while fall-applied AA + KAS-771G77 had the lowest (43%) RE<sub>N</sub> (Table 4.4). Recovery efficiency of spring applied AA + KAS-771G77 was 30% greater than its fall application timing. Spring and fall followed by spring applied AN were 31 and 39% greater than fall applied AA + KAS-771G77. Moreover, fall followed by spring applied AN resulted 33% greater RE<sub>N</sub> than fall applied PCU. Altogether, as with total tissue N uptake, spring applied treatments generally resulted in greater N recovery efficiencies.

Grain moisture of the non-treated control (156 g kg<sup>-1</sup>) was lower than spring applied AA with or without nitrapyrin (Table 4.4). At harvest, spring applied treatments

such as AA + KAS-771G77 ( $179 \text{ g kg}^{-1}$ ) had the highest in grain moisture content which was  $14 \text{ g kg}^{-1}$  and  $15 \text{ g kg}^{-1}$  greater than fall applications of AA + KAS-771G77 and AA + nitrapyrin, respectively. Anhydrous ammonia was applied in bands 76 cm wide which indicated some striping in the field especially when it was applied in the spring (visual observation). This could affect the overall biomass production for a spring compared to a fall application and could have affected overall maturity of the later planted wheat (Table 4.4) since spring applied AA treatments had greater grain moisture concentration.

All treatments had grain N concentrations that were greater than the non-treated control ( $15 \text{ g kg}^{-1}$ ) (Table 4.4). Grain total N uptake was greater with all N treatments compared to the non-treated control ( $91 \text{ kg N ha}^{-1}$ ) and fall applied PCU ( $141 \text{ kg N ha}^{-1}$ ) (Table 4.4). Fall applied AA + KAS-771G77 ( $162 \text{ kg ha}^{-1}$ ) had the greatest total grain N uptake of all N treatments. Grain N uptake of fall or fall followed by spring applications of AN were similar to treatments of AA with or without nitrapyrin.

Grain yield for the non-treated control ( $3,190 \text{ kg ha}^{-1}$ ) was less than all N treatments indicating a yield response to all N treatments (Table 4.4). Fall applied AA + KAS-771G77 resulted in the highest yield ( $5,030 \text{ kg ha}^{-1}$ ) which was 8% ( $413 \text{ kg ha}^{-1}$ ) greater than fall applied PCU. Fall applications of AA prior to late planted wheat in the presence of a nitrification inhibitor (nitrapyrin or KAS-771G77) resulted in higher average grain yields compared to their equivalent spring applications. In addition, a preplant fall application of AA + KAS-771G77 had greater overall grain yields compared to preplant fall applied PCU, spring applied AN, or AA + nitrapyrin.

For early planted winter wheat, both NIs (nitrapyrin and KAS-771G77) had a positive impact on increasing SPAD meter readings because all the treatments with a NI

had greater SPAD values compared to treatments without a NI. In comparison, AA + KAS-771G77 was greener in late planted winter wheat compared to nitrapyrin at both fall and spring application timings. Nitrapyrin was injected in the same manner as KAS-771G77 which may have affected its efficacy. Typically, nitrapyrin is mixed in the anhydrous ammonia stream through a direct injection system (Nash et al., 2012b; Nelson et al., 2014). Both these results indicate an important factor that application of NIs with N fertilizer increased SPAD readings and hence chlorophyll content of plants which could translate into higher yields.

The overall positive impact of high SPAD readings in NI treatments can be related to the potential increase in partial  $\text{NH}_4^+$  nutrition. Trenkel (2010) reported that when soils were supplemented with NIs,  $\text{NH}_4^+$  ions in soils remained adsorbed to soil particles making them available for plant uptake (partial  $\text{NH}_4^+$  nutrition) and protected from N losses, such as leaching and denitrification. Kidwaro and Kephart (1998) also showed a greater soil ammonium concentration when applied with anhydrous ammonia in central Missouri. For early planted winter wheat, KAS-771G77 with AA in the spring produced the greatest yields. Similarly, spring applied AA + KAS-771G77 also resulted in significant 10.3% ( $525 \text{ kg ha}^{-1}$ ) and 9.3% ( $474 \text{ kg ha}^{-1}$ ) over spring and fall followed by spring application of AN, respectively.

This supports the idea of using a NI with AA for enhancing productivity of wheat in this production system. Generally, early planted winter wheat with an AA + NI application in the spring resulted in higher grain yields compared to its fall application with these additives suggesting the usefulness of spring applications of AA with or without NI over fall applications. The fall application to existing wheat using the HSLD

applicator caused limited damage in the fall and spring (visual observation), but the damage and potential N loss in the fall may have caused a reduction in yield because seedling wheat plants may be more susceptible to physical damage.

Among fall applied treatments, PCU resulted in the highest yield (4,920 kg ha<sup>-1</sup>) which was similar to the AA treatments. Polymer-coated urea applied in the fall has worked well in other research (Nelson et al., 2009b; Nash et al., 2012c). This increased yield of PCU over AN can be attributed to its ability to reduce potential gaseous N losses (Halvorson et al., 2010b) and NO<sub>3</sub><sup>-</sup>-N leaching (Trenkel, 2010) that may have occurred with AN. However, fall applied PCU yields were similar to spring applied AA + NI treatments indicating this treatment was a viable option for farmers with concerns about applying AA in a timely manner. This also suggests that for early planted winter wheat an application of AA +/- NI in spring is recommended over a fall application. However sometimes, due to inconvenient field conditions in spring, spring applications become difficult. However, higher yields with AA may be due to placement of N below the soil surface compared to a broadcast application of AN to the soils surface, especially a single application of AN in the spring. Therefore, growers may consider applying PCU in fall when AN single application in spring or split application in fall followed by spring is difficult due to any underlying reasons which may make fall application inevitable. However, when conditions are suitable and spring applications are possible using the HSLD application technology and/or affordable by the growers, application of AA + NI (nitrapyrin or KAS-771G77) may be ideal to obtain the highest yields.

Late planted winter wheat responded differently which could be due to the stage of wheat development causing lack of injury in the fall, or the overall yield potential of

planting date. For late planted winter wheat, fall applied AA + KAS-771G77 had the greatest grain yields. It also had grain total N uptake, SPAD values, and yields similar to spring applications of N. Applying KAS-771G77 with preplant fall application of AA is recommended over a spring application of AA or AN for late planted wheat.

## **CONCLUSIONS**

Yields of winter wheat were affected by application timings, presence of nitrification inhibitor, and wheat planting date between the two experiments. Application timings of AA affected grain moisture at harvest and overall N uptake and tissue  $RE_N$ . An application of AA + KAS-771G77 had the highest yields when spring applied following early planted wheat, and when fall or spring applied following late planted wheat, while PCU had the highest yields when fall applied following early planted wheat. Within the application timings (fall or spring), yields were similar among AA treatments +/- NIs. However, recommendations for an AA application may differ depending on the relative planting date since early planted wheat generally had greater yields from a spring application of AA and late planted wheat had greater yields from a preplant application of AA.

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Table 4.1. Selected initial soil characteristics for early and late planted wheat experiments.

Year <sup>†</sup>	pH	NA	CEC	Bray I P	Ca	Mg	K	OM	NO <sub>3</sub> -N	NH <sub>4</sub> -N
		---- cmol <sub>c</sub> kg <sup>-1</sup> ----		----- kg ha <sup>-1</sup> -----				g kg <sup>-1</sup>	----- mg kg <sup>-1</sup> -----	
Early planted										
2013	5.5	3.5	15.6	65	4643	381	309	37	--- <sup>‡</sup>	---
2014	6.7	0.5	11.9	140	4138	369	704	36	---	---
2015	4.8	6.0	16.5	122	3702	470	439	30	6	31
2016	5.5	3.0	13.3	109	3665	378	615	32	14	4
		---- cmol <sub>c</sub> kg <sup>-1</sup> ----		----- kg ha <sup>-1</sup> -----				g kg <sup>-1</sup>	----- mg kg <sup>-1</sup> -----	
Late planted										
2013	6.3	1.1	16.0	85	5670	493	395	3.6	---	---
2014	5.3	3.3	12.9	223	3116	378	1012	36	---	---
2015	6.7	0.1	14.0	58	5264	483	352	32	7.5	2.7
2016	5.6	3.6	16.4	130	4530	541	622	38	11.8	4.5

<sup>†</sup>Abbreviations: CEC, cation exchange capacity; NA, neutralizable acidity; P, phosphorus; Ca, exchangeable calcium; Mg, exchangeable magnesium; K, potassium; OM, organic matter; NO<sub>3</sub>-N, nitrate nitrogen; NH<sub>4</sub>-N, ammonium nitrogen

<sup>‡</sup>Data for NO<sub>3</sub>-N and NH<sub>4</sub>-N were not collected in 2013 and 2014.

Table 4.2. Crop management and timeline of evaluations for early and late planted wheat harvested in 2013, 2014, 2015, and 2016.

Management	Year			
	2013	2014	2015	2016
Planting dates				
Early planted experiment	10 Oct. 2012	11 Oct. 2013	21 Oct. 2014	17 Sep. 2015
Late planted experiment	1 Nov. 2012	28 Oct. 2013	7 Nov. 2014	10 Nov. 2015
Cultivar	MFA2525	MFA2525	MFA2525	MFA2449
Seed rate (kg ha <sup>-1</sup> )	112	112	134	112
Nitrogen application				
Fall	1 Nov. 2012	28 Oct. 2013	7 Nov. 2014	9 Nov. 2015
Spring	4 Apr.	26 Mar.	18 Mar.	29 Feb.
Soil series <sup>†</sup>	Kilwinning	Kilwinning	Putnam	Putnam
Plot Size (m)	3.1 by 15.2	3.1 by 15.2	3.1 by 18.3	3.1 by 21.3
Replications	4	6	4	4
Soil maintenance fertilizer				
Date	3 Oct. 2012	18 Oct. 2013	NA	NA
N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O (kg ha <sup>-1</sup> )	31-80-120	23-110-120	NA	NA
Crop protection <sup>‡</sup>				
Date/timing		23 May 2014, Foliar preventative	21 Oct. 2014, Burndown	
Product		Azoxystrobin	Glyphosate + 2,4-D	
Amount (kg ai ha <sup>-1</sup> )		0.15	1.17 + 0.35	
Date/timing			13 May 2015, Foliar preventative	
Product			Azoxystrobin	
Amount (kg ai ha <sup>-1</sup> )			0.15	
Timeline of evaluations				
Initial soil sampling	1 Nov. 2012	21 Nov. 2013	21 Oct. 2014	11 Nov. 2015
Spad				
Early planted experiment	23 May	3 June	27 May	24 May
Late planted experiment	30 May	3 June	27 May	25 May
Tissue sampling				
Early planted experiment	19 June	10 June	3 June	31 May
Late planted experiment	25 June	10 June	2 June	31 May
Harvest				
Early planted experiment	3 July	3 July	7 July	24 June
Late planted experiment	10 July	7 July	7 July	24 June

<sup>†</sup>Kilwinning silt loam (fine, smectitic, mesic Vertic Epiaqualfs); Putnam silt loam (fine, smectitic, mesic Vertic Albaqualfs).

<sup>‡</sup>Chemical names: Azoxystrobin, methyl (E)-2-[2-[6-(2-cyanophenoxy) pyrimidine-4-yloxy] phenyl]-3-methoxycrylate; glyphosate, N-(phosphonomethyl) glycine; 2,4-D, Isooctyl (2-ethylhexyl) ester of 2,4-Dichlorophenoxyacetic acid.

Table 4.3. SPAD meter reading, tissue biomass, tissue nitrogen (N) uptake, and grain moisture, grain N uptake, grain N recovery efficiency (RE<sub>N</sub>), and yield for early planted winter wheat

Nitrogen timing and treatment <sup>†</sup>	SPAD	Tissue biomass	Tissue N uptake	Grain moisture	Grain N uptake	Grain RE <sub>N</sub>	Grain yield
	SPAD units	kg ha <sup>-1</sup>	g kg <sup>-1</sup>	----- g kg <sup>-1</sup> -----		%	kg ha <sup>-1</sup>
Non-treated	36	11,680	10	146	15		3,370
Fall							
AA + KAS-771G77	46	19,120	13	149	18	30	4,860
AA + UAN	45	20,850	13	149	18	31	4,860
AA + nitrapyrin	46	19,440	14	151	18	29	4,710
PCU	44	18,650	13	145	18	30	4,920
Spring							
AA + KAS-771G77	48	19,310	14	147	19	40	5,120
AA + UAN	48	19,180	14	145	19	36	5,040
AA + nitrapyrin	49	19,550	14	147	18	35	5,110
AN	45	20,910	14	142	19	28	4,600
Fall fb spring							
AN at 34 kg N ha <sup>-1</sup> fb	45	18,970	13	142	18	27	4,650
AN at 78 kg N ha <sup>-1</sup>							
LSD ( <i>P</i> = 0.01)	3	3,630	2	9	2	11	380

<sup>†</sup>Abbreviations: AA, anhydrous ammonia; UAN, urea ammonium nitrate; PCU, polymer-coated urea; AN, ammonium nitrate; fb, followed by

Table 4.4. SPAD meter reading, tissue biomass, tissue nitrogen (N) uptake, tissue N uptake, tissue N recovery efficiency ( $RE_N$ ), and grain moisture, grain N, grain N uptake, and yield for late planted winter wheat.

Nitrogen timings and treatments <sup>†</sup>	SPAD	Tissue biomass	Tissue N	Tissue N uptake	Tissue $RE_N$	Grain moisture	Grain N	Grain N uptake	Grain yield
	SPAD units	kg ha <sup>-1</sup>	g kg <sup>-1</sup>	kg ha <sup>-1</sup>	%	----- g kg <sup>-1</sup> -----	-----	----- kg ha <sup>-1</sup> -----	-----
Non-treated	37	8,570	10	77		156	15	91	3,190
Fall									
AA + KAS-771G77	47	11,400	12	125	43	165	18	162	5,030
AA + UAN	45	11,690	13	135	51	168	17	153	4,970
AA + nitrapyrin	46	11,910	12	141	57	164	18	158	4,910
PCU	44	11,740	12	131	49	167	17	141	4,620
Spring									
AA + KAS-771G77	48	11,680	14	159	73	179	18	157	4,920
AA + UAN	47	10,910	13	144	60	174	18	159	4,850
AA + nitrapyrin	46	11,690	14	150	65	174	18	156	4,750
AN	45	12,930	13	161	74	166	18	154	4,750
Fall fb Spring									
AN at 34 kg N ha <sup>-1</sup> fb	47	12,140	15	170	82	166	18	161	4,830
AN at 78 kg N ha <sup>-1</sup>									
LSD ( $P = 0.01$ )	3	1,940	2	31	28	14	1	12	290

<sup>†</sup>Abbreviations: AA, anhydrous ammonia; UAN, urea ammonium nitrate; PCU, polymer-coated urea; AN, ammonium nitrate; fb, followed by

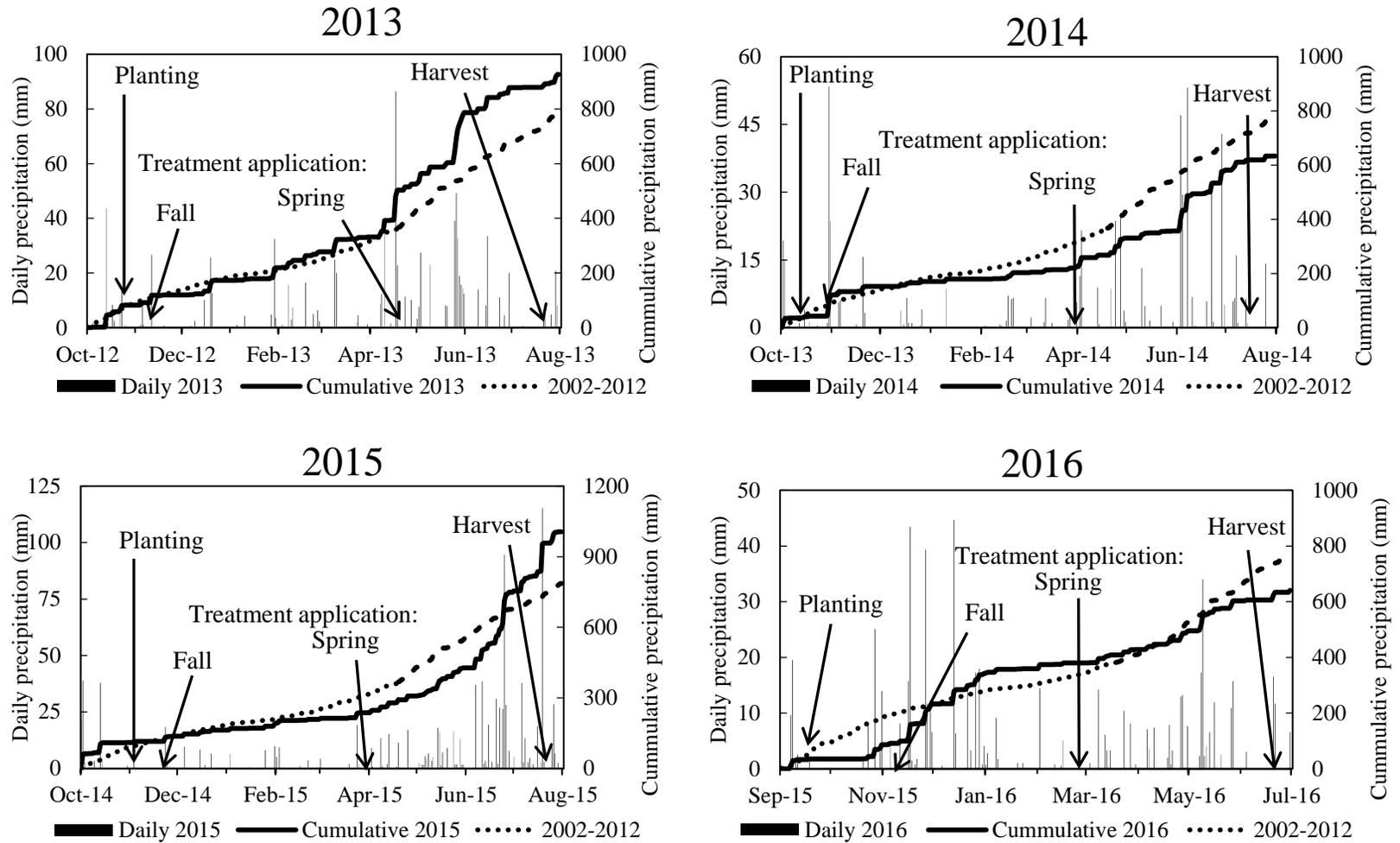


Figure 4.1. Precipitation and production practices for the growing seasons of 2013, 2014, 2015 and 2016 for early planted wheat. The bars represent daily precipitation (mm). The solid line represents cumulative precipitation (mm) over the season and the dotted-line represent cumulative precipitation (mm) from 2002 to 2012.

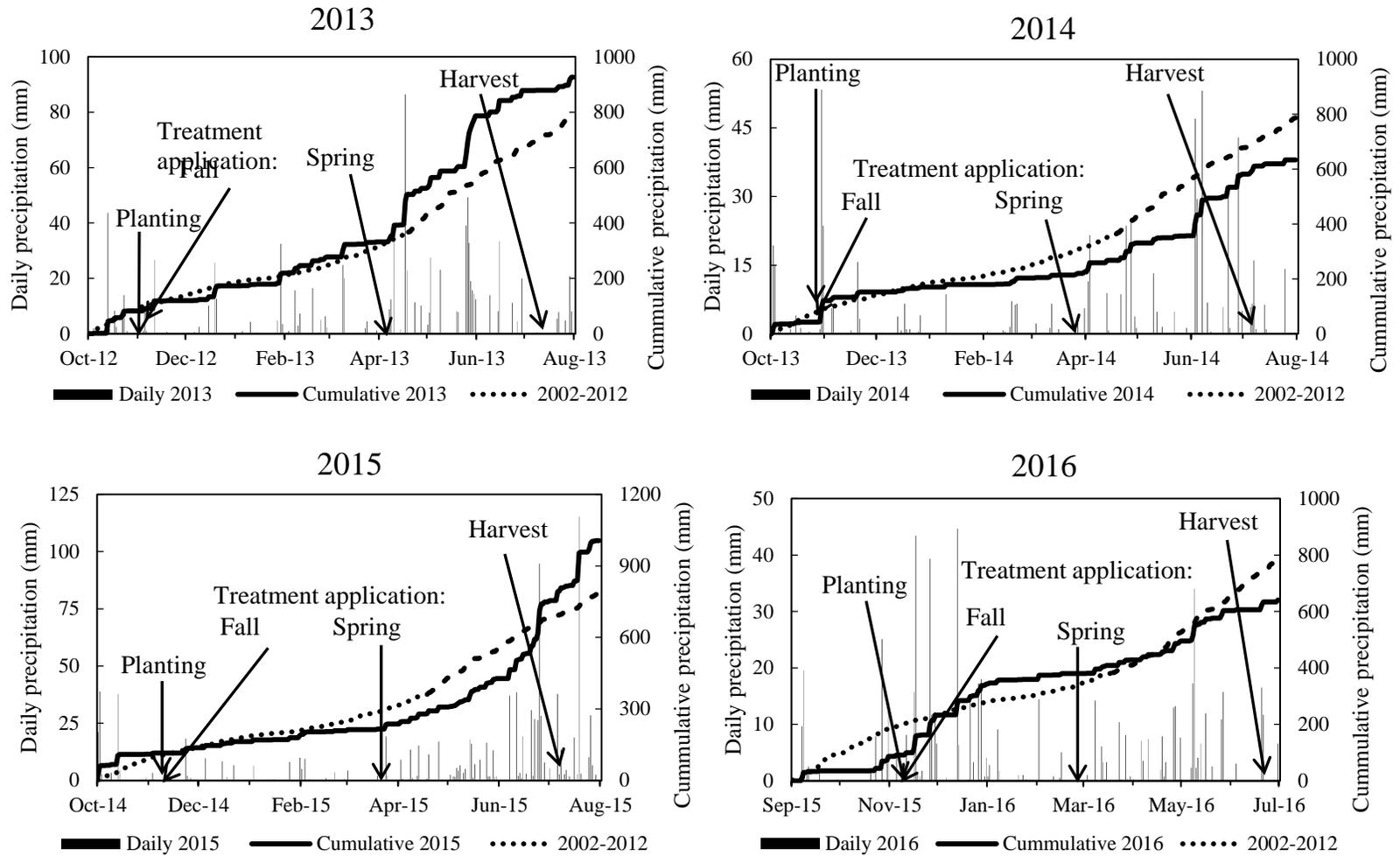


Figure 4.2. Precipitation and production practices for the growing seasons of 2013, 2014, 2015 and 2016 late planted wheat. The bars represent daily precipitation (mm). The solid line represents cumulative precipitation (mm) over the season and the dotted-line represent cumulative precipitation (mm) from 2002 to 2012.

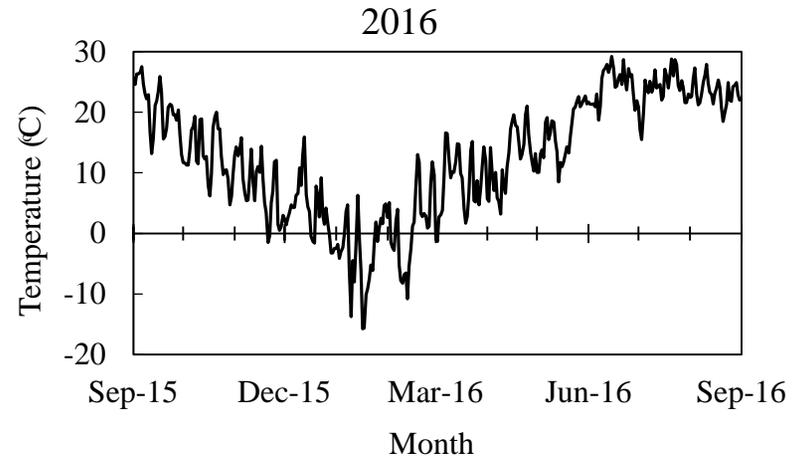
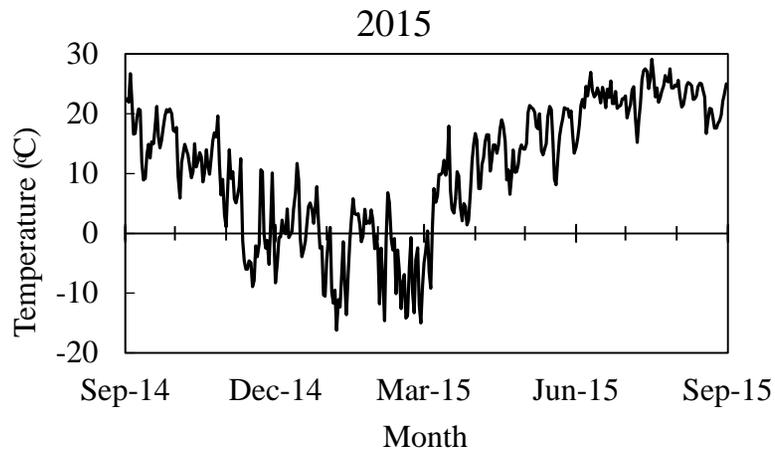
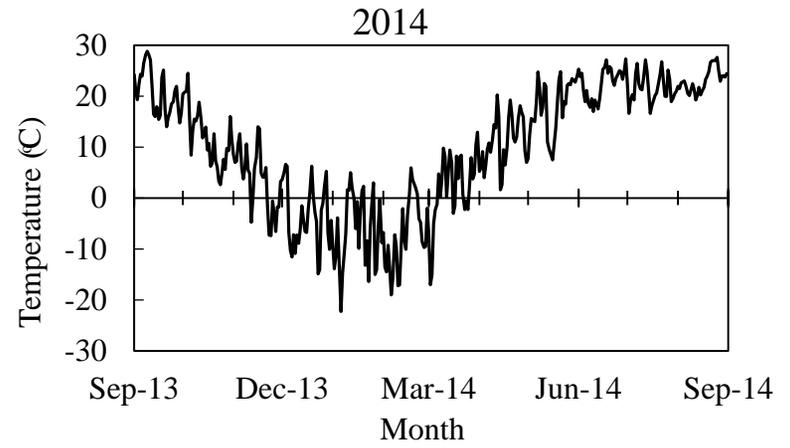
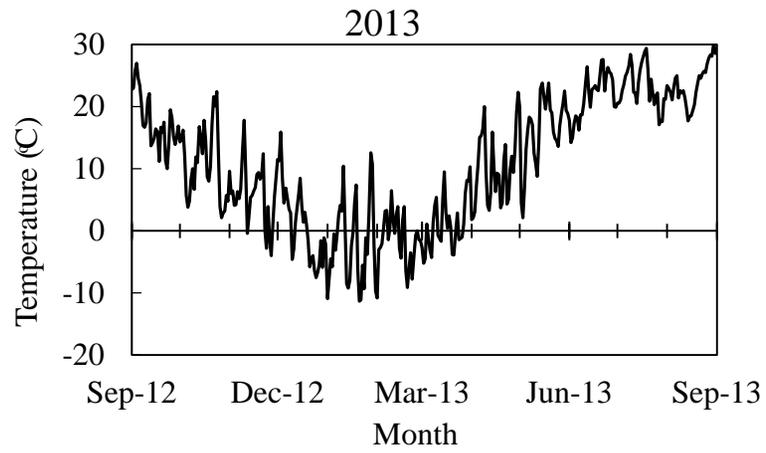


Figure 4.3. Daily average air temperature (°C) for the years of 2013, 2014, 2015 and 2016.

## CHAPTER 5 OVERALL CONCLUSION

The overall objective of this research was to evaluate if nitrification inhibitors (NI) and/or N management would increase crop production for corn and wheat in poorly drained claypan soils.

One study evaluated whether application of nitrapyrin with UAN source of N, application rates (143 and 168 kg ha<sup>-1</sup>) and application timings (pre-emergence and V3 growth stage) could regulate soil N status during the growing season and increase corn production in a four-year research trial. Nitrapyrin applied with UAN at 143 kg N ha<sup>-1</sup> at V3 growth stage of corn development produced the highest yield of 8.6 Mg ha<sup>-1</sup>. It was followed by 7.7 Mg ha<sup>-1</sup> yield of nitrapyrin with UAN at 168 kg N ha<sup>-1</sup> applied pre-emergence. A lower rate of UAN with nitrapyrin as a side-dress application would be beneficial during wet growing seasons, which increases potential N loss mechanisms. However, if side-dress application during season is not possible, pre-emergence application of a higher rate of UAN with nitrapyrin is an alternative option to achieve high corn yields. Soil N status, NO<sub>3</sub>-N and NH<sub>4</sub>-N, during the season was generally a factor of UAN application timing and application rate rather than application of nitrapyrin.

However, these results were not consistent. One possible reason was probably the difference in the amount of daily precipitation events, their distribution within the season, and the difference in cumulative daily precipitation between seasons. Further investigation into evaluating these interactions may be needed in claypan soils. Workload on growers before planting or early during growing season, excessive wet field conditions in early spring, reduced N fertilizer use efficiencies due to uncertain climatic

conditions during growing season, and environmental concerns of pollution from N escaping from agriculture production systems may provide an incentive to growers and policy makers to increase the use of NIs in the future.

A second study evaluated the effectiveness of nitrapyrin and KAS-771G77 on winter wheat grain, soil and plant N status applying two split-applied (40:60%) UAN application rates (79 and 112 kg ha<sup>-1</sup>), and two application timings of the NI (none, NI with the first application of UAN, or NI with the second application of UAN) of nitrapyrin or KAS-771G77. Nitrapyrin and KAS-771G77 did not affect soil N status compared to non-treated UAN in the two years of this research. Application timing of KAS-771G77 and application rate of UAN affected tissue biomass the greatest. UAN application rate increased tissue N concentration and tissue total N uptake, but there was no effect of the NIs or timing of the NI. Nitrapyrin had the highest tissue RE<sub>N</sub> in 2015, but no effect was observed in 2016. Plant population and grain moisture were not affected by UAN rate, NI and NI timing. Nitrification inhibitors had similar grain test weights although differences were observed between 2015 and 2016. Yields were similar (3,550 to 3686 kg ha<sup>-1</sup>) in 2015 between UAN application rates. However, UAN at 112 kg N ha<sup>-1</sup> increased yield 551 kg ha<sup>-1</sup> over UAN at 79 kg N ha<sup>-1</sup> (4,871 kg ha<sup>-1</sup>) in 2016. Application of nitrapyrin or KAS-771G77 or their application timings with split applied UAN rate (low or high) did not affect winter wheat yields. However, use of nitrification inhibitors is a risk management strategy for unpredictable wet field conditions. In optimal growing conditions, split application of a high UAN application rate could be adjusted accordingly.

A third study evaluated the efficacy of a new NI (KAS-771G77) on claypan soils for winter wheat yield using different anhydrous ammonia (AA) application timings using a HSLD AA applicator. This research was conducted in two separate experiments over four years. Nitrogen treatments included AA treatments (with nitrapyrin, KAS-771G77, or urea ammonium nitrate (UAN)) applied in the fall and spring, fall applied polymer coated urea (PCU), spring applied ammonium nitrate (AN), and fall-spring split-applied AN at a total of 112 kg N ha<sup>-1</sup>. A non-treated control was also included. Treatments were applied in two separate experiments 1) in the fall after wheat emergence followed by spring sidedress (early planted wheat) and 2) in the fall prior to wheat planting followed by spring sidedress (late planted wheat). Spring applied AA + KAS-771G77 produced the highest yields (5,120 kg ha<sup>-1</sup>) for early planted wheat. For late planted wheat, fall applied AA + KAS-771G77 (5030 kg ha<sup>-1</sup>) produced the highest yields with an 8% (413 kg ha<sup>-1</sup>) greater yield than fall applied PCU. Spring application of AA + KAS-771G77 is recommended over its fall application after wheat emergence. While in contrast, preplant fall application of AA + KAS-771G77 is recommended over its spring application. Other crop response variables including SPAD meter readings were affected differently by different N fertilizer sources, application timings and NIs.

Nitrapyrin affected soil N status during corn production on claypan soils. However, for wheat production, neither nitrapyrin nor KAS-771G77 significantly changed soil nitrate-N and ammonium-N concentration in soil. Future research may need to investigate the effect of claypan soil properties on the efficiency of nitrapyrin and KAS-771G77. A single application of UAN with nitrapyrin or KAS-771G77 may be needed to evaluate winter wheat grain yields in claypan soils. This will help assess

whether split applications in the presence of nitrapyrin or KAS-771G77 are economical compared to a single application of UAN applied with NI. Relatively, KAS-771G77 performed similar or better for winter grain yields and/or other crop response variables. Future research on alternative management systems on claypan soils should include KAS-771G77 to compare its performance in diverse management systems across claypan soils.