# EFFICACY OF UREASE INHIBITOR WITH AND WITHOUT NITRIFICATION INHIBITOR IN IMPROVING EFFICIENCY OF UREA-BASED FERTILIZERS

by

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## A Thesis

Submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirements for the degree of

### DOCTOR OF PHILOSOPHY

Department of Soil Science

University of Manitoba

Winnipeg, Manitoba

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#### ABSTRACT

Lasisi, Ahmed Agboola. Ph.D., The University of Manitoba, October, 2020. <u>Efficacy of Urease</u> <u>Inhibitor with and without Nitrification Inhibitors in Improving Efficiency of Urea-Based</u> <u>Fertilizers</u>. Major Professor; Olalekan Oluwole Akinremi.

Nitrogen (N) losses in the form of ammonia volatilization limit the efficiency of urea-based fertilizers world-wide. Urease inhibitor, N-(n-butyl) thiophosphoric triamide (NBPT) is a promising additive to reduce this N loss. Studies were conducted to test the efficacy of a relatively new NBPT formulation, ARM U (18% NBPT m/v), containing a proprietary polymer that allows for a low NBPT application rate in reducing ammonia volatilization from urea-based fertilizers. The results showed that ARM U reduced ammonia volatilization by an average of 85% across three soils and its effectiveness was not significantly different from other commercial NBPT formulations whose NBPT concentrations were 33-67% greater than ARM U.

Further studies showed that delaying N fertilizer application until late-fall, as recommended on the Canadian prairies, did not stop ammonia volatilization from urea-based fertilizers. Across 4 site-year, total ammonia volatilization (% of applied N) from untreated urea-based fertilizers applied in the fall and spring were 14 and 16%, respectively. We found that the reduction of ammonia volatilization by NBPT was significantly greater in the fall (65%) than in the spring (40%) across 4 site-year and the addition of NI with NBPT (double inhibitor, DI) reduced the efficacy of NBPT in decreasing ammonia volatilization from spring-applied urea by 27%. The conserved N by NBPT and DI improved the crop N use efficiency at one of the two sites in this study.

Investigation on the interaction between NBPT and NI on urea hydrolysis in five soils at 21 °C and six soils at 5, 15, and 25 °C showed that while NBPT reduced the rate of urea hydrolysis across soils and temperatures, the addition of NI reduced the inhibitory effect of NBPT on urea hydrolysis. Hence, reduction in ammonia volatilization is less with DI than only NBPT.

Overall, the research shows that (i) NBPT effectively reduced ammonia volatilization even at a low concentration (ii) significant ammonia volatilization could occur from urea-based fertilizers even at soil temperature below 3 °C (iii) use of NBPT to bridge crop urea use efficiency is site-specific (iv) NI impaired the effectiveness of NBPT in suppressing urea hydrolysis and ammonia volatilization.

#### **FOREWARD**

This thesis was written in a manuscript format in compliance with the Department of Soil Science, University of Manitoba guidelines. The thesis consists of eight chapters. Chapter 1 is a general introduction of the thesis, Chapter 2 to 7 are research chapters prepared and submitted (some have been published) as manuscripts to different refereed journals and Chapter 8 is the overall synthesis of the thesis that includes the implications of the study and recommendations for future studies. Although the manuscripts were prepared for different journals, they were all reformatted to one style as stated in the Department of Soil Science, University of Manitoba thesis guidelines.

Chapter 2 was based on testing the efficacy of a relatively new urease inhibitor formulation in reducing ammonia volatilization from urea-based fertilizers under greenhouse and field conditions. Chapter 3 tested the effect of storage time of urease inhibitor treated urea-based fertilizers on the efficacy of the urease inhibitor in reducing ammonia volatilization. Chapters 4 and 5 reported on the efficacy of urease inhibitor with and without nitrification inhibitor in reducing ammonia volatilization and bridging nitrogen use efficiency gap between fall and spring-applied urea-based fertilizers in two contrasting soils. Chapter 6 and 7 investigated the interaction among temperature, urease inhibitor, and nitrification inhibitor on kinetics and thermodynamics of urea hydrolysis in soils.

Chapter 2 has been published as:

Lasisi, A.A., Akinremi, O.O. and Kumaragamage, D. 2019. Efficacy of a new N-(n-butyl) thiophosphoric triamide formulation in reducing ammonia volatilization from urea-based fertilizers. Can. J. Soil Sci. 99: 395-405. DOI:10.1139/cjss-2018-0072.

Chapter 3 has been published as:

Lasisi, A.A., Akinremi, O.O. and Kumaragamage, D. 2020. Stability of stored N-(n-butyl) thiophosphoric triamide (NBPT) treated urea-based fertilizers. Commun. Soil Sci. Plant Anal. 51: 911-918. DOI:10.1080/00103624.2020.1744632

Chapter 4 has been published as:

Lasisi, A.A., Akinremi, O.O., Zhang Q. and Kumaragamage, D. 2020. Efficiency of fall versus spring applied urea-based fertilizers treated with urease and nitrification inhibitors I. Ammonia volatilization and mitigation by NBPT. Soil Sci. Soc. Am. J. 84: 949-962. DOI: 10.1002/saj2.20062

Chapter 5 has been accepted as:

Lasisi, A.A., Akinremi, O.O. and Kumaragamage, D. 2020. Efficiency of fall versus spring applied urea-based fertilizers treated with urease and nitrification inhibitors II. Crop yield and nitrogen use efficiency. Soil Sci. Soc. Am. J. DOI: 10.1002/saj2.20126

Chapter 6 has been accepted as:

Lasisi, A.A., Akinremi, O.O. and Kumaragamage, D. 2020. Nitrification inhibitor reduces the inhibitory effect of N-(n-butyl) thiophosphoric triamide (NBPT) on the hydrolysis of urea. Soil Sci. Soc. Am. J. DOI: 10.1002/saj2.20122

Chapter 7 has been submitted as:

Lasisi, A.A. and Akinremi, O.O. 2020. Kinetics and thermodynamics of urea hydrolysis in the presence of urease and nitrification inhibitors. Can. J. Soil Sci. [Manuscript ID: CJSS-2020-0044]

#### ACKNOWLEDGMENTS

"He who is not grateful to people is not grateful to God" (Muhammad, 570-632 CE). It is with my deepest respect and responsibility that I express my gratitude to my supervisor, Dr. Olalekan Oluwole Akinremi for giving me the opportunity to be tutored, mentored, and guided by his wealth of experience during my graduate programme. I have learnt from you not only academic skills but also "happy living skills". I extend my profound gratitude to members of my advisory committee: Dr. Darshani Kumaragamage and Dr. Qiang Zhang. Your advice and suggestion are the fuel that propelled this thesis. I am honoured to have both of you on my committee.

This research work would not have been possible without the funding from Natural Sciences and Engineering Research Council and Active AgriScience Inc. BC or awards that I received from the University of Manitoba Graduate Fellowship, Faculty of Graduate Studies Travel Awards, International Graduate Student Scholarship, Faculty of Graduate Studies Research Completion Scholarship, Canadian Society of Soil Science Student Travel Award, and Murphy Foundation Incorporated Graduate Student Awards. Thank you all for your support.

Special thanks to Rob Ellis, Justin Soucie, Curtis Caver, George Dyck, Veses Indraratne, and Dr. Timi Ojo for your help in data collection. Justin Soucie, your technical support and commitment to go out to collect samples even on public holidays without hesitation are forever appreciated. I acknowledge the proprietary contribution of Dr. Ranil Waliwitiya to the development of ARM U and ARM U Advanced used in this study. Thanks to Dr. Francis Zvomuya, Dr. Nick Jeke, and Adesanya Theresa for your statistical help and discussion.

I use this medium to thank all those who have taught me; from Mr. Samuel (my primary one class teacher) to Dr. Akinremi (my Ph.D. supervisor). Special thanks to Dr. Olushola Adesanwo for easing the beginning of my journey into a graduate programme. I am very grateful to all staff and graduate students in the Department of Soil Science, University of Manitoba. Theresa Adesanya and Mayowa Adelekun, your support and friendship are boldly written on my heart.

Certainly, a thousand words can not describe how happy and grateful to have my family support for this programme. Thanks to my parents and siblings for your prayer. To my wonderful kids, Ameerah Ikeoluwa and Aliyyah Aanuoluwa, who wonder what I do on the computer every day, I say thank you for your patience. To my beloved spouse, Amina Oluwaseun, thank you for your patience, love, support, and sacrifices in getting this thesis done. You are the backbone of this thesis. My heart is full of appreciation as I write this last word, Amina!

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#### **1.0 INTRODUCTION**

#### **1.1 Urea-Based Fertilizers**

Globally, urea-based fertilizers account for more than one-half of the nitrogen (N) fertilizers use on agricultural fields. Urea-based fertilizers are commonly sold as granular urea (46-0-0) and urea ammonium nitrate solutions (UAN, 28-0-0 or 32-0-0). Urea and UAN are widely used because they are relatively safe, contain high N content (especially the granular urea), relatively cheap per unit N, easy to handle and store with little or no fire hazard (Li et al. 2015). According to the International Fertilizer Association, the demand for urea is estimated to increase by 17 MT from 2016 to 2021 (IFA 2017).

#### 1.2 Hydrolysis of Urea

Urea is a highly stable compound in the absence of urease enzymes. In aqueous solution, the halflife of uncatalyzed urea is approximately 40 years at 25 °C (Sigurdarson et al. 2018). When ureabased fertilizers are applied to the soil, they undergo hydrolysis to produce two molecules of ammonia. Hydrolysis of urea is a two-stage process: (i) breaking down of urea into ammonia and carbamate ion by urease enzymes (ii) rapid decomposition of carbamate ion into bicarbonate and another molecule of ammonia (Mazzei et al. 2019). The schematic diagram of the hydrolysis of urea is represented in Figure 1.1.

The urease which catalyzes the first stage of urea hydrolysis (Figure 1.1) is a nickel-dependent enzyme (Mobley and Hausinger 1989). Urease is regarded as a proficient enzyme that is ubiquitous. Urease originates from plant root exudates, plant residues and rupture of ureolytic soil microorganisms (Marshall 1990). It is found in soil and a variety of living organisms such as



Figure 1.1 Schematic diagram of urea hydrolysis in soil.

bacteria, fungi, algae, and plants (Mobley and Hausinger 1989; Krajewska 2009). The two nickel (Ni) ions in the urease active sites bind with urea molecules via an oxygen atom and an amide group.

The rate of urea hydrolysis is reported to increase with an increase in temperature, the quantity of urea, and moisture content around field capacity as a result of increasing activities of urease enzymes (Singh and Nye 1984; Lei et al. 2017). The overall process of urea hydrolysis results in an increase in soil pH around the urea granules (Rochette et al. 2009). Since the equilibrium between ammonium and ammonia is pH dependent, high soil pH caused by urea hydrolysis shifts the equilibrium towards ammonia; hence ammonia volatilization (Chin and Kroontje 1963; Caicedo et al. 2000).

#### **1.3 Ammonia Volatilization from Urea-Based Fertilizers**

The high amount of N losses in form ammonia volatilization is a major challenge that limits the N use efficiency of urea-based fertilizers. The ammonia volatilization from urea-based fertilizers could be greater than 20% of applied N especially when urea-based fertilizers are surface-applied (Rawluk et al. 2001; Soares et al. 2012). The amount of ammonia volatilization from surface-

applied urea-based fertilizers is dependent on several factors such as soil pH, soil texture, soil surface moisture, temperature, relative humidity, and wind intensity (Chien et al. 2009; Kissel et al. 2013). Soils with high pH have been reported to increase the potential for ammonia volatilization from surface-applied urea-based fertilizers (He et al. 1999). The presence of calcareous soils with neutral to alkaline pH on the Canadian prairies increases the potential of ammonia volatilization if not properly managed. Application of urea to soils with high moisture content followed by high relative humidity resulted in higher ammonia volatilization than to soils with lower initial moisture content (Cabrera et al. 2010). In contrast, when surface application of urea is immediately followed by rainfall or irrigation greater than 14.6 mm, the potential for ammonia volatilization is significantly reduced as a result of urea incorporation into the soil (Holcomb et al. 2011). At high temperatures, N losses in the form of ammonia volatilization tend to be high because of rapid urea hydrolysis (Watson et al. 2008). Also, soils with high sand content and low cation exchange capacity (CEC) have a greater potential for ammonia volatilization than soil with a low sand content and high CEC due to their lower retention of ammonium on the soil colloid (Watson et al. 1994; San Francisco et al. 2011). The agronomic consequence of ammonia volatilization is a low N use efficiency by crops. In Canada, ammonia volatilization from livestock and fertilizers accounts for over 80% of atmospheric ammonia contamination (Environment and Climate Change Canada 2017). The volatilized ammonia can react with acidic gases in the atmosphere such as sulphur dioxide to form droplets of ammonium sulphate or other ammonium salt solutions which when dried will form an aerosol particle small enough to be inhaled resulting in serious health effect (Sheppard et al. 2010; Behera et al. 2013).

#### 1.4 Measurement of Ammonia Volatilization

Over the years, several methods have been used to measure ammonia volatilization from ureabased fertilizers (McGinn and Janzen 1998; Misselbrook et al. 2005; Chapman et al. 2016; Baldé et al. 2019). The methods include dynamic chamber/wind tunnel, micrometeorological, semi-open static chambers, and dosi tubes. In general, micrometeorology methods are considered as the most accurate methods because they do not disrupt the environmental conditions during measurement. However, they are expensive to install, require large areas of land, highly technical, and they are not feasible for evaluating several treatments simultaneously (Cabrera et al. 2001). On the other hand, wind tunnel and static chamber methods require modification of environmental conditions during measurement. While wind tunnels allow for movement of wind across the soil, static chamber methods restrict wind movement across the soil. As such, studies that compared static chamber versus wind tunnel have reported that static chamber methods underestimated ammonia volatilization measurement (Smith et al. 2007; Miola et al. 2015). Despite this shortcoming, the static chamber methods are often used because they are relatively cheap and easy to construct (Figure 1.2), require little technical skill to use, and allow for multiple treatments comparison.



Figure 1.2 Schematic diagram of the ammonia volatilization static chamber set up.

#### 1.5 Mitigation of Ammonia Volatilization from Urea-Based Fertilizers

To mitigate ammonia volatilization, sub-surface instead of surface application is usually recommended for urea-based fertilizer application. However, due to constraints such as labour shortage, soil condition, time, and/or established tree crops or forages at the time of application, sub-surface fertilizer application may not be feasible.

Several products have been developed and studied over the years to reduce ammonia volatilization from urea-based fertilizers (Chien et al. 2009). The most studied of these products is a urease inhibitor whose active ingredient is N-(n-butyl) thiophosphoric triamide (NBPT) (Cantarella et al. 2018). The urease inhibitor, NBPT, has been reported to reduce ammonia volatilization from surface-applied urea-based fertilizers by a global average of 52% (Silva et al. 2017; Cantarella et al. 2018). The NBPT slows down urea hydrolysis by first being rapidly transformed into either N-(n-butyl) thiophosphoric diamide (NBPD) or N-(n-butyl) phosphoric triamide (NBPTO) (Creason et al. 1990; Mazzei et al. 2019). The NBPD or NBPTO then becomes hydrolyzed to monoamido thiophosphoric acid (MATP) or diamido phosphoric acid (DAP), respectively. Either the MATP or DAP prevents contact between urea and urease by binding with the two nickel ions of urease active sites via two oxygen atoms and an amide group (Figure 1.3; Sigurdarson et al. 2018; Mazzei et al. 2019).

The inhibitory action of NBPT is most effective during the first week after urea application to the soil when urea hydrolysis is expected to be at maxima (Rawluk et al. 2001; Sanz-Cobena et al.



Figure 1.3 Schematic description of inhibition of urea hydrolysis by NBPT

2008). Urease inhibitor thereby allows sufficient time for urea molecules to move below the soil surface where ammonia volatilization will be greatly reduced. The NBPT is sold under different trade names. Common NBPT formulations and concentrations include Agrotain Advanced (30% NBPT m/v; Koch Agronomic Services LLC, Wichita, KS), Factor (24.5% NBPT m/v; Rosen's Inc., Liberty, MO), Arborite (24% NBPT m/v; Weyerhaeuser Co., Olympia, WA), and N-Veil (26.7% NBPT m/v; Innvictis Crop Care LLC, CO). Each of the formulations has unique qualities that have bearing on its efficacy. A review by Cantarella et al. (2018) suggested that the stability and longevity of NBPT formulations depended on the solvent used by different manufactures in their formulations. This explains the reasons why many manufacturers continuously change the solvent and compositions of their formulations. A relatively new NBPT formulation known as ARM U (18% NBPT m/v; Active AgriScience Inc., BC) had been claimed by its manufacturer to contain a proprietary polymer that enables lower use of NBPT concentration without

compromising the efficacy of NBPT to reduce ammonia volatilization from urea-based fertilizers. Thus far, this claim is yet to be independently verified.

#### 1.6 Effect of NBPT on N Use Efficiency of Urea-Based Fertilizers

The efficiency of fertilizer N applied to soils has been reported to be an average of 50% (Delgado 2002). The remaining 50% that is not harvested in the above-ground biomass is either lost to the environment as ammonia, nitrate, nitrous oxide, and/or dinitrogen gas; becomes immobilized in the soil, stored in the soil, or stored in the root. In addition to the use of NBPT to improve yield through the reduction of ammonia volatilization, nitrification inhibitors (NI) such as nitrapyrin, dicyandiamide, and 3,4 dimethyl pyrazole phosphate have been used to reduce N losses in form of nitrate leaching and nitrous oxide emission (Subbarao et al. 2006). Despite the use of NBPT with and without NI to reduces N losses, the conserved N by the inhibitors has not consistently increased grain yield in the northern Great Plain. While studies such as Mohammed et al. (2016) found a significant increase in grain yield of cereals due to coating of urea with NBPT or NBPT+NI (double inhibitor, DI), other studies such as McKenzie et al. (2010) and Grant (2014) did not find any significant effect of NBPT on grain yield of cereals.

A survey of Manitoba agronomist showed that fall N application is preferred to spring due to the cheaper cost of fertilizer in the fall and overall efficient time management (Amiro et al. 2017). However, the efficiency of fall-applied N is less than spring-applied N fertilizer on grain yield (Ridley 1977; Nyborg and Malhi 1986; Jaynes 2015). The lower efficiency of fall-applied N is in part due to the time gap between fall N application and crop N need in the spring. To reduce the time difference between N application in the fall and crop need during the growing season, farmers

are advised to delay their N application to mid- (early October) or late-fall (mid-October) when the soil temperature is below 5 °C at 10 cm depth (MAFRI 2007). The delayed N application is expected to reduce N losses in the form of nitrate leaching and denitrification. However, delaying N application until late-fall did not consistently increase grain yield of fall-applied N relative to spring (Tiessen et al. 2005). Also, there is a dearth of information on the quantity of ammonia volatilization from surface-applied urea-based fertilizers applied in the late fall as well as the benefit of NBPT on the N use efficiency of late fall-applied urea-based fertilizers.

#### 1.7 Objectives

The overall objective of this study was to evaluate the efficacy of urease inhibitor, specifically NBPT, with and without nitrification inhibitor in improving the efficiency of urea-based fertilizers. Specific objectives were to (i) evaluate the efficacy of a new NBPT formulation, ARM U (18% NBPT m/v), in reducing ammonia volatilization from surface-applied urea-based fertilizers in comparison to Agrotain Advanced (30% NBPT m/v) and Arborite (24% NBPT m/v) under greenhouse and field conditions (Chapter 2); (ii) determine the effect of storage time of urea and UAN treated with a new formulation of NBPT, ARM U, on the effectiveness of NBPT in reducing ammonia volatilization (Chapter 3); (iii) quantify and contrast the efficacies of NBPT with and without NI in reducing ammonia volatilization from fall and spring-applied urea-based fertilizers in two contrasting soils (Chapter 4); (iv) evaluate the efficacies of NBPT with and without NI in improving crop yield, N removal and N uptake from fall versus spring-applied urea-based fertilizers in two contrasting soils (Chapter 5); (v) evaluate the interaction between NBPT and NI

on the hydrolysis of urea (Chapter 6); (vi) evaluate the interactive effect of temperature, NBPT, and NI on the kinetics and thermodynamics parameters of urea hydrolysis (Chapter 7).

#### **1.8 Thesis Outline**

The layout of this thesis followed the thesis guidelines of the Department of Soil Science, University of Manitoba. Chapters 2 to 7 were prepared in manuscript formats with the titles as follows:

**Chapter 2:** Efficacy of a new N-(n-butyl) thiophosphoric triamide (NBPT) formulation in reducing ammonia volatilization from urea-based fertilizers.

**Chapter 3:** Stability of stored N-(N-Butyl) thiophosphoric triamide (NBPT) treated urea-based fertilizers.

**Chapter 4:** Efficiency of fall versus spring-applied urea-based fertilizers treated with urease and nitrification inhibitors I. Ammonia volatilization and mitigation by NBPT.

**Chapter 5:** Efficiency of fall versus spring-applied urea-based fertilizers treated with urease and nitrification inhibitors II. Crop yield and nitrogen use efficiency.

**Chapter 6:** Nitrification inhibitor reduces the inhibitory effect of N-(n-butyl) thiophosphoric triamide (NBPT) on the hydrolysis of urea.

**Chapter 7:** Kinetics and thermodynamics of urea hydrolysis in the presence of urease and nitrification inhibitors.

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#### **CHAPTER 2**

## 2 EFFICACY OF A NEW N-(n-BUTYL) THIOPHOSPHORIC TRIAMIDE (NBPT) FORMULATION IN REDUCING AMMONIA VOLATILIZATION FROM UREA-BASED FERTILIZER

#### 2.1 Abstract

N-(n-butyl) thiophosphoric triamide (NBPT) has been reported to reduce ammonia volatilization from surface-applied urea and urea ammonium nitrate (UAN). A new NBPT formulation (ARM U<sup>TM</sup>, 18% NBPT) that contains a polymer allowing for lower application rate of NBPT was evaluated for its efficacy relative to Agrotain Advanced (30% NBPT) and Arborite (24% NBPT). Trials consisted of (i) a greenhouse study that compared two rates of ARM U treated urea (360 and 540 mg NBPT kg<sup>-1</sup> urea) with Arborite or Agrotain Advanced treated urea (480 and 600 mg NBPT kg<sup>-1</sup> urea, respectively); (ii) a field study that compared urea and UAN treated with either ARM U (360 mg NBPT kg<sup>-1</sup> urea) or Agrotain Advanced (600 mg NBPT kg<sup>-1</sup> urea) at two sites. Static chambers fitted with acid-charged discs were used to measure ammonia volatilization at six or seven dates over 28 d. In the greenhouse study, ammonia volatilization was reduced by 96% with either ARM U or Agrotain Advanced and 95% with Arborite. In the field study, ARM U and Agrotain Advanced reduced ammonia volatilization from urea by 80 and 66%, respectively across sites. Similarly, ammonia volatilization from UAN was reduced by 46 and 60% with ARM U and Agrotain Advanced, respectively. Despite the lower NBPT application rates with ARM U, ammonia reduction by ARM U, Agrotain Advanced, and Arborite were not significantly different. The addition of ARM U to urea and UAN enabled a lower application rate of NBPT without compromising its efficacy.

#### **2.2 Introduction**

Urea and urea ammonium nitrate (UAN) are urea-based nitrogen (N) fertilizers that are commonly used to supply N to crops. Urea is the most widely used N fertilizer in most agricultural systems due to its ease of application, high N content, and relatively low cost (Behera et al. 2013; Li et al. 2015). However, the potential for substantial N loss as a result of ammonia volatilization can reduce the N use efficiency of urea-based fertilizers. Ammonia volatilization from urea-based fertilizers occurs during the hydrolysis of urea [(NH<sub>2</sub>)<sub>2</sub>CO] to ammonium and carbonate ions in the presence of urease enzyme which increases soil pH around the urea granules (Bremner 1995; Chien et al. 2009; Soares et al. 2012). The high soil pH caused by urea hydrolysis shifts the equilibrium between ammonium and ammonia to ammonia, thereby increasing the risk of ammonia volatilization. The predominantly calcareous soils with neutral to alkaline pH on the Canadian prairies (Michalyna et al. 1988) increase the potential of ammonia volatilization from urea-based fertilizers in this region.

The magnitude of ammonia volatilization from urea-based fertilizers depends on several factors including soil pH, texture, organic matter, moisture, temperature, relative humidity, and wind intensity (Chien et al. 2009; Cabrera et al. 2010). For example, soils with high sand content and low organic matter generally have greater ammonia volatilization than those with low sand content and high organic matter (Carmona et al. 1990; Watson et al. 1994). Also, ammonia volatilization increases as soil temperature increases (Yan et al. 2016) especially in moist soils. This is because an increase in temperature increases the rate of urea hydrolysis with a greater risk of ammonia volatilization. In addition to soil and environment factors, methods of measurement influence the
magnitude of ammonia volatilization. Static chamber method has been reported to underestimate ammonia volatilization when compared to dynamic methods such as wind tunnel and micrometeorological methods (Miola et al. 2015; Smith et al. 2007). The reasons for the underestimation with static chambers include ammonia gradient and wind restriction across the soil surface. Despite the limitations with the static chamber method, it is still commonly used in many studies because (i) it is relatively cheap to construct (ii) it requires little logistics compared to wind tunnel (iii) it allows for ease of several treatments comparison (iv) it allows for easy replications.

Nitrogen losses due to ammonia volatilization can be greater than 35% of applied N (Rawluk et al. 2001; Cai et al. 2002; Soares et al. 2012) when conditions are favourable. The consequence of these losses is a reduction in crop N use efficiency of urea-based fertilizers. In addition to its negative agronomic consequence, the volatilized ammonia can react with acidic gases in the atmosphere to form droplets of ammonium salt that are detrimental to human health (Sheppard et al. 2010).

To mitigate ammonia volatilization from urea-based fertilizers, farmers are advised to incorporate surface-applied urea-based fertilizers immediately after application. The incorporation practice should position urea granules at least 5 cm below the surface; otherwise, a remarkable amount of ammonia loss may result if conditions are favourable for volatilization (Rochette et al. 2013). Immediate irrigation or rainfall greater than 14.6 mm after fertilization has been reported to reduce ammonia volatilization by moving urea molecules beneath the soil surface (Holcomb et al. 2011). However, due to constraints such as labour and time shortage, the immediate incorporation of urea-

based fertilizers is not usually feasible. Several products have been developed and tested over the years to reduce ammonia volatilization from urea-based fertilizers (Chien et al. 2009). The most studied of these products is a urease inhibitor whose active ingredient is N-(n-butyl) thiophosphoric triamide (NBPT) (Watson et al. 2008; Chien et al. 2009). The NBPT slows down urea hydrolysis by being rapidly transformed into its oxygen analogue N-(n-butyl) phosphoric triamide (NBPTO), which then competes with urea for active sites on the urease enzyme (Christianson et al. 1990; Creason et al. 1990). The use of NBPT provides an opportunity to change the source of N fertilizer in agreement with the "4R nutrient stewardship" of using the right source at the right time, at the right rate in the right placement (Johnston and Bruulsema 2014). Recent meta-analyses and reviews have shown that treating urea with NBPT reduced ammonia volatilization from surface-applied urea-based fertilizers across different soil properties and environmental conditions (Pan et al. 2016; Silva et al. 2017; Cantarella et al. 2018). The reduction relative to untreated urea may exceed 70% during the first 7 d of fertilization when ammonia volatilization from urea is at a maximum (Rawluk et al. 2001; Engel et al. 2011; Soares et al. 2012).

In recent years, several formulations of NBPT such as Agrotain Advanced (Koch Agronomic Services LLC, KS), Arborite (Weyerhaeuser Co., WA), N-Veil, (Innvictis Crop Care LLC, CO), and Nitrain (Loveland Product Inc. ON) have been developed to reduce ammonia volatilization from urea-based fertilizers. Each of the formulations has unique qualities that have bearing on its efficacy (Sanders 2007; Franzen et al. 2011; Goos 2013; Peng et al. 2015). A new formulation of NBPT known as ARM U (18% NBPT m/v) was developed by Active AgriScience Inc. (Abbotsford, BC). The unique attributes and proprietary formulations of ARM U include (i) the

presence of polymers that enable uniform coverage of urea granules, thereby allowing for lower application rate of NBPT per kg of urea (ii) low temperature fluidity that allows for easy handling at temperature as low as -15 °C without freezing. We are not aware of any study that has tested the efficacy of ARM U formulation in reducing ammonia volatilization from urea-based fertilizers. The objective of this study was to evaluate the efficacy of ARM U in reducing ammonia volatilization from surface-applied urea-based fertilizers in comparison to Agrotain Advanced (30% NBPT m/v) and Arborite (24% NBPT m/v) under greenhouse and field conditions. We hypothesized that ARM U with a lower rate of NBPT application would have similar effectiveness in reducing ammonia volatilization compared to Agrotain Advanced or Arborite with a higher rate of NBPT application, thereby improving the efficiency of NBPT application.

#### 2.3 Materials and Methods

# 2.3.1 Greenhouse Study: Soil Description, Treatment Applications, and Experimental Design

The Dezwood soil used for this study was an Orthic Dark Gray Chernozem in the Canadian soil classification system (Langman 1986) which is comparable to Boralfic Boroll subgroup in the USDA classification system and to Greyzem in WRB/FAO classification system (Agriculture and Agri-Food Canada 1998). The soil properties such as electrical conductivity and pH (Hendershot et al. 2008), organic matter (Walkley and Black 1934), bulk density (Hao et al. 2008), volumetric water content (Cassel and Nielsen 1986), and soil texture (Gee and Bauder 1986) at 0 - 15 cm are presented in Table 2.1.

	Greenhouse	Fie	eld
Soil properties	Dezwood	Carman	High Bluff
Soil series	Dezwood	Hibsin	High Bluff
Soil pH (soil/water, 1:1)	7.4	5.8	7.7
Electrical conductivity ( $\mu S m^{-1}$ )	453	191	465
Organic matter (g kg <sup>-1</sup> )	40	39	47
Volumetric water content at field capacity (m m <sup>-3</sup> )	0.35	0.28	0.37
Bulk density (Mg m <sup>-3</sup> )	1.1	1.2	1.2
Soil texture	Loam	Sandy loam	Loam
Sand content (g kg <sup>-1</sup> )	465	680	450
Silt content (g kg <sup>-1</sup> )	318	120	310
Clay content (g kg <sup>-1</sup> )	217	200	240

Table 2.1 Characteristics of Dezwood, Carman, and High Bluff soils.

Treatments consisted of urea treated either ARM U, Agrotain Advanced, or Arborite applied at a rate of 2 L per 1000 kg urea, urea treated with ARM U applied at a rate of 3 L per 1000 kg urea, and untreated urea (Table 2.2). These treatments were set up as a randomized complete block design with four replications.

Ammonia volatilization was measured with white polyvinyl chloride cylindrical static chambers (20 cm in height by 15 cm internal diameter) following a method described by Grant et al. (1996) and Jantalia et al. (2012). The chambers were sealed at the bottom with flat plastic plates. This was followed by filling the chamber with air-dried and sieved (< 2 mm) soil up to 5 cm height from the bottom at a bulk density of 1.1 Mg m<sup>-3</sup>. The soils in the chambers were moistened to 75% field capacity, covered with paper plates and allowed to stand for twenty-four hours in the greenhouse

	Treatment <sup>a</sup>	Concentration of NBPT <sup>b</sup>
		(mg kg <sup>-1</sup> urea or mg L <sup>-1</sup> UAN)
Greenhouse	UR	
	UR <sub>ARM</sub>	360
	UR <sub>AG</sub>	600
	UR <sub>ARB</sub>	480
	UR <sub>ARM2</sub>	540
Field	UR	
	UR <sub>ARM</sub>	360
	UR <sub>AG</sub>	600
	UAN	
	UAN <sub>ARM</sub>	180
	UANAG	300

Table 2.2 Types of treatments and concentrations of inhibitors applied in greenhouse and field studies.

<sup>*a*</sup> UR, untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>ARB</sub> are urea treated with ARM U, Agrotain Advanced, and Arborite at 2 L formulation per 1000 kg urea respectively; UR<sub>ARM2</sub>, urea treated with ARM U at 3 L formulation per 1000 kg urea; UAN, untreated urea ammonium nitrate; UAN<sub>ARM</sub> and UAN<sub>AG</sub> are UAN treated with ARM U and Agrotain Advanced at 1 L formulation per 1000 L UAN, respectively. ARM U, Agrotain Advanced, and Arborite are NBPT formulations. <sup>*b*</sup> NBPT, N-(n-butyl) thiophosphoric triamide (urease inhibitor); mg kg<sup>-1</sup> urea is for the urea granule

treatments while mg  $L^{-1}$  UAN is for the UAN solution treatments.

for the soil and water to equilibrate. Twenty-four hours after wetting, treatments were surfaceapplied to the centre of the chamber to provide a rate of 100 kg N ha<sup>-1</sup> based on the surface area of the chamber. Agrotain Advanced and Arborite were sourced from a farm input dealer while ARM U was sourced directly from its manufacturer. Urea was coated with an inhibitor a day before application by spraying 1 kg of urea in a jar with an appropriate volume of inhibitor (2 or 3 mL). The urea in the jar was immediately mixed vigorously to ensure thorough and uniform coating of the urea with the inhibitors. Despite the low inhibitor volume to urea quantity ratio, coating urea with the inhibitors was relatively uniform across the NBPT formulations. As claimed by the manufacture, NBPT formulations are designed to allow enough time for coating even at a lower rate (volume) before they dry off.

Immediately after treatment application, two acid-charged polyfoam discs (upper and lower discs) of 2.5 cm thick and 16 cm diameter were fitted into the chambers to absorb ammonia. The discs were designed to tightly fit into the chambers and reduce air exchange with outside air. The discs were charged by washing in 0.001 M H<sub>2</sub>SO<sub>4</sub> solution twice. The H<sub>2</sub>SO<sub>4</sub> washed discs were then immersed twice in glycerol-phosphoric acid solution (40 mL 14.7 M H<sub>3</sub>PO<sub>4</sub> and 50 mL glycerol in 1 L de-ionized water). The prepared charged discs were wrung to reduce the amount of solution in them and transferred through an air-tight bag to the greenhouse where they were fitted into the chambers. The lower discs were fitted at 5 cm above the surface of the treated soils to trap volatilized ammonia while the upper discs were fitted at 2 cm below the top of the chambers to prevent atmospheric ammonia contamination of lower discs.

# 2.3.2 Field Study: Site Description, Treatment Applications, and Experimental Design

A complimentary field study was conducted at two sites seeded to canola (*Brassica napus* L.; cv, Liberty Link) in the spring of 2016. The two sites were Carman (49° 29′ 6″ N, 98° 02′ 2″ W) and High Bluff (50° 01′ 2″ N, 98° 08′ 9″ W). The soil at Carman was an Orthic Black Chernozem in the Canadian soil classification system (Mills and Haluschak 1993) which is comparable to the Udic Boroll subgroup in the USDA classification system and Chernozem in WRB/FAO classification system (Agriculture and Agri-Food Canada 1998). The soil at High Bluff is a Gleyed Cumulic Regosol (Michalyna and Smith 1972) which is comparable to Entisol in the USDA

classification system and Regosol in WRB/FAO classification system (Agriculture and Agri-Food Canada 1998). The properties of the soils (0 - 15 cm) at the two sites are shown in Table 2.1.

The treatments for the field study consisted of two sources of N (urea and UAN, 28-0-0) and two sources of urease inhibitors (ARM U and Agrotain Advanced) to give the following seven treatments: urea treated with either ARM U or Agrotain Advanced at a rate of 2 L per 1000 kg urea, UAN with either ARM U or Agrotain Advanced at a rate of 1 L per 1000 L UAN, untreated urea, untreated UAN, and a control with no N amendment (Table 2.2). Urea granule was coated with the inhibitor as described above. UAN was treated with the inhibitor by adding 10 mL of the inhibitor to 10 L of UAN in a jug. The UAN was then thoroughly mixed.

Chambers (20 cm in height by 15 cm internal diameter) were installed to a depth of 5 cm at the northwest corner of each 5 m x 3 m plot in a randomized complete block design. Seedlings, as well as crop residues within the internal area of the chambers were removed. Rainfall events from 1 to 3 days before fertilization at both sites provided sufficient water to keep the soil moist for treatment application (Figure 2.1). Treatment application followed the same method and rate (100 kg N ha<sup>-1</sup> of total N) described for the greenhouse study. UAN was applied to the soil surface with a pipette. Disc preparation and fitting followed the same procedure that was described for the greenhouse study. Chambers were covered with puck board sheets positioned at about 30 cm above ground level to prevent rainfall from falling directly into the chambers but still permit air circulation above chambers.

# 2.3.3 Ammonia Volatilization Measurement

Discs were sampled and replaced with newly prepared discs on 1, 2, 4, 7, 14, 21, and 28 days after fertilization (d) in the greenhouse and field studies (except no sampling on 1 d in the field). The lower discs were placed directly in a zip-lock bag of known weight for ammonium extraction. In the greenhouse study, soil moisture was maintained at 75% field capacity by weighing chambers and adding water around the interior chamber edges after sampling events on 4, 7, 14, and 21 d (Franzen et al. 2011). In the field study, water was not added to the chambers after sampling or after rainfall. However, the initially moist soil condition within the chamber and possible lateral and upward movement of water within the chamber following rainfall events provided a moist soil condition necessary for urea hydrolysis. Daily mean temperature in the greenhouse was monitored with Watchdog 2000 series weather station (model 2900ET; Spectrum Technologies Inc., IL). For the field study, daily rainfall and temperature data were collected from the nearest Environment Canada weather station during the sampling periods.

In the laboratory, each zip-lock bag containing disc was weighed to calculate the amount of absorbing solution trapped in the foam disc. The ammonia trapped in the discs was extracted with 250 mL of 0.5 M KCl solution. Ammonium concentration in the KCl extract was determined colorimetrically using the AQ2 Discrete Analyzer (SEAL Analytical Inc., WI). Ammonia volatilization (kg N ha<sup>-1</sup>) was calculated as follows:

$$NH_{3} loss = \frac{(Extractant (mL) + absorbent in disc (mL)) \times NH_{3}(mg N mL^{-1})}{Area of chamber (ha) \times 10^{6}}$$
(2.1)

Cumulative ammonia volatilization was calculated by summing ammonia losses between the sampling periods. Total ammonia volatilization was calculated as the sum of ammonia volatilization during the sampling periods. Percentage ammonia volatilization was calculated as a ratio of ammonia loss to total N applied. Percentage reduction of ammonia volatilization from urea and UAN treated with inhibitors was calculated as a percentage of ammonia volatilization in untreated urea and UAN, respectively.

### 2.3.4 Statistical Analysis

Analysis of variance (ANOVA) was conducted with PROC GLIMMIX (SAS Institute, version 9.4) for repeated measure analysis to determine the significance of fertilizer treatments, time, and fertilizer treatment by time interaction on daily ammonia volatilization. Covariance structures that accounted for the unequal sampling intervals were compared and the one with the lowest Akaike's information criterion was used for the model (Littell et al. 2006). Fertilizer treatment and time were fixed effects while block (replicate) was a random effect. Because most losses occurred during the first 7 d after fertilization in untreated urea and UAN and after the first 7 d in urea and UAN treated with urease inhibitors (Rochette et al. 2009; Soares et al. 2012), cumulative ammonia volatilization was analyzed at three-time intervals (0 - 7 d, 7 - 28 d, and 0 - 28 d) with PROC GLIMMIX in which fertilizer treatment is a fixed effect and block (replicate) is a random effect. Prior to PROC GLIMMIX, data were checked for the assumption of a normal distribution with PROC UNIVARIATE (Shapiro-Wilk test). Data that were not normally distributed were specified for lognormal transformation in the model. Percentage reduction of ammonia volatilization by the inhibitors was also compared using PROC GLIMMIX (beta distribution) in which fertilizer

treatment was a fixed effect and block (replicate) was a random effect. Fisher's protected least significant difference (LSD) procedure at a probability level of < 0.05 was used for the comparison of treatment means.

# 2.4 Results

# 2.4.1 Greenhouse Study

Daily mean air temperature in the greenhouse ranged from 19.6 to 29.4 °C (Figure 2.1). Addition of water to the chambers from 4 d helped to keep the soil moist throughout the sampling periods for biological activities to proceed.

Repeated measure ANOVA for ammonia volatilization showed that there was significant (P < 0.0001) fertilizer treatment by time interaction. Ammonia volatilization was significantly greater in untreated urea than the inhibitor treated urea on 1, 2, 4, 7, and 14 d (except UR<sub>AB</sub> on 14 d) whereas there were no significant differences between untreated and inhibitor treated urea on 21 and 28 d (Figure 2.2). Ammonia volatilization peaked on 4 d in untreated urea (15.8 kg N ha<sup>-1</sup>)



Figure 2.1 Daily (a) rainfall and (b) mean air temperature in the greenhouse and field.



Figure 2.2 Daily ammonia volatilization between sampling days following surface application of urea with and without NBPT formulations in the greenhouse. Insert graph contains results from all treatments except untreated urea. Error bars are standard errors of the mean. Note: UR, untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>ARB</sub> are urea treated with ARM U, Agrotain Advanced, and Arborite at 2 L formulation per 1000 kg urea respectively; UR<sub>ARM2</sub>, urea treated with ARM U at 3 L formulation per 1000 kg urea.

whereas the peak of ammonia volatilization was delayed until 14 d in urea treated with urease inhibitors (0.3 to 0.7 kg N ha<sup>-1</sup>; Figure 2.2 'insert'). Daily ammonia volatilization was not significantly different among the urea treated with inhibitors except on 7 and 14 d when UR<sub>AB</sub> had significantly greater volatilization than UR<sub>ARM2</sub> (Figure 2.2 'insert').

Cumulative ammonia volatilization was significantly greater in UR than in other treatments by 7 d when UR had lost a cumulative of 21.6 kg N ha<sup>-1</sup> compared to a loss of less than 0.5 kg N ha<sup>-1</sup>

in urea treated with inhibitors (Table 2.3). The addition of ARM U, Agrotain Advanced, and Arborite to urea reduced ammonia volatilization by at least 98% at the end of 7 d. Cumulative ammonia volatilization was not significantly different between the two rates of ARM U by 7 d (Table 2.3). Similarly, cumulative ammonia volatilization from UR<sub>ARM</sub>, UR<sub>ARM2</sub>, UR<sub>AG</sub>, and UR<sub>AB</sub> were not significantly different after 7 d (Table 2.3).

Treatment <sup>a</sup>	Cumulative ammonia volatilization		Total ammonia volatilization	Reduction
	0 - 7 d	7 - 28 d	0 - 28 d	
		—— kg N ha <sup>-1</sup> —		%
UR	21.6a	0.9a	22.5a	
UR <sub>ARM</sub>	0.4b	0.6ab	1.0bc	96a
UR <sub>AG</sub>	0.3b	0.6ab	0.9bc	96a
UR <sub>AB</sub>	0.4b	0.8ab	1.2b	95a
UR <sub>ARM2</sub>	0.3b	0.5b	0.8c	96a
ANOVA	< 0.0001	0.1729	< 0.0001	0.3249

Table 2.3 Effect of NBPT formulation on cumulative and total ammonia volatilization and percentage reduction of ammonia volatilization from surface-applied urea in the greenhouse.

<sup>*a*</sup> UR is untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>AB</sub> are urea treated with ARM U, Agrotain Advanced and Arborite, respectively at a rate of 2 L per 1000 kg of urea; UR<sub>ARM2</sub> is urea treated with ARM U at the rate of 3 L per 1000 kg of urea; ANOVA, analysis of variance Means with different letters within a column are significantly different at a probability of < 0.05 Fisher protected LSD.

Cumulative ammonia volatilization from 7 - 28 d was not significantly different among the treatments except between UR and  $UR_{ARM2}$  where  $UR_{ARM2}$  had significantly lower cumulative ammonia volatilization than UR (Table 2.3).

At the end of 28 d, total ammonia volatilization was significantly greater in untreated urea than in NBPT treated urea. Untreated urea lost a total of 22.5% of applied N by the end of 28 d whereas  $UR_{ARM}$  and  $UR_{ARM2}$  lost 1.0 and 0.8% of applied N, respectively and  $UR_{AG}$  and  $UR_{AB}$  lost 0.9 and 1.2% of applied N, respectively (Table 2.3). Total ammonia volatilization was not significantly different between  $UR_{ARM}$  and  $UR_{ARM2}$  (Table 2.3). Total ammonia volatilization from  $UR_{ARM}$ ,  $UR_{AG}$ , and  $UR_{AB}$  that contained the same volume of formulation but different concentrations of NBPT were not significantly different from one another. At the end of 28 d, ammonia volatilization reduction was not significantly affected (P = 0.3249) by the concentrations of NBPT or types of formulations (Table 2.3). Ammonia volatilization from urea was reduced by 96, 96, and 95% with ARM U, Agrotain Advanced, and Arborite, respectively.

#### 2.4.2 Field Study

Daily mean temperature and rainfall were similar between Carman and High Bluff during the sampling period (Figure 2.1). Daily mean air temperatures ranged from 13.1 to 22.4 °C at Carman and from 15.2 to 22.7 °C at High Bluff. Daily rainfall ranged from 0 to 33 mm at Carman and 0 to 29 mm at High Bluff. At both sites, the largest amount of precipitation was received on 5 d (Figure 2.1).

There was a significant treatment by time interaction (P < 0.0001) for ammonia volatilization at both sites. Expectedly, daily ammonia volatilization was significantly smaller in control plots without N amendment at each site throughout the sampling period (Figure 2.3). Ammonia volatilization peaked on 4 d in untreated urea at each site and on 4 and 7 d in untreated UAN at



Figure 2.3 Daily ammonia volatilization between sampling days following surface application of urea (upper graphs) and UAN (lower graphs) treated with and without NBPT formulations in the field. Error bars are standard errors of the mean.

Note: UR, untreated urea; UR<sub>ARM</sub> and UR<sub>AG</sub>, are urea treated with ARM U and Agrotain Advanced at 2 L formulation per 1000 kg urea respectively; UAN, untreated urea ammonium nitrate; UAN<sub>ARM</sub> and UAN<sub>AG</sub> are UAN treated with ARM U and Agrotain Advanced at 1 L formulation per 1000 L UAN, respectively.

High Bluff and Carman, respectively (Figure 2.3). At Carman, the peak of ammonia volatilization occurred at 14 d for urea and UAN treated with inhibitors. At High Bluff, peak ammonia volatilization occurred at 7 and 14 d for UAN and urea treated with inhibitors, respectively (Figure 2.3).

Cumulative ammonia volatilization by 7 d from UR was 15.4 kg N ha<sup>-1</sup> at Carman and 18.5 kg N ha<sup>-1</sup> at High Bluff whereas it was 0.7 and 2.9 kg N ha<sup>-1</sup> in UR<sub>ARM</sub> and UR<sub>AG</sub>, respectively at Carman and 3.1 kg N ha<sup>-1</sup> in both UR<sub>ARM</sub> and UR<sub>AG</sub> at High Bluff (Table 2.4). Cumulative ammonia volatilization from untreated UAN by 7 d was 4.2 kg N ha<sup>-1</sup> at Carman and 12.8 kg N ha<sup>-1</sup> at High Bluff while it was 0.8 kg N ha<sup>-1</sup> from both UAN<sub>ARM</sub> and UAN<sub>AG</sub> at Carman and 6.1 and 5.8 kg N ha<sup>-1</sup> from UAN<sub>ARM</sub> and UAN<sub>AG</sub>, respectively at High Bluff (Table 2.4). Cumulative ammonia volatilization from UR<sub>ARM</sub>, UAN<sub>ARM</sub>, and UAN<sub>AG</sub> at High Bluff (Table 2.4). Cumulative ammonia volatilization from UR<sub>ARM</sub>, UAN<sub>ARM</sub>, and UAN<sub>AG</sub> at High Bluff was at least four times the cumulative ammonia volatilization at Carman by 7 d. Cumulative ammonia volatilization in urea and UAN treated with ARM U were not significantly different from cumulative ammonia volatilization in their corresponding urea and UAN treated with Agrotain Advanced at each site (Table 2.4). When averaged across sites, ARM U and Agrotain Advanced reduced ammonia volatilization by 89 and 82%, respectively in urea and 59 and 61%, respectively in UAN by 7 d.

Cumulative ammonia volatilization was significantly different among the treatments during 7 - 28 d period at each site (Table 2.4). At Carman, a significant difference existed among the N amended treatments. However, the significant difference at High Bluff was due to the significantly low cumulative ammonia volatilization in control treatment with no N amendment and not due to the differences in N amended treatments. Cumulative ammonia volatilization during the 7 - 28 d period from UR<sub>ARM</sub> and UR<sub>AG</sub> were not significantly different from UR at each site (Table 2.4). Similarly, cumulative ammonia volatilization from UAN<sub>ARM</sub> and UAN<sub>AG</sub> were not significantly different from the cumulative ammonia volatilization in UAN during the 7 - 28 d period at each site (Table 2.4).

Treatment <sup>a</sup>	Cumulative	e ammonia	Total ammonia	Reduction
_	volatili	zation	volatilization	
	0 - 7 d	7 - 28 d	0 - 28 d	
		—— kg N ha <sup>-</sup>	1	(%)
Carman				
UR	15.4a	1.4ab	16.8a	
UR <sub>ARM</sub>	0.7c	0.8ab	1.5cd	91a
UR <sub>AG</sub>	2.9bc	4.4a	7.3bc	57ab
UAN	4.2b	0.7b	4.9b	
UANARM	0.8c	1.7ab	2.5bcd	49b
UAN <sub>AG</sub>	0.8c	0.7b	1.5d	69ab
Control	0.1d	0.1c	0.2e	
ANOVA	< 0.0001	0.003	< 0.0001	0.1991
High Bluff				
UR	18.5a	3.0a	21.6a	
UR <sub>ARM</sub>	3.1c	3.7a	6.7c	69ab
UR <sub>AG</sub>	3.1c	2.6a	5.7c	74a
UAN	12.8a	1.2a	14.0b	
UANARM	6.1b	1.9a	8.0c	42c
UAN <sub>AG</sub>	5.8b	1.2a	7.0c	50bc
Control	0.2d	0.1b	0.3d	
ANOVA	< 0.0001	< 0.0001	< 0.0001	0.0203

Table 2.4 Effect of NBPT formulation on cumulative ammonia volatilization and percentage reduction of ammonia volatilization from surface-applied urea and UAN in the field.

<sup>*a*</sup> UR and UAN are untreated urea and UAN, respectively; UR<sub>ARM</sub>, UR<sub>AG</sub> are urea treated with ARM U and Agrotain Advanced, respectively; UAN<sub>ARM</sub>, UAN<sub>AG</sub> are UAN treated with ARM U and Agrotain Advanced, respectively at a rate of 2 L per 1000 kg of urea or 1 L per 1000 L of UAN; ANOVA, analysis of variance.

Means with different letters within a column are significantly different at a probability level < 0.05 Fisher protected LSD.

Total ammonia volatilization (0 - 28 d) was significantly greater from UR than from the other treatments at Carman and High Bluff (Table 2.4). Total ammonia volatilization in UR was 16.8% of applied N at Carman and 21.6% of applied N at High Bluff. Total ammonia volatilization from

UR<sub>ARM</sub> and UR<sub>AG</sub> was 1.5 and 7.3% of applied N, respectively at Carman whereas total ammonia volatilization was 6.7 and 5.7% of applied N from UR<sub>ARM</sub> and UR<sub>AG</sub>, respectively at High Bluff (Table 2.4). Total ammonia volatilization in untreated UAN was 4.9% of applied total N at Carman and 14.0% of applied total N at High Bluff. The corresponding values for UANARM and UANAG were 2.5 and 1.5% of applied total N, respectively at Carman, and 8.0 and 7.0% of applied total N, respectively at High Bluff (Table 2.4). Based on the proportion of urea-N fraction, untreated UAN lost 1.3 times more ammonia than untreated urea at High Bluff and 1.7 times less ammonia than untreated urea at Carman. Total ammonia volatilization from URARM and UANARM were not significantly different from total ammonia volatilization in the corresponding  $UR_{AG}$  and  $UAN_{AG}$ at each site. Total ammonia volatilization from urea and UAN treated with and without urease inhibitors were generally greater at High Bluff than at Carman except in URAG. At the end of 28 d, ammonia volatilization reduction by ARM U and Agrotain Advanced was not significantly different on urea (P = 0.0777) and UAN (P = 0.2911) at Carman (Table 2.4). Similarly, ammonia volatilization reduction by ARM U and Agrotain Advanced was not significantly different on urea (P = 0.5631) and UAN (P = 0.4405) at High Bluff (Table 2.4). ARM U and Agrotain Advanced significantly reduced ammonia volatilization from urea by 57 to 91% at Carman and 69 to 74% at High Bluff and from UAN by 49 to 69% at Carman and 42 to 50% at High Bluff (Table 2.4).

# **2.5 Discussion**

The pattern of daily ammonia fluxes in the field was similar to the greenhouse, especially at Carman. The minimal amount of ammonia losses by 1 d in the greenhouse was because even under favorable conditions, hydrolysis of urea to ammonium and carbonate ions takes about 2 d

(Cantarella et al. 2008; Behera et al. 2013). Logistics and minimal ammonia losses on 1 d in the greenhouse contributed to a lack of sampling on 1 d in the field. Ammonia volatilization measured on 2 d in the field did not suggest that a large amount of ammonia volatilization occurred on 1 d in urea treatments. In the UAN treatments, ammonia volatilization on 2 d was relatively high when compared to the total ammonia volatilization especially at High Bluff (32 to 34% of total ammonia volatilization). The relatively high ammonia volatilization at High Bluff on 2 d was similar to the study of Woodley et al. (2018) that found 33 and ~ 50% of the total ammonia volatilization from surface-applied UAN to have occurred within 1 and 2 d, respectively. Peak ammonia volatilization from untreated urea in the greenhouse and field trials was similar to other studies (Rawluk et al. 2001; Cantarella et al. 2008; Rochette et al. 2009) that showed that most of the ammonia volatilization in untreated urea occurred between 2 and 5 d. The lower peak of ammonia volatilization in untreated UAN compared to untreated urea may be due to lower urea-N fraction in UAN. The lower peak from untreated UAN relative to untreated urea, however, contradicts the findings reported by Viero et al. (2014) where peak ammonia volatilization in untreated UAN was found to be similar or greater than peak ammonia volatilization of untreated urea. Also, some of the volatilized ammonia from UAN during the early sampling days will be from the ammonium fraction of the UAN which is prone to be lost as ammonia on neutral to alkaline soils.

The pattern of ammonia volatilization from urea treated with and without NBPT formulations was similar in the greenhouse and field. Although the amount of losses from urea treated with urease inhibitors was smaller in the greenhouse than in the field, the amount of ammonia volatilization from untreated urea was greater in the greenhouse than in the field. The period of peak ammonia volatilization in urea and UAN treated with NBPT formulations showed that the hydrolysis of urea was delayed beyond 7 d with the addition of urease inhibitors, which thereafter resulted in a significant overall reduction in ammonia volatilization. The time of maximum losses of ammonia in urea and UAN treated with NBPT formulations in our study was similar to previous studies that showed most of the ammonia volatilization from urea treated with urease inhibitor occurred after 7 d (Rawluk et al. 2001; Zaman and Blennerhassett 2010; Engel et al. 2011; Viero et al. 2014).

The amount of ammonia volatilization from urea and UAN treated with NBPT formulations after 7 d showed clearly that the inhibitory action of this urease inhibitor decreased with time. The delayed hydrolysis of urea and UAN treated with urease inhibitors may extend the period of ammonia volatilization from urea and UAN beyond 14 d since urea hydrolysis may not have been completed. Engel et al. (2011) measured as much as 50% of applied urea from NBPT treated urea after 20 d due to insufficient precipitation, while urea concentration was very small in untreated urea. This implies that ammonia volatilization from urea treated with inhibitor may be appreciable after 7 d depending on environmental conditions. Cantarella et al. (2008) found that ammonia volatilization from urea treated with urease inhibitor was similar to ammonia volatilization from untreated urea in a dry environment. They attributed the similarity in ammonia volatilization between the urea treated with and without urease inhibitor in a dry condition to insufficient rainfall needed to incorporate the remaining urea into the soil after the effective period of urease inhibitor. Although rainfall or irrigation events can help to mitigate ammonia volatilization from urea and UAN treated with inhibitor, the amount of rainfall or irrigation may affect the magnitude of ammonia volatilization, as inadequate rainfall may exacerbate ammonia volatilization. Holcomb et al. (2011) observed that rainfall or irrigation event that is less than 7.6 mm did not significantly reduce ammonia volatilization from surface-applied urea. Under field condition or open chamber

condition without the puck board that was used in this study, the amount of rainfall (32.5 mm at Carman and 28.6 mm at High Bluff) on 5 d was sufficient to move the urea below the soil surface where ammonia volatilization will be significantly reduced. As such, most of the losses in urea and UAN treated with NBPT in the field study would have been avoided by the rain event whereas the losses from the untreated urea and UAN would still be large, as much of the volatilization occurred before the rainfall event. Also, under field conditions without a chamber, the canopy from canola leaves may provide conditions that would reduce ammonia volatilization from unhydrolyzed urea during the later days of sampling (Sommer and Olesen 2000). However, this would not have any effect on ammonia volatilization from untreated urea as most of the losses had occurred before the leaves were large enough to provide such canopy.

The magnitude of ammonia volatilization depends on soil and environmental conditions (Rachhpal-Singh and Nye 1986; Kissel et al. 2004; Behera et al. 2013). For example, higher ammonia volatilization from urea and UAN treated with and without NBPT at High Bluff than at Carman during the first 7 d would have been due to its higher soil moisture as a result of greater water holding capacity of loam over sandy loam following precipitation that occurred before fertilizer application. Also, higher ammonia volatilization at High Bluff than at Carman might be due to its higher soil pH, which promoted ammonia volatilization by shifting the equilibrium between ammonium and ammonia in favour of ammonia. The greater total ammonia volatilization from untreated UAN than untreated urea based upon urea-N composition at High Bluff was probably due to the ammonium fractions of the UAN on an alkaline soil.

The lack of water addition to the chambers after rain events (to move some urea downward as it is in an open field condition) might have overestimated the ammonia volatilization from urea and UAN treated with urease inhibitors in this study. However, this overestimation due to lack of water addition might have been partly compensated for by underestimation of the losses due to chamber enclosure and reduced wind movement in the chamber (Harper 2005; Smith et al. 2007; Miola et al. 2015). The magnitude of ammonia volatilization in our greenhouse and field studies is however lower than the ammonia volatilization measured in studies (Frame et al. 2012; Soares et al. 2012) that allowed air exchange between the chambers and the environment. The lower magnitude of ammonia volatilization in our studies agreed with the result of Smith et al. (2007) that found static chamber methods to underestimate ammonia volatilization by 95 to 99% after 6 d of manure application when compared to dynamic methods. However, the underestimation by the static chamber method was reported to decrease with an increase in the duration of measurement (Miola et al. 2015). Miola et al. (2015) reported that the underestimation by a static chamber method decreased from 74% by 1 d to 23% by 22 d.

The lack of significant difference in total ammonia volatilization between  $UR_{ARM}$  (360 mg NBPT kg<sup>-1</sup> urea) and  $UR_{ARM2}$  (540 mg NBPT kg<sup>-1</sup> urea) and among  $UR_{ARM}$ ,  $UR_{AG}$  (600 mg NBPT kg<sup>-1</sup> urea), and  $UR_{AB}$  (480 mg NBPT kg<sup>-1</sup> urea) despite different concentrations of NBPT kg<sup>-1</sup> urea in the greenhouse showed that NBPT effectively reduced ammonia volatilization from urea even at a lower concentration. The lack of differences in ammonia volatilization among the different concentrations of NBPT in our study is in agreement with Watson et al. (2008) that compared ammonia volatilization from different concentrations of NBPT and found no significant benefit at concentrations greater than 250 mg NBPT kg<sup>-1</sup> urea. Also, Frame et al. (2012) that compared

ammonia volatilization from different rates of NBPT (200, 400, 600, and 800 mg NBPT kg<sup>-1</sup> urea) found no significant benefit of additional NBPT above 200 mg NBPT kg<sup>-1</sup> urea.

# **2.6 Conclusion**

Coating urea or mixing UAN fertilizers with urease inhibitors will not only delay ammonia volatilization from urea-based fertilizers, it will also significantly reduce ammonia volatilization. The most effective period for NBPT was during the 0 - 7 d when ammonia volatilization from urea and UAN were the greatest. Ammonia volatilization was reduced by 69 to 96% when urea was coated with ARM U and by 42 to 49% when UAN was mixed with ARM U during 28 d periods. The greenhouse and field studies showed that a lower rate of NBPT with ARM U (360 mg NBPT kg<sup>-1</sup> urea) was as effective as a higher rate of NBPT with Agrotain Advanced (600 mg NBPT kg<sup>-1</sup> urea). The new NBPT formulation, ARM U, has the same efficacy as other available products. Our study showed that optimum ammonium reduction can be achieved with a low concentration of NBPT even though almost all NBPT formulations in the market including the ones used in this study recommended above 360 mg NBPT kg<sup>-1</sup> urea. In conclusion, addition of ARM U to urea and UAN enabled a lower application rate of NBPT without compromising its efficacy.

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#### **CHAPTER 3**

# 3 STABILITY OF STORED N-(n-BUTYL) THIOPHOSPHORIC TRIAMIDE (NBPT) TREATED UREA-BASED FERTILIZERS

#### **3.1 Abstract**

The effectiveness of N-(n-butyl) thiophosphoric triamide (NBPT) in reducing ammonia volatilization from urea-based fertilizers has been thoroughly investigated. However, the stability of this inhibitor during the storage of NBPT treated urea and urea ammonium nitrate (UAN) needs further investigation. We compared ammonia volatilization from NBPT treated urea (360 mg NBPT kg<sup>-1</sup> urea) and UAN (180 mg NBPT L<sup>-1</sup> UAN) that were stored at room temperature for six, three and zero months. We measured ammonia volatilization with cylindrical chambers fitted with acid-charged discs at five times for 21 d. Total ammonia volatilization (% of applied total nitrogen) was significantly greater in untreated urea and UAN (32 to 33%) than those in NBPT treated urea and UAN (6 to 12%). Reduction of ammonia volatilization was not significantly different among NBPT treated urea (73 to 81%) and UAN (63 to 73%) irrespective of storage time. This implies that farmers can mix their urea-based fertilizers with NBPT formulation six months prior to fertilization without compromising the ammonia volatilization reducing property of the NBPT.

# **3.2 Introduction**

Ammonia volatilization from surface-applied urea-based fertilizers is a major contributor to atmospheric ammonia contamination (Environment and Climate Change Canada 2017). When urea-based fertilizers are applied to the soil, the urease enzyme hydrolyzes the urea into ammonia and carbonate ion with an increase in soil pH around the urea granule (Bremner 1995). This

increase in pH promotes ammonia volatilization from urea-based fertilizers by shifting the equilibrium between ammonium and ammonia towards ammonia. These losses are greatest when urea-based fertilizers are surface-applied to soils with neutral to alkaline pH and low diffusion capacity, under continuous wetting and drying conditions (Christianson et al. 1993; Chien et al. 2009).

Urease inhibitor containing N-(n-butyl) thiophosphoric triamide (NBPT) has been found to effectively reduce ammonia volatilization from urea-based fertilizers (Sanz-Cobena et al. 2008). The NBPT is commercially available to farmers under different formulation names such as Agrotain Advanced (Koch Agronomic Services LLC, KS), Arborite (Weyerhaeuser Co., WA), and ARM U (Active AgriScience Inc., BC). Studies have shown that coating or mixing urea and urea ammonium nitrate (UAN) with NBPT can reduce ammonia volatilization from urea and UAN by over 50% and it is most effective during the first week of fertilization (Engel et al. 2011; Frame 2017).

The spring season is a busy period for farmers as a result of pre-planting operations that have to be carried out. Farmers may prefer to buy and mix their urea and urea ammonium nitrate (UAN) with NBPT formulation at any time during the off growing season and store until application time. The stability of the NBPT with time following its mixing with urea and UAN is important to maximize the benefits of urease inhibitors by farmers. Most studies (Sanz-Cobena et al. 2008; Engel et al. 2011; Frame 2017) have focussed on the inhibitory effect of urease inhibitors that are mixed with urea-based fertilizers at the time of fertilizer application. Watson et al. (2008) studied the stability of NBPT treated urea and UAN that were stored for nine months under various storage

temperatures (4, 15, and 25 °C). The study reported that the stability of NBPT was correlated to storage temperature as the NBPT recovery was lowest at 25 °C. However, the study of Watson et al. (2008) did not determine the efficacy of stored NBPT treated urea and UAN in reducing ammonia volatilization. A study by Cantarella et al. (2016) compared ammonia volatilization from urea that was freshly treated with NBPT to urea that was treated and stored for various periods of time. The study found that ammonia volatilization in freshly treated urea was not significantly different from NBPT treated urea that was stored for up to six months. However, the study of Cantarella et al. (2016) was limited to granular urea fertilizers, and the effect of storage on other urea-based fertilizers such as UAN was not documented.

A recent review by Cantarella et al. (2018) suggested that the longevity and stability of NBPT formulations applied to urea depend on the additives including solvent used by different manufactures. ARM U (18% NBPT, m/v) is a relatively new NBPT formulation with a claim by its manufacturer that it contains a polymer (proprietary information) that allows for a low application rate of NBPT without compromising its efficacy. Greenhouse and field studies to evaluate the efficacy of ARM U (Chapter 2) showed that a lower NBPT rate with ARM U (360 mg NBPT kg<sup>-1</sup> urea) was as effective as a higher NBPT rate with ARM U (540 mg NBPT kg<sup>-1</sup> urea), Arborite (480 mg NBPT kg<sup>-1</sup> urea), and Agrotain Advanced (600 mg NBPT kg<sup>-1</sup> urea). However, there is a dearth of information on the effect of storage time of NBPT treated urea and UAN on the efficacy of new NBPT formulations such as ARM U. Therefore, our study was conducted to determine the effect of storage time of urea and UAN treated with a new formulation of NBPT, ARM U, on the effectiveness of NBPT in reducing ammonia volatilization.

#### **3.3 Materials and Methods**

#### **3.3.1 Soil Description and Experimental Design**

This study was conducted in a greenhouse facility at the University of Manitoba, Manitoba, Canada. The soil (0 - 15 cm) used for this study was a High Bluff series classified as Gleyed Cumulic Regosol (Michalyna and Smith 1972). The soil texture (Gee and Bauder 1986) is a loam (sand is 450 g kg<sup>-1</sup>, silt is 310 g kg<sup>-1</sup>, and clay is 240 g kg<sup>-1</sup>) with organic matter (Walkley and Black 1934) of 47 g kg<sup>-1</sup>, inorganic nitrogen (N) of 7.4 mg kg<sup>-1</sup> (Maynard et al. 2008), volumetric water content at field capacity (Cassel and Nielsen 1986) of 0.37 m<sup>3</sup> m<sup>-3</sup>, and soil pH<sub>water</sub> (1:1) of 7.7.

The experimental set up was a randomized complete block design with four replications. Two sources of N, urea (46-0-0) and UAN (28-0-0), were used for this study. There were nine fertilizer treatments which were urea treated with NBPT at six ( $UR_{NBPT(6)}$ ), three ( $UR_{NBPT(3)}$ ) and zero ( $UR_{NBPT(0)}$ ) months before the start of the study; UAN treated with NBPT at six ( $UAN_{NBPT(6)}$ ), three ( $UAN_{NBPT(3)}$ ) and zero ( $UAN_{NBPT(0)}$ ) months before the start of the study; UAN treated with NBPT at six ( $UAN_{NBPT(6)}$ ), three ( $UAN_{NBPT(3)}$ ) and zero ( $UAN_{NBPT(0)}$ ) months before the start of the study; untreated urea, untreated UAN and a control (bare soil) with neither urea nor UAN (Table 3.1). The NBPT formulation used was ARM U (18% NBPT m/v). Urea was coated with ARM U at 2 L per 1000 kg urea and ARM U was mixed with UAN at 1 L per 1000 L UAN. The coating of ARM U with urea granule gave a concentration of 360 mg NBPT kg<sup>-1</sup> urea while the mixing of ARM U with UAN gave a concentration of 180 mg NBPT L<sup>-1</sup> UAN which is an equivalent of 360 mg NBPT kg<sup>-1</sup> urea (based on urea fraction of UAN). The NBPT treated urea and UAN were stored in a

sealed plastic container at room temperature until the start of the experiment. The  $UR_{NBPT(0)}$  and  $UAN_{NBPT(0)}$  were prepared a day before the start of the study.

Treatment <sup>a</sup>	Concentration of NBPT <sup>b</sup>	
	(mg kg <sup>-1</sup> urea or mg L <sup>-1</sup> UAN)	
Untreated urea		
UR <sub>NBPT(6)</sub>	360	
UR <sub>NBPT(3)</sub>	360	
UR <sub>NBPT(0)</sub>	360	
Untreated UAN		
UAN <sub>NBPT(6)</sub>	180	
UAN <sub>NBPT(3)</sub>	180	
UAN <sub>NBPT(0)</sub>	180	

Table 3.1 Types of treatments and concentration of inhibitor.

<sup>*a*</sup> UR<sub>NBPT(6)</sub>, UR<sub>NBPT(3)</sub>, and UR<sub>NBPT(0)</sub> are stored NBPT treated urea for 6, 3, and 0 months, respectively; UAN<sub>NBPT(6)</sub>, UAN<sub>NBPT(3)</sub>, and UAN<sub>NBPT(0)</sub> are stored NBPT treated UAN for 6, 3, and 0 months, respectively; NBPT is N-(n-butyl) thiophosphoric triamide.

<sup>*b*</sup> NBPT, N-(n-butyl) thiophosphoric triamide (urease inhibitor); mg kg<sup>-1</sup> urea is for the urea granule treatments while mg  $L^{-1}$  UAN is for the UAN solution treatments.

# 3.3.2 Chamber Installation and Ammonia Measurement

Ammonia volatilization was measured with cylindrical chambers (20 cm in height by 15 cm i.d) as described in chapter 2 (Grant et al. 1996). Briefly, the bottoms of the chambers were sealed with flat plastic plates followed by filling the chambers with soil up to 5 cm at a bulk density of 1.1 Mg m<sup>-3</sup>. Water was added to the soil in the chambers to bring the water content to 75% field capacity. The chambers were covered with plates for twenty-four hours for the water to equilibrate after which treatments (100 kg N ha<sup>-1</sup>) were applied to the centre of the chambers. This was immediately followed by tightly fitting the chambers with acid-charged discs (mass of 24.5 g, thickness of 2.5

cm, and diameter of 16 cm) at 5 cm above the soil surface (lower disc) and 2 cm from the top of the chamber (upper disc). The discs were charged by thoroughly rinsing and wringing in 0.001 M sulphuric acid and then glycerol-phosphoric acid solution (40 mL 14.7 M phosphoric acid and 50 mL glycerol in 1 L distilled water). The lower discs trapped volatilized ammonia from the soil surface and the upper disc prevented atmospheric contamination of the lower disc.

The acid-charged discs were sampled and replaced on 2, 4, 7, 14, and 21 days after fertilization (d) to measure the volatilized ammonia. Each lower disc was placed in a pre-labelled zip-lock bag and transferred to the laboratory for extraction on the same day. After sampling, the chambers were weighed to determine moisture loss on 4, 7, and 14 d and the weight difference was made up by adding the required amount of water. Daily mean air and soil temperature were monitored with Watchdog 2000 series weather station (model 146 2900ET; Spectrum Technologies Inc., IL).

In the laboratory, the amount of acid solution in the lower disc was determined by weighing the disc before it was extracted for ammonia. Ammonia trapped in the disc was extracted by dispensing 250 mL of 0.5 M potassium chloride (KCl) on the disc in the zip-lock bag. This disc in zip-lock bag was squeezed thoroughly and the aliquot was decanted into a vial. The aliquot of the resulting solution was stored in the refrigerator (4 °C) and analyzed within two days of extraction for ammonium-N concentration using AQ2 Discrete Analyzer (SEAL Analytical Inc. WI, USA).

Ammonia volatilization (kg N ha<sup>-1</sup>) was calculated as follows:

$$NH_{3} loss = \frac{(Extractant (mL) + absorbent in disc (mL)) \times NH_{3}(mg N mL^{-1})}{Area of chamber (ha) \times 10^{6}}$$
(3.1)
Cumulative ammonia volatilization was calculated by summing ammonia volatilization measured between sampling days. Total ammonia volatilization (measured as a % of applied total N) was calculated by summing ammonia volatilization measured between sampling days for the 21 d after deducting ammonia volatilization from bare soil.

At the end of the study (21 d), soil in each chamber was thoroughly mixed. Six grams of each soil was sampled and extracted with 25 mL of 2 M KCl to determine ammonium and nitrate concentrations (Maynard et al. 2008). Also, soil samples were collected from each chamber to determine the gravimetric moisture content.

# **3.3.3 Statistical Analysis**

GLIMMIX procedure for repeated measure analysis in SAS 9.4 (SAS Institute Inc. 2014) was used to determine the effect of treatments and day on cumulative ammonia volatilization. In the GLIMMIX model, treatments and time were fixed effects and block (replicate) is a random effect. GLIMMIX procedure was also used to determine the effect of treatments on ammonium and nitrate concentrations remaining in the soil (after deducting inorganic N from the bare soil) at the end of the study. Prior to using GLIMMIX procedure, data were checked for the assumption of normal distribution (Shapiro-wilk test). All data except nitrate concentrations were lognormally distributed and a lognormal distribution was specified in their models. The effect of treatment on total ammonia volatilization and storage time of NBPT treated urea and UAN on percentage reduction of ammonia volatilization were tested with GLIMMIX procedure (beta distribution). Treatment means comparison was performed with Fisher's protected least significant difference (LSD) at a probability level (P) < 0.05.

# **3.4 Results and Discussion**

Daily mean air temperature ranged from 13.4 to 24.7 °C (Figure 3.1). Similarly, daily mean soil temperature ranged from 14.4 to 24.5 °C (Figure 3.1). Except on 12 d, the daily mean air and soil temperatures were above 17 °C.



Figure 3.1. Daily mean air and soil (at 2 cm) temperatures during the study

# 3.4.1 Ammonia Volatilization

There was a significant treatment by time interaction on cumulative ammonia volatilization (P = 0.0017). Cumulative ammonia volatilization was significantly greater in untreated urea and UAN than NBPT treated urea and UAN throughout the sampling period (Figure 3.2). Cumulative

ammonia volatilization was not significantly different between the untreated urea and UAN throughout the study. When the ammonia volatilization was compared based on the source of N, cumulative ammonia volatilization among NBPT treated urea was significantly different from 2 to 7 d but not after 7 d (Figure 3.2). Cumulative ammonia volatilization from UR<sub>NBPT(6)</sub> was significantly greater than cumulative ammonia volatilization in UR<sub>NBPT(0)</sub> from 2 to 7 d but not greater than cumulative ammonia volatilization from UR<sub>NBPT(3)</sub>. After 7 d, cumulative ammonia volatilization among the NBPT treated urea was not significantly different. In the case of UAN source, cumulative ammonia volatilization among the NBPT treated urea was not significantly different. In the case of UAN source, cumulative ammonia volatilization among the NBPT treated UAN was not significantly different throughout the sampling period.



Figure 3.2. Cumulative ammonia volatilization following surface application of (a) urea and (b) UAN treated with and without NBPT. Error bars are standard errors of the mean. Note: UR<sub>NBPT(6)</sub>, UR<sub>NBPT(3)</sub>, and UR<sub>NBPT(0)</sub> are stored NBPT treated urea for 6, 3 and 0 months, respectively; UAN<sub>NBPT(6)</sub>, UAN<sub>NBPT(3)</sub>, and UAN<sub>NBPT(0)</sub> are stored NBPT treated UAN for 6, 3, and 0 months, respectively; UR and UAN are untreated urea and UAN respectively; NBPT is N-(n-butyl) thiophosphoric triamide.

Greatest ammonia volatilization from untreated urea (16.9 kg N ha<sup>-1</sup>) was measured on 4 d which resulted in cumulative ammonia volatilization of 20.4% of applied N from 0 to 4 d (Figure 3.2). In contrast, the greatest amount of ammonia volatilization in NBPT treated urea (4.5 to 6.7 kg N ha<sup>-1</sup>) was measured on 14 d to give cumulative ammonia volatilization of 5.5 to 8.5% of applied N from 0 to 14 d (Figure 3.2). With the UAN treatments, the greatest ammonia volatilization from untreated UAN (10.1 kg N ha<sup>-1</sup>) was measured on 14 d to give cumulative ammonia volatilization of 31.3% of applied N from the day the treatment was applied. In the case of NBPT treated UAN, greatest amount of ammonia volatilization also measured on 14 d (2.8 to 3.6 kg N ha<sup>-1</sup>) to give cumulative ammonia volatilization of 8.0 to 10.5% of applied N from 0 to 14 d (Figure 3.2). The time of greatest ammonia volatilization among the treatments was similar to other studies (Engel et al. 2011; Viero et al. 2014) that showed ammonia volatilization peaked within the first week of fertilization in untreated urea and after the first week of fertilization in NBPT treated urea and UAN.

There was a significant effect (P < 0.0001) of treatment on total ammonia volatilization (Table 3.2). Untreated urea and UAN lost significantly greater amounts of ammonia than NBPT treated urea and UAN at the end of the study (Table 3.2). Total ammonia volatilization was not significantly different among NBPT treated urea and UAN irrespective of the time of coating or mixing with NBPT (Table 3.2). Total volatilized ammonia in NBPT treated urea ranged from 6.3 to 8.9%, while it ranged from 8.5 to 11.8% in NPBT treated UAN. Conversely, total ammonia volatilization was 33.0% in untreated urea and 31.7% in untreated urea occurred during the second and third week period (Figure 3.2). Unlike the NBPT treated urea, 87% of total ammonia

volatilization in untreated urea occurred within 1 week after fertilization. This agrees with other studies that showed that most of the ammonia volatilization in untreated urea occurred during the first week of fertilization whereas it occurred after the first week of fertilization in NBPT treated urea (Goos 2013; Tian et al. 2015). In addition, the quantity of total ammonia volatilization from urea treated with and without NBPT in our study was similar to the total ammonia volatilization measured by Frame (2017).

Table 3.2 Total ammonia volatilization, percentage reduction of ammonia volatilization, soil ammonium, and nitrate concentrations from surface-applied urea and UAN treated with and without NBPT.

Treatment <sup>a</sup>	Total ammonia	Reduction	Soil ammonium	Soil nitrate
	volatilization			
	(%)		(mg N kg <sup>-1</sup> )	
Untreated urea	33.0a		2.5a	117.1b
UR <sub>NBPT(6)</sub>	8.9bc	73ab	4.5a	145.2ab
UR <sub>NBPT(3)</sub>	6.3c	81a	8.3a	117.1b
UR <sub>NBPT(0)</sub>	8.6bc	74ab	2.6a	168.5a
Untreated UAN	31.7a		3.5a	154.9ab
UAN <sub>NBPT(6)</sub>	8.5bc	73ab	9.5a	178.9a
UAN <sub>NBPT(3)</sub>	9.3bc	71ab	12.0a	167.4a
UAN <sub>NBPT(0)</sub>	11.8b	63b	8.3a	171.3a

<sup>*a*</sup> UR<sub>NBPT(6)</sub>, UR<sub>NBPT(3)</sub>, and UR<sub>NBPT(0)</sub> are stored NBPT treated urea for 6, 3, and 0 months, respectively; UAN<sub>NBPT(6)</sub>, UAN<sub>NBPT(3)</sub>, and UAN<sub>NBPT(0)</sub> are stored NBPT treated UAN for 6, 3, and 0 months, respectively; NBPT is N-(n-butyl) thiophosphoric triamide. Means with different letters within a column are significantly different at a probability level < 0.05 LSD mean separation.

Similar to total ammonia volatilization, there was no significant effect of storage time among NBPT treated urea as well as among NBPT treated UAN on the effectiveness of NBPT in reducing

ammonia volatilization (Table 3.2). Treating urea and UAN with NBPT reduced ammonia volatilization by 73 to 81% in urea and by 63 to73% in UAN at the end of 21 d, regardless of when the coating or mixing took place. The amount of ammonia volatilization reduction from urea and UAN due to NBPT from this study were similar to previous studies that reported over 50% less ammonia volatilization from urea and UAN with NBPT (Turner et al. 2010; Engel et al. 2011). The lack of significant differences in the percentage reduction of ammonia volatilization from stored NBPT treated urea and UAN showed that NBPT retained its effectiveness in reducing ammonia volatilization irrespective of the time of storage for up to six months after treatment. Our result from stored NBPT treated urea in this study was similar to that of Cantarella et al. (2016) that found no significant difference in ammonia volatilization between fresh NBPT treated urea and stored NBPT treated urea up to six months. Although Watson et al. (2008) reported the half-life of NBPT in NBPT treated urea to be 74 and 188 d at 25 and 15 °C, respectively, this reported degradation did not have any significant effect on the effectiveness of stored NBPT in reducing ammonia volatilization in our study.

#### 3.4.2 Soil Ammonium and Nitrate Concentrations

There was no significant effect (P = 0.7471) of treatments on ammonium concentrations (Table 3.2). Despite UAN<sub>NBPT(3)</sub> having the highest ammonium concentration (12.0 mg N kg<sup>-1</sup>), its ammonium concentration was not significantly different from those in untreated urea and UR<sub>NBPT(0)</sub> (2.6 mg N kg<sup>-1</sup>). In contrast, there was a significant treatment effect (P = 0.0243) on the concentration of nitrate. Among the urea treatments, UR<sub>NBPT(0)</sub> had the highest nitrate concentration (168.5 mg N kg<sup>-1</sup>) while untreated urea and UR<sub>NBPT(3)</sub> had the smallest nitrate

concentrations (117.1 mg N kg<sup>-1</sup>). Nitrate concentrations were not significantly different among the UAN treatments.

The expectation was for soil available N concentration to increase as percentage reduction of ammonia volatilization increases or as total ammonia volatilization decreases. However, the soil available N of some treatments did not reflect these expected concentrations on a statistical basis (Table 3.2). The lack of significant difference in available N is possibly due to variability in the ammonium concentrations (coefficient of variation ranged from 73 to 144%, LSD = 11.1). The inconsistency between the total ammonia volatilization and soil available N concentrations is not unique to this study. Previous studies that measured ammonia volatilization and soil available N concentrations from urea treated with and without NBPT and nitrification inhibitor found recovered soil available N concentrations to be higher in some plots with higher ammonia volatilization than where ammonia volatilization was lower (Soares et al. 2012; Frame 2017). It is possible that the measurement of other pathways of N disappearance from the available pool such as denitrification and immobilization may account for this inconsistency. Potential N losses due to immobilization (if any) will later be mineralized to become available N. Dinitrogen gas emission during denitrification may result in significant amount of N losses. In contrast, N losses in form of nitrous oxide emission during nitrification and denitrification neither present any agronomic benefit to the farmers nor represent a significant portion of applied N asnitrous oxide emission is an average of 1% of applied N according to the Intergovernmental Panel on Climate Change (2006). However, nitrous oxide emission has a serious negative environmental consequence (Harty et al. 2016).

### **3.5 Conclusion**

The new NBPT formulation, ARM U, significantly reduced ammonia volatilization from urea and UAN. Reduction of ammonia volatilization among NBPT treated urea (360 mg NBPT kg<sup>-1</sup> urea) and UAN (180 mg NBPT L<sup>-1</sup> UAN) were not significantly different for treatments that were stored at room temperature for periods that ranged from 0 to 6 months. This study shows that farmers can treat their urea-based fertilizers with NBPT formulation at least six months prior to application and store at room temperature without compromising the effectiveness of NBPT. On the other hand, producers of NBPT formulations can coat urea fertilizers and sell these to farmers without compromising its efficacy.

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#### **CHAPTER 4**

# 4 EFFICIENCY OF FALL VERSUS SPRING APPLIED UREA-BASED FERTILIZERS TREATED WITH UREASE AND NITRIFICATION INHIBITORS I. AMMONIA VOLATILIZATION AND MITIGATION BY NBPT

# 4.1 Abstract

Mid- or late-fall nitrogen (N) fertilization is usually recommended on the Canadian prairies to reduce N losses from fall-applied N. Yet, N fertilizers are less efficient when applied in mid- or late-fall than in spring on crop yield. Ammonia volatilization from N fertilizers is one of the reasons for the low N use efficiency. We conducted a two-year study to quantify and contrast the efficacy of a urease inhibitor, N-(n-butyl) thiophosphoric triamide (NBPT), with and without a nitrification inhibitor, 3,4- dimethylpyrazole phosphate (NI), in reducing ammonia volatilization from fall and spring-applied urea-based fertilizers in two contrasting soils (Carman sandy loam and Portage clay loam). Treatments consisted of surface-applied urea and UAN with and without NBPT or NBPT+NI (double inhibitor, DI). We measured ammonia volatilization with acidcharged discs during 21 (fall) or 28 (spring) d. Total ammonia volatilization (% of applied N) from untreated urea and UAN across sites ranged from 7 to 22% with fall application and from 3 to 29% with spring application. Addition of NBPT only or DI to urea or UAN reduced ammonia volatilization by 61 to 74% in the fall and 4 to 69% in the spring across site-year. We found that the reduction of ammonia volatilization by NBPT was significantly greater in the fall (65%) than in the spring (40%) and with urea (61%) than with UAN (43%). Our study showed that (i) significant ammonia volatilization could occur from surface-applied urea-based fertilizers even under late-fall temperature (ii) NBPT reduced ammonia volatilization from fall- as well as springapplied urea-based fertilizers. Therefore, farmers are encouraged to treat their urea-based fertilizers with NBPT when considering surface application.

# **4.1 Introduction**

Urea-based fertilizers contribute to more than one-half of the global agricultural N input and their demand is expected to rise by 17 Mt from 2016 to 2021 (IFA 2017). Urea-based fertilizers are commonly sold as granular urea (46% N) and urea ammonium nitrate solution (UAN, 28 to 32% N). The continuous rise in urea-based fertilizer usage can be attributed to their relative ease of storage, handling, application, and sometimes low cost per unit N (Li et al. 2015). Despite these benefits of urea-based fertilizers, the risk of N losses through ammonia volatilization in addition to other losses such as nitrate leaching, nitrous oxide emission, dinitrogen gas, and immobilization that are common to other N fertilizers is a major concern (Woodley et al. 2018). Ammonia volatilization from urea-based fertilizers occurs during hydrolysis of urea into carbonate and ammonium ions with an increase in soil pH around the urea granule (Singh and Nye 1984; Chien et al. 2009). The resulting localized increase in soil pH is usually higher in soil with low pH and lower buffering capacity than in soil with high pH and higher buffering capacity (Rochette et al. 2009; Jones et al. 2013). The increase in pH shifts the equilibrium between ammonium and ammonia towards ammonia, leading to emission of ammonia to the environment. The magnitude of ammonia volatilization may be greater than 15% of applied N especially when urea-based fertilizers are broadcasted without incorporation (Grant et al. 1996; Rawluk et al. 2001). Ammonia volatilization reduces the agronomic efficiency of urea-based fertilizers and could lead to soil acidification when volatilized ammonia is returned to the ground (Sheppard et al. 2010; Sigurdarson et al. 2018).

To reduce ammonia volatilization and maximize agronomic efficiency of surface-applied ureabased fertilizers, farmers may treat their urea-based fertilizers with urease inhibitors. The most widely tested and used urease inhibitor is N-(n-butyl) thiophosphoric triamide (NBPT) (Cantarella et al. 2018; Modolo et al. 2018). This inhibitor has been reported to consistently reduce ammonia volatilization from urea-based fertilizers by slowing down the hydrolysis of urea (Watson et al. 2008; Sigurdarson et al. 2018). The NBPT is found to be most effective during the first week of fertilization when ammonia volatilization is at maximum (Soares et al. 2012; Keshavarz et al. 2018). The NBPT is sold under different trade names with different concentrations such as ARM U (18% NBPT m/v; Active AgriScience Inc., BC), Agrotain Advanced (30% NBPT m/v; Koch Agronomic Services LLC, Wichita, KS), Factor (24.5% NBPT m/v; Rosen's Inc., Liberty, MO), and Arborite (24% NBPT m/v; Weyerhaeuser Co., Olympia, WA).

In addition to the use of NBPT to reduce ammonia volatilization, farmers may also use nitrification inhibitors (NI) to mitigate nitrate leaching, dinitrogen gas emission, and nitrous oxide emission. Common NI that have been widely used in agricultural and horticultural systems include 2-Chloro-6-(trichloromethyl) pyridine commonly known as nitrapyrin, 3,4- dimethyl pyrazole phosphate (DMPP), dicyandiamide (DCD) and 2-amino-4-chloro-6-methyl pyrimidine (Subbarao et al. 2006). Studies have reported DMPP to be a better NI than nitrapyrin and DCD due to its effectiveness at a low rate, inhibitory action over a longer period, and relative immobility (Wissemeier et al. 2001; Zerulla et al. 2001; Subbarao et al. 2006). Nitrification inhibitors may be

sold in a combined formulation with NBPT. Common combined NBPT and NI (also known as a double inhibitor, DI) formulations include Agrotain plus (6.5% NBPT and 81.2% DCD m/v; Koch Agronomic Services LLC, Wichita, KS), and ARM U Advanced (24% NBPT and 6% DMPP m/v; Active AgriScience Inc., BC).

The time of fertilizer application is critical in maximizing the efficiency of urea-based fertilizers. Studies have shown that fall N application is less efficient than the spring N application due to the time interval between fertilizer application and crop uptake in the Canadian prairies (Nyborg and Malhi 1986; Tiessen et al. 2005). Nevertheless, a survey among Manitoba agronomists showed a preference for fall N application because of lower cost and easier time management (Amiro et al. 2017). As such, farmers on the Canadian prairies who prefer to apply fertilizers in the fall are advised to wait until mid (early October) or late (from mid-October) fall when soil temperature at 10 cm is below 5 °C (Tiessen et al. 2005; MAFRI 2007; Tenuta et al. 2016). According to The Manitoba Water Protection Act (2008), late-fall fertilizer application can be done until November 10 except otherwise extended. Delaying fall applications is based on the premise that low temperature in the late-fall will reduce the transformation of applied N fertilizers thereby reducing the risk of ammonia volatilization, dinitrogen gas emission, nitrous oxide emission, and nitrate leaching. A study by Tiessen et al. (2006) showed that delaying the application of urea in the fall, reduced nitrate accumulation and improved recovery of N as ammonium in the order of late-fall > mid-fall > early fall application. However, a study by Engel et al. (2011) in the United States showed that a significant amount of ammonia volatilization from urea may still occur even at low soil temperatures (< 5 °C). Delaying fertilizer application until the soil temperature is below 5 °C may be complicated by snow events in the Canadian prairie region. Despite the recommendation given to farmers to apply N fertilizers when the soil temperature is below 5 °C on the Canadian prairies, there is a dearth of information on the magnitude of ammonia volatilization from fall versus spring-applied urea-based fertilizers. Therefore, the objective of the study was to quantify and contrast the efficacies of NBPT with and without NI in reducing ammonia volatilization from fall and spring-applied urea-based fertilizers in two contrasting soils.

#### 4.3 Materials and Methods

# **4.3.1 Sites Characteristics**

We conducted field trials in the fall and spring of 2016/2017 (first year) and 2017/2018 (second year) growing seasons at two locations of contrasting soils. The locations were (i) University of Manitoba-Ian Morrison Research station, Carman, Manitoba (49° 29′ 04″ N, 98° 02′ 01″ W) (ii) Canada-Manitoba Crop Diversification Centre, Portage la Prairie, Manitoba (49° 57′ 9″ N, 98° 16′ 0″ W). The soil at Carman is mapped as an Orthic Black Chernozem while the soil at Portage la Prairie (Portage) is mapped as a Gleyed Rego Black Chernozem in the Canadian soil classification system (Michalyna and Smith 1972; Mills and Haluschak 1993). The Black Chernozem is equivalent to Udic Boroll subgroup in the USDA classification system (Agriculture and Agri-Food Canada 1998). Prior to study establishment in the fall of 2016, we collected soils from the two locations (Carman and Portage) from 0 - 15 cm. The soils were air-dried and sieved (< 2mm) for soil characterization. The air-dried soils were characterized for soil texture (Gee and Bauder 1986), organic matter (Walkley and Black 1934), pH (soil/water, 1:1), electrical conductivity, cation exchange capacity (Hendershot et al. 2008), urease activity (Tabatabai and Bremner 1972), available N (Maynard et al. 2008), and Olsen P (Olsen et al. 1954) (Table 4.1).

Property	Carman	Portage
Soil series	Hibsin	Neurhorst
Soil pH <sub>water</sub>	6.78	7.96
Electrical conductivity ( $\mu$ S cm <sup>-1</sup> )	387	596
Organic matter (g kg <sup>-1</sup> )	32	71
Field capacity (m <sup>3</sup> m <sup>-3</sup> )	0.35	0.44
CEC (cmol kg <sup>-1</sup> )	16	36
Urease activity (mg NH4 <sup>+</sup> -N kg <sup>-1</sup> soil hr <sup>-1</sup> )	24	88
Available N (mg kg <sup>-1</sup> )	14	11
Bulk density (Mg m <sup>-3</sup> )	1.3	1.1
Soil texture	Sandy loam	Clay loam
Sand (g kg <sup>-1</sup> )	799	269
Silt (g kg <sup>-1</sup> )	47	343
Clay $(g kg^{-1})$	154	388

Table 4.1 Physical and chemical properties of soil (0 - 15 cm) at Carman and Portage sites.

# **4.3.2 Experimental Design and Treatment Preparation**

In the fall of 2016 and 2017, the plots were tilled to kill weeds and bury residues at Carman while tillage was carried out at Portage only in the fall of 2017. Portage was not tilled in the fall of 2016 due to logistics (equipment, labour, and time for the start of the study). In the spring of 2017 and 2018, the plots were cultivated for seeding at both sites. The experimental design was a randomized complete block design with four replications. The plot layout was a season (fall versus spring) by fertilizer treatment factorial layout. Our fertilizer treatments consisted of urea treated with either ARM U or Agrotain Advanced at a recommended rate of 2 L per 1000 kg urea; urea treated with ARM U Advanced at the rate of 1.5 L per 1000 kg urea; UAN treated with either ARM U or ARM U advanced at the rate of 1.5 L per 1000 kg urea; UAN treated with either ARM U or ARM at the rate of 1 L per 1000 L UAN; untreated urea (UR); untreated UAN (UAN); and a control with neither urea nor UAN (Table 4.2).

Season	Treatment <sup>a</sup>	Concentrations of NBPT/DMPP in		First year	Second year
Season		urea an	d UAN <sup>b</sup>	First year	Second year
		NBPT	DMPP	N appli	cation rate
		mg kg <sup>-1</sup> urea or mg L <sup>-1</sup> UAN		kg ha <sup>-1</sup>	
Fall			-	_	
	Bare soil	0	0	0	0
	UR(100)	0	0	100	NA
	UR <sub>ARM(100)</sub>	360	0	100	NA
	UR <sub>AG(100)</sub>	600	0	100	NA
	UR <sub>DI(100)</sub>	360	90	100	NA
	UAN(100)	0	0	100	NA
	UANARM(100)	180	0	100	NA
	UAN <sub>DI(100)</sub>	240	60	100	NA
	UR <sub>(75)</sub>	0	0	NA	75
	UR <sub>ARM(75)</sub>	360	0	NA	75
	UR <sub>AG(75)</sub>	600	0	NA	75
	$UR_{DI(75)}$	360	90	NA	75
	UAN(75)	0	0	NA	75
	UAN <sub>ARM(75)</sub>	180	0	NA	75
	UAN <sub>DI(75)</sub>	240	60	NA	75
Spring					
	Bare soil	NA	NA	NA	0
	UR <sub>(75)</sub>	0	0	75	75
	UR <sub>ARM(75)</sub>	360	0	75	75
	$UR_{(100)}$	0	0	100	NA
	UR <sub>ARM(100)</sub>	360	0	100	NA
	UR <sub>AG(75)</sub>	NA	NA	NA	75
	UR <sub>DI(75)</sub>	360	90	75	75
	UAN(75)	0	0	75	75
	UAN <sub>ARM(75)</sub>	180	0	75	75
	UAN <sub>DI(75)</sub>	240	60	75	75

Table 4.2 Types and rates of treatment applied during the first (2016/2017 growing season) and second (2017/2018 growing season) years of study.

<sup>*a*</sup> UR, untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>DI</sub> are urea treated with ARM U, Agrotain Advanced, and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript; UAN, untreated urea ammonium nitrate; UAN<sub>ARM</sub> and UAN<sub>DI</sub> are UAN treated with ARM U and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript. ARM U and Agrotain Advanced are NBPT formulations while ARM U Advanced is a double inhibitor formulation (combined NBPT and DMPP). NA, treatment is not applicable.

<sup>*b*</sup> NBPT, N-(n-butyl) thiophosphoric triamide (urease inhibitor); DMPP, 3,4- dimethylpyrazole phosphate (nitrification inhibitor); mg kg<sup>-1</sup> urea is for the urea granule treatments while mg  $L^{-1}$  UAN is for the UAN solution treatments.

# **4.3.3 Treatment Application**

In the first year of study, the N fertilizers were fall-applied in 2016 at a rate of 100 kg N ha<sup>-1</sup>. However, we changed the N rate to 75 kg N ha<sup>-1</sup> during spring application in 2017 based on the results of a greenhouse study conducted, which showed 75 kg N ha<sup>-1</sup> as the optimum rate for crop response when urea was coated with NBPT (results not shown). In order to make comparisons between fall and spring-applied untreated urea and urea treated with ARM U, we included additional treatments, untreated urea and urea treated with ARM U applied at 100 kg N ha<sup>-1</sup>, in the spring of 2017, instead of having urea treated with Agrotain Advanced and bare soil treatments. In the second year of the study (fall of 2017 and spring of 2018), all the seven N amended treatments listed earlier were applied at 75 kg N ha<sup>-1</sup> in fall and spring. Table 4.2 provides details of the types and rates of treatment application in the two years of the study.

# 4.3.4 Chamber Installation and Ammonia Measurement

We measured ammonia volatilization with acid-charged discs (2.5 cm thick, 16 cm diameter and 24.5 g in mass) fitted in cylindrical static chambers (i.d. of 15 cm and height of 20 cm) constructed with polyvinyl chloride (Grant et al. 1996). Chambers (Figure 4.1) were installed (inserted to a soil depth of 5 cm) at the northwest corner of the 5 m by 3 m plot that was seeded to wheat (*Triticum aestivum L.* 'Brandon') and canola (*Brassica napus L.* 'Invigor L233P'), in the spring of

the first and the second years, respectively. We installed the chambers to the fall plots after land preparation and the chambers to the spring plots after seeding in the spring. Crop residues and seedlings within the internal area of the chamber were removed to eliminate any effect of residues or growing crops on urea hydrolysis.



Figure 4.1 Schematic diagram of the ammonia volatilization chamber set up.

Fertilizer treatments were applied to the soil surface in the centre of each chamber. The quantity of fertilizer applied to the chambers was calculated based on the area of the chamber. The amount of urea granule needed for each chamber was weighed into a vial and then manually applied to the centre of the chamber. In the case of UAN amendments, the volume of the UAN needed was applied to the centre of the chamber with a pipette. Fall treatment application to the chambers occurred on 19 October 2016 (first year) and 16 October 2017 (second year). In the spring, treatment application to the chambers occurred on 23 May 2017 (first year) and 15 May 2018 (second year). In each season, one day after the chamber was fertilized, the entire plot to which a chamber was installed received the same treatment as the chamber (as a separate operation,

Chapter 5). The chambers were sheltered from direct rainfall and/or snow with puck board sheets (40 by 40 cm) positioned at about 30 cm above the ground and supported on rebars (driven into the soil) as shown in Figure 4.1.

Following treatment application to the chamber, we fitted two (lower and upper) acid-charged discs to the chambers to trap ammonia. We designed the discs to tightly fit into the chambers. These discs were charged in the laboratory by washing and wringing them twice in distilled water, followed by dipping and wringing the discs twice in 0.001 M H<sub>2</sub>SO<sub>4</sub>. Finally, the H<sub>2</sub>SO<sub>4</sub> acid-washed discs were rinsed and wrung twice in glycerol-phosphoric acid solution (500 mL glycerol and 400 mL 14.7 M H<sub>3</sub>PO<sub>4</sub> in 10 L of water). The charged discs were transported to the field in air-tight bags to be fitted to the chambers. The lower disc was placed at 5 cm above the soil surface to trap volatilized ammonia and the upper disc was located at 2 cm from the top of the chamber to prevent atmospheric contamination of the lower disc (Figure 4.1).

# 4.3.5 Sampling and Laboratory Procedures

We sampled and replaced the acid-charged discs on 2, 4, 7, 14, and 21 d after treatment application in the fall and 2, 4, 7, 14, 21, and 28 d in the spring. The lower discs were placed directly in prelabeled zip-lock bags for ammonia extraction and analysis in the laboratory. In the laboratory, each zip-lock bag containing a sampled disc was weighed (two decimal points) to determine the quantity of absorbing solution trapped in the disc. This was followed by dispensing 250 mL of 0.5 M KCl on the discs in the zip-lock bag. The zip-lock bags were sealed, squeezed several times and about 20 mL of the aliquot was decanted into scintillating vials to determine ammonium-N concentration on the AQ2 Discrete Analyzer (SEAL Analytical Inc., WI). The ammonium-N concentration of the aliquot was used to calculate ammonia volatilization (NH<sub>3</sub> loss; % of applied N) as follows:

$$NH_{3} loss = \frac{(Extractant (mL) + absorbent in disc (mL)) \times NH_{3}(mg N mL^{-1})}{Area of chamber (ha) \times 10^{6} \times applied N (kg ha^{-1})} \times 100$$
(4.1)

Total ammonia volatilization (% of applied N) in each trial was calculated as the sum of ammonia volatilization between sampling days during the trial. The efficacy of the NBPT which is the ammonia volatilization reduction ( $NH_{3R}$ ) by the NBPT was calculated as:

$$NH_{3R} = \frac{NH_{3u} - NH_{3i}}{NH_{3u}} \times 100$$
(4.2)

Where  $NH_{3u}$  is the % ammonia volatilization in untreated urea or UAN and  $NH_{3i}$  is the % ammonia volatilization in urea or UAN treated with inhibitor.

Environmental data (temperature and precipitation) were collected from Manitoba Agriculture weather stations located about 200 m from the study site at Carman and about 900 m from the study site at Portage.

#### **4.3.6 Statistical Analysis**

Statistical analysis was conducted with PROC GLIMMIX (SAS Institute, version 9.4). Except where otherwise stated, we separately analyzed by site and year. In agreement with the experimental design, analysis of variance (ANOVA) was performed in two stages (except in spring of the first year): (i) comparison between N amended treatments versus bare soil; (ii) comparison among N amended treatments. In the first year of the study, a one-way ANOVA was used to

determine the significant effect of fertilizer treatments on total ammonia volatilization for each season because of the differences in the rate of N and type of treatments in the fall and spring. In the second year where we had the same number and types of treatments in both seasons, we used a two-way ANOVA to determine the significant effects of fertilizer treatments, seasons and their interactions on total ammonia volatilization. Treatment and season were fixed effects while block (replicates) and its interaction with fixed effects were random effects. Also, a two-way ANOVA was used to analyze the percentage reduction in ammonia volatilization from urea and UAN by the inhibitors [NBPT (ARM U and Agrotain Advanced) and DI] at each site. Inhibitors and season were fixed effects while block and year were random effects. Fisher's protected least significant difference (LSD) procedure at a probability level of < 0.05 was used for treatment means comparison.

# 4.4 Results

# **4.4.1 Environmental Conditions**

The two sites had similar daily mean air and soil temperature during the fall and spring seasons (Figure 4.2). Daily mean air temperature during the two years of study ranged from -6.7 to 13.9 °C in the fall sampling period (October and November) and from 7.7 to 26.6 °C in the spring sampling period (May and June). Similarly, daily mean soil temperature (measured at 5 cm depth) ranged from 0.6 to 11.4 °C during the fall sampling period (October and November) and from 7.9 to 23.2 °C during the spring sampling period (May and June). The daily mean air temperature during sampling periods in the first year and during the spring sampling period in the second year



Figure 4.2 Daily mean air and soil temperature and precipitation during the first (A; top 4 graphs) and second (B; bottom 4 graphs) year of study. Arrows indicate dates of fertilizer application.

was greater than the 30-year daily mean air temperature at both sites for about two-thirds of the sampling periods. In the fall of the second year, daily mean air temperature during the first 10 d sampling period was greater than the 30-year daily mean air temperature at both sites whereas daily mean air temperature after the first 10 d was lower than the 30-year daily mean air temperature. According to Manitoba Agriculture weather data, Carman and Portage have climate normal precipitation of 91 and 83 mm, respectively, during this study sampling period in the fall and 33 mm each during this study sampling period in the spring. However, the total precipitation received at both sites during the sampling periods in the two years of study was lower than the climate normal precipitation. It should be noted that precipitation during the latter part of the fall sampling period was in the form of snow (Figure 4.2). Also, the use of puck board sheets as shelter prevented subsequent rain and snow from directly getting into the chambers but the lateral and upward movement of water prevented the soil from drying and ensured urea hydrolysis.

#### 4.4.2 First Year (2016/2017 Growing Season) Ammonia Volatilization

In the fall of the first year, ammonia volatilization measured from untreated urea was greater than any other treatment in the first 14 d at both sites (Figure 4.3). Ammonia volatilization from urea and UAN treated with inhibitors increased linearly until 21 d at both sites whereas losses from untreated urea and UAN followed a quadratic pattern with a peak on 7 and 14 d in Portage clay loam (PCL) and Carman sandy loam (CSL), respectively.

In the spring, the two N rates of untreated urea peaked on 4 d in CSL, but the peak was delayed until 14 d in PCL (Figure 4.3). Ammonia volatilization was negligible in urea treated with inhibitors until after 4 d at both sites. In CSL, most of the ammonia volatilization from urea and

UAN treated with inhibitors occurred between 7 and 14 d whereas, in PCL, a greater portion of the ammonia volatilization occurred over 7 to 21 d (Figure 4.3).



Figure 4.3 Ammonia volatilization between sampling days in the first year (2016/2017) in Carman sandy loam (A & B) and Portage clay loam (C & D). Error bars are standard errors of the means. Note that the left legend is for the fall-applied treatments (left graphs: A & C) while the right legend is for spring-applied treatments (right graphs: B & D). UR, untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>DI</sub> are urea treated with ARM U, Agrotain Advanced, and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript; UAN, untreated urea ammonium nitrate;

UAN<sub>ARM</sub> and UAN<sub>DI</sub> are UAN treated with ARM U and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript. ARM U and Agrotain Advanced are NBPT formulations while ARM U Advanced is a double inhibitor formulation (combined NBPT and nitrification inhibitor).

Total ammonia volatilization was significantly different among the fall-applied N amended treatments in CSL and PCL (Table 4.3). Untreated urea had the greatest ammonia volatilization in CSL (16.7%) and PCL (12.1%). The total ammonia volatilization from urea treated with inhibitors at both sites ranged from 3.2 to 11.1% (Table 4.3). With the exception of  $UR_{AG(100)}$  in CSL, the total ammonia volatilization from urea treated with inhibitors was significantly lower than the total ammonia volatilization from untreated urea at each site. With UAN amendments, the total ammonia volatilization across the two sites from untreated UAN (6.8 to 10.3%) was significantly greater than the total ammonia volatilization from UAN treated with inhibitors (1.7 to 5.5%) (Table 4.3).

In the spring, the total ammonia volatilization across both sites ranged from 10.8 to 28.7% in untreated urea whereas total ammonia volatilization was 3.1 to 13.9% in untreated UAN (Table 4.3). The total ammonia volatilization from urea treated with inhibitors ranged from 2.6 to 16.8% across the two sites. In CSL, there was no significant effect of NBPT on the total ammonia volatilization from urea-N applied at the lower rate (75 kg N ha<sup>-1</sup>). In contrast, there was a significant effect of NBPT on the total ammonia volatilization from urea in PCL (Table 4.3). In the UAN amendment, the total ammonia volatilization was not significantly different among UAN, UAN<sub>ARM</sub>, and UAN<sub>DI</sub> for each site (Table 4.3).

	Treatment <sup>a</sup>	Total ammonia volatilization			
		Carman sandy	Portage clay	Average	
		loam	loam	Average	
Fall		% of applied N			
	Bare soil	0.5f	0.5d	0.5d	
	UR(100)	16.7a	12.1a	14.4a	
	URARM(100)	5.0cd	3.2c	4.1c	
	UR <sub>AG(100)</sub>	11.1ab	5.3b	8.2b	
	UR <sub>DI(100)</sub>	4.2cd	3.2c	3.7c	
	UAN(100)	6.8bc	10.3a	8.6b	
	UAN <sub>ARM(100)</sub>	3.4de	5.5b	4.5c	
	UAN <sub>DI(100)</sub>	1.7e	5.1b	3.4c	
Model effect	et <sup>b</sup>	Probability values			
$\pm N$		< 0.0001	< 0.0001	< 0.0001	
+N		< 0.0001	< 0.0001	< 0.0001	
Spring					
			% of applied N—		
	UR(75)	10.8b	28.7a	19.8a	
	UR <sub>ARM(75)</sub>	7.3b	16.8bc	12.1ab	
	UR(100)	18.9a	20.4ab	19.7a	
	UR <sub>ARM(100)</sub>	2.6c	7.6c	5.1b	
	UR <sub>DI(75)</sub>	8.5b	12.6bc	10.6	
	UAN(75)	3.1c	13.9bc	8.5b	
	UAN <sub>ARM(75)</sub>	1.3c	11.3bc	6.3b	
	UAN <sub>DI(75)</sub>	1.9c	16.1bc	9.0b	
Model effect		Probability values			
+N		< 0.0001	0.0209	0.0028	

Table 4.3 Effect of treatment on total ammonia volatilization in each season of the first (2016/2017 growing season) year.

<sup>*a*</sup> UR, UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>DI</sub> are untreated urea, urea treated with ARM U, Agrotain Advanced, and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript; UAN, UAN<sub>ARM</sub>, and UAN<sub>DI</sub> are untreated urea ammonium nitrate, UAN treated with ARM U and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript. ARM U and Agrotain Advanced are NBPT formulations while ARM U Advanced is a double inhibitor formulation (combined NBPT and nitrification inhibitor).

 $^{b} \pm N$ , model effect comparison between N amended treatments versus bare soil; +N, model effect comparison among N amended treatments.

Means with different letters within a column in each season are significantly different at a probability level of < 0.05 Fisher protected LSD.

# 4.4.3 Second Year (2017/2018 Growing Season) Ammonia Volatilization

In the second year, the trends of ammonia volatilization measured between sampling days at both sites were similar to that in the first year (Figure 4.4). A greater portion of the total ammonia volatilization from the untreated urea and UAN occurred between 2 and 14 d in the fall and spring at both sites. In the fall season, ammonia volatilization from urea and UAN treated with inhibitors persisted until 21 d despite daily mean air and soil temperatures being less than 3 °C from 12 d (Figure 4.2 & 4.4).

In CSL, there was a significant treatment by season interaction on the total ammonia volatilization (Table 4.4). While there was no significant effect of season on the total ammonia volatilization from  $UR_{(75)}$ ,  $UR_{AG(75)}$ , and  $UAN_{(75)}$ , there was a significant effect of season on the total ammonia volatilization from  $UR_{ARM(75)}$ ,  $UR_{DI(75)}$ ,  $UAN_{ARM(75)}$ , and  $UAN_{DI(75)}$  (Table 4.4). The untreated urea had the greatest total ammonia volatilization (19.0 to 22.2%) which was significantly greater than total ammonia volatilization from  $UR_{ARM(75)}$  and  $UR_{AG(75)}$  (6.6 to 12.4%). However, the total ammonia volatilization from spring-applied  $UR_{DI(75)}$  (12.9%) was not significantly different from total ammonia volatilization from fall and spring-applied  $UR_{(75)}$ . In the UAN amendment, there was a significant effect of inhibitor on the total ammonia volatilization of fall-applied UAN

amendments, whereas, in the spring, the total ammonia volatilization between  $UAN_{(75)}$  and  $UAN_{ARM(75)}$  was not significantly different (Table 4.4).



Figure 4.4 Ammonia volatilization between sampling days in the second year (2017/2018) in Carman sandy loam (A & B) and Portage clay loam (C & D). Error bars are standard errors of the means.

Note: UR, untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>DI</sub> are urea treated with ARM U, Agrotain Advanced, and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the

subscript; UAN, untreated urea ammonium nitrate; UAN<sub>ARM</sub> and UAN<sub>DI</sub> are UAN treated with ARM U and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript. ARM U and Agrotain Advanced are NBPT formulations while ARM U Advanced is a double inhibitor formulation (combined NBPT and nitrification inhibitor).

Season x Treatment <sup>a</sup>		Total ammonia volatilization		
		Carman	Portage	Average
		sandy loam	clay loam	
		%	of applied N-	
Fall	Bare soil	0.0g	0.0k	0.0i
	UR(75)	22.2a	20.1a	21.2a
	URARM(75)	12.4b	3.9hi	8.2cd
	UR <sub>AG(75)</sub>	6.7cd	2.8i	4.8efg
	UR <sub>DI(75)</sub>	7.3cd	4.2hi	5.8defg
	UAN(75)	8.6bc	15.8ab	12.2b
	UAN <sub>ARM(75)</sub>	1.6e	7.0efg	4.3g
	UAN <sub>DI(75)</sub>	4.2d	4.6ghi	4.4efg
Spring	Bare soil	0.5f	0.4j	0.5h
	UR(75)	19.9a	24.2a	22.1a
	UR <sub>ARM(75)</sub>	6.6cd	11.7bc	9.2bc
	URAG(75)	8.9bc	5.7fgh	7.3cde
	UR <sub>DI(75)</sub>	12.9ab	12.0bc	12.5b
	UAN(75)	5.4cd	10.4bcde	7.9cd
	UAN <sub>ARM(75)</sub>	5.6cd	7.6efg	6.6cdef
	UAN <sub>DI(75)</sub>	1.6e	8.0cdef	4.8efg
Model effect <sup>b</sup>		Probability values		
$\pm$ N		< 0.0001	< 0.0001	< 0.0001
+N		< 0.0001	< 0.0001	< 0.0001
Season (S)		< 0.0001	< 0.0001	< 0.0001
+N x S		0.0001	0.0003	0.0042

Table 4.4 Effect of season and treatment on total ammonia volatilization in the second (2017/2018 growing season) year.

<sup>&</sup>lt;sup>*a*</sup> UR, untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>DI</sub> are urea treated with ARM U, Agrotain Advanced, and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript; UAN, untreated urea ammonium nitrate; UAN<sub>ARM</sub>, and UAN<sub>DI</sub> are UAN treated with ARM U and ARM

U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript. ARM U and Agrotain Advanced are NBPT formulations while ARM U Advanced is a double inhibitor formulation (combined NBPT and nitrification inhibitor).

 $^{b} \pm N$ , model effect comparison between N amended treatments versus bare soil; +N, model effect comparison among N amended treatments.

Means with different letters within a column are significantly different at probability level of < 0.05 Fisher protected LSD.

In the PCL, there was also a significant season by treatment interaction on the total ammonia volatilization (Table 4.4). The interaction was because there was no significant effect of season on total ammonia volatilization from  $UR_{(75)}$ ,  $UAN_{(75)}$ , and  $UAN_{ARM(75)}$  while the total ammonia volatilization was significantly greater in spring than fall-applied  $UR_{ARM(75)}$ ,  $UR_{AG(75)}$ ,  $UR_{DI(75)}$ , and  $UAN_{DI(75)}$  (Table 4.4). The total ammonia volatilization from untreated urea (20.1 to 24.2%) was significantly greater than the total ammonia volatilization from urea treated with inhibitors (2.8 to 12.0%). While the total ammonia volatilization from fall-applied untreated UAN was significantly greater than total ammonia volatilization from fall-applied UAN treated with inhibitor, there was no significant difference between spring-applied UAN treated with and without inhibitors (Table 4.4).

# 4.4.4 Mitigation of Ammonia Volatilization by NBPT

Combining the percentage reduction by either NBPT (ARM U and Agrotain Advanced) or DI (ARM U Advanced) from urea and UAN for the two years at each site, there was a significant season by treatment (N source + inhibitor type) interaction on ammonia reduction in CSL (P = 0.0272; Figure 4.5). The significant season by treatment interaction in CSLwas because there was

no significant difference in the effectiveness of NBPT and DI on urea and UAN in the fall whereas there was a significant effect of N source (urea and UAN) and inhibitor type (NBPT and DI) on the reduction of ammonia volatilization in the spring (Figure 4.5). The use of NBPT and DI reduced ammonia volatilization from urea and UAN by 56 to 71% in the fall and 27 to 64% in the spring. The addition of NI with NBPT on urea significantly reduced the efficacy of spring-applied NBPT on urea by 38%. Also, when N was applied as UAN instead of urea, the effectiveness of spring-applied NBPT was significantly reduced by 58%.



Figure 4.5 Percentage reduction of ammonia volatilization by NBPT and double inhibitor (combined NBPT and nitrification inhibitor) on urea and UAN in Carman sandy loam (A) and Portage clay loam (B) in the two years of study. Error bars are standard errors of the mean. Bars with different letters are significantly different at a probability level of < 0.05 Fisher protected LSD.

UR<sub>NBPT</sub>, urea treated with NBPT only (average reduction in UR<sub>ARM</sub> and UR<sub>AG</sub> where UR<sub>ARM</sub> and UR<sub>AG</sub> are urea treated with ARM U and Agrotain Advanced formulations); UR<sub>DI</sub>, urea treated with double inhibitor (ARM U Advanced formulation); UAN<sub>ARM</sub>, UAN treated with NBPT (ARM U formulation); UAN<sub>DI</sub>, UAN treated with double inhibitor (ARM U Advanced formulation).

In PCL, there was no significant season by treatment interaction on percentage ammonia reduction (P = 0.2758). However, there were significant effects of season (P < 0.0001) and treatment (P < 0.0001) on ammonia reduction. Fall-applied inhibitors on either urea or UAN were significantly more effective in reducing ammonia volatilization (51 to 76%) than their corresponding spring-applied urea and UAN treated with inhibitors (4 to 57%; Figure 4.5). The inhibitors were more effective on urea than on the corresponding UAN in each season in PCL (Figure 4.5).

### 4.5 Discussion

In this study, and as observed in some previous studies (Cantarella et al. 2008; Mira et al. 2017), we found that measurable (or significant) ammonia volatilization following urea application frequently took two or more days to occur when conditions for hydrolysis were not limiting. In contrast, a study by Forrestal et al. (2016) has found that a significant portion of total ammonia volatilization from surface-applied urea could occur within one day after fertilization possibly because their urea was applied on grassland with thatches which have a higher urease activity than bare soil. Despite the differences in temperature between fall and spring seasons, the delayed peak of ammonia volatilization from urea and UAN treated with inhibitors in both seasons were similar and followed the commonly observed patterns of delayed peak losses of ammonia to second week after fertilization (Cantarella et al. 2008; Soares et al. 2012). Our results showed that the cold soil temperature during the fall season (<  $3 \,^{\circ}$ C) did not stop ammonia volatilization from urea-based fertilizers. Our results are consistent with those of Bremner and Zantua (1975) who found that urease enzyme was active at temperatures below 0 °C; Chantigny et al. (2019) who found that cold

and frozen soils during late-fall and winter (at temperatures below 0 °C) did not stop microbial transformation of applied fertilizer N, and Engel et al. (2011, 2017) who observed generally larger ammonia loss from urea applied during cold weather months (November-March) relative to the spring applications. A cold and frozen soil possibly allows a high zone of ammonia, ammonium, and pH following urea hydrolysis to persist longer on the soil surface and reduces diffusion thereby allowing the persistent ammonia to volatilize.

On average across season and years, the ammonia volatilization from untreated urea at each site was greater than the global average of 16% (Cantarella et al. 2018). However, the ammonia volatilization from untreated urea at both sites was still within the range of 20 to 26% that was measured by Rawluk et al. (2001) on the Canadian Prairie using the same static chamber method. Also, the average ammonia volatilization from spring-applied untreated urea in CSL(17%) was similar to that from our previous study (Chapter 2) conducted at this field location. While greater urease activity in the clay loam soil at Portage than the sandy loam soil at Carman has the potential of increasing ammonia volatilization at Portage, this might have been counterbalanced by the higher CEC and clay content at Portage. A study of 79 soils in the United States found that CEC was the most important soil variable that accounted for the quantity of ammonia volatilization (negative correlation) from urea fertilizers (Sunderlage and Cook 2018). The greater percentage of applied N that was lost as ammonia from lower than higher N rate of spring-applied urea treated with NBPT only at both sites was in contrast to an earlier study (Overrein and Moe 1967) that reported an increase in ammonia volatilization as the rate of applied urea-N increased.

The quantity of ammonia volatilization measured is influenced by the methods of measurement. The static chamber method used in our study was reported by Smith et al. (2007) to underestimate ammonia volatilization compared to wind tunnel and micrometeorological methods. The reasons for the underestimation of ammonia volatilization include a lack of direct ammonia gradient, and wind restriction across the soil surface in the chamber. The underestimation of ammonia volatilization by static chamber compared to wind tunnel methods was reported to decrease with deployment or measurement time with an average of 23% over 22 d (Miola et al. 2015). However, the underestimation of ammonia volatilization by the chambers may be partially compensated by the overestimation of ammonia volatilization as a result of the possible greater relative humidity in the chamber than the critical humidity of urea (Dempsey et al. 2017). As such, the ammonia volatilization measured in this study is relative among the treatments. Nevertheless, the static chamber method is still widely used for its simplicity, ease of construction, relatively inexpensive, ease of replication, and allows for multiple treatments comparison.

Generally, NBPT is known for reducing ammonia volatilization from urea-based fertilizers by inhibiting urea hydrolysis process especially during the first week of fertilization (Keshavarz et al., 2018). Interestingly, the reduction of ammonia by NBPT averaged 52% in our study and was identical to the global review and meta-analysis (Silva et al. 2017; Cantarella et al. 2018). The efficacy of NBPT depends on how soon the unhydrolyzed fraction of the urea gets incorporated into the soil after 7 d of fertilization (Rawluk et al. 2001; Cantarella et al. 2008). As the efficacy of NBPT declines, the difference in total ammonia volatilization between urea treated with and without NBPT also declines, particularly when NBPT treated urea is not incorporated into the soil in the latter days. In our study, upward and lateral water movement following rainfall would
provide sufficient water for hydrolysis, but the rain shelter prevented the downward movement of urea by the infiltrating rain. This may potentially elevate ammonia volatilization from the remaining NBPT treated urea and UAN during the later days after fertilization. The lower efficacy of NBPT on UAN especially in PCL was because of the relatively high soil pH at Portage which predisposed the ammonium fraction of both treated and untreated UAN to greater levels of ammonia volatilization (Sommer and Ersboll 1996; He et al. 1999). Although not statistically significant except with spring-applied urea in CSL, the efficacy of NBPT when combined with NI was reduced. This may be due to the persistence of ammonium-N by the NI which then increased the potential for N losses via ammonia volatilization. Several studies had also reported that the addition of NI with urease inhibitor reduces the effectiveness of urease inhibitor (Gioacchini et al. 2002; Soares et al. 2012). Also, despite the higher NBPT per kg urea with Agrotain Advanced treated urea than ARM U treated urea, the amount of ammonia volatilization across the seasonsite-year was not related to the NBPT concentrations. Our earlier greenhouse and field studies that compared these two formulations at the concentrations used in this study did not find any significant difference in their ammonia volatilization (Chapter 2). Overall, we found that NBPT was less effective in spring than fall-applied urea-based fertilizers. Higher soil temperature in the spring than in the fall resulted in rapid hydrolysis of urea and degradation of the NBPT in the spring thereby reducing its efficacy (Carmona et al. 1990; Watson et al. 2008).

# 4.6 Conclusion

The potential for ammonia volatilization from surface-applied urea-based fertilizer is high irrespective of the season of application. Our two-year study at two sites found that total ammonia

volatilization from untreated urea and UAN ranged from 7 to 22% and 3 to 29% in fall and spring, respectively. Low mean air temperature in the fall (sometimes < 0 °C) did not prevent ammonia volatilization from urea-based fertilizers. Addition of NBPT reduced ammonia volatilization from urea-based fertilizers by 65 and 40% for fall and spring applications, respectively. We found that the addition of NI with NBPT reduced the efficacy of NBPT on spring-applied urea by 27%. Our study showed that significant ammonia volatilization could occur when farmers broadcast urea-based fertilizers in mid- or late-fall as recommended for the Canadian prairie region. As such, the use of NBPT should not be limited to spring application alone, rather, both fall and spring applications of urea-based fertilizer could benefit from NBPT.

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#### **CHAPTER 5**

# 5 EFFICIENCY OF FALL VERSUS SPRING APPLIED UREA-BASED FERTILIZERS TREATED WITH UREASE AND NITRIFICATION INHIBITORS II. CROP YIELD AND NITROGEN USE EFFICIENCY

### **5.1 Abstract**

Urease inhibitor [N-(n-butyl) thiophosphoric triamide (NBPT)] and nitrification inhibitor [3,4dimethyl pyrazole phosphate (NI)] have been reported to conserve urea-based nitrogen (N) fertilizers by reducing N losses. However, their effects on crop yield and N uptake are inconsistent. In addition, fall-applied N fertilizer is usually less efficient than spring application. We conducted a two-year field study in two contrasting soils [Carman sandy loam (CSL) and Portage clay loam (PCL)] to evaluate the efficiency of NBPT with and without NI in increasing grain yield, N removal, and N uptake from fall and spring surface-applied urea-based fertilizers. Treatments (75 or 100 kg N ha<sup>-1</sup>) consisted of urea and urea ammonium nitrate (UAN) treated with and without NBPT or NBPT+NI. Canola and wheat grain yields, N removal, and N uptake were not consistently greater from urea and UAN treated with inhibitors than untreated urea and UAN. A significant effect of inhibitors on grain yield and N uptake was observed in urea treated with NBPT in CSL but not in PCL. In PCL, agronomic efficiency (kg grain kg<sup>-1</sup> N) was significantly greater from spring-applied untreated urea or UAN than fall-applied urea or UAN treated with and without inhibitors. However, there was no significant difference between fall-applied urea or UAN treated with inhibitor and spring-applied untreated urea or UAN in CSL. The conserved N by the inhibitors did not show up in the soil as nitrate-N. We conclude that while the inhibitors may safeguard the environment through reduced N losses, their use to increase crop yield and bridge efficiency gap between fall and spring-applied urea-based fertilizers may be site specific.

#### **5.2 Introduction**

Urea-based fertilizers, especially urea granule, is the dominant source of commercial nitrogen (N) fertilizers worldwide. As the world population increases, the demand for urea fertilizer is estimated to increase by 17 Mt from 2016 to 2021 (IFA 2017). The continuous increase in the demand for urea-based fertilizers is primarily due to its ease of handling, storage, and application. However, the amount of N losses via ammonia volatilization, nitrous oxide emission, dinitrogen gas emission, and nitrate leaching is of major agronomic and environmental concerns (Aneja et al. 2008; Cantarella et al. 2018). The potential and magnitude for ammonia volatilization from urea are a function of soil and environmental conditions as well as management practices (Cai et al. 2002; Kissel et al. 2009; Pelster et al. 2018). Ammonia volatilization from urea-based fertilizers is relatively greater on calcareous soil with neutral to alkaline pH and may exceed 15% of applied N when broadcasted without incorporation (He et al. 1999).

While sub-surface application is one of the ways of reducing ammonia volatilization from ureabased fertilizers (Cai et al. 2002), sub-surface application may not be feasible or desirable in fields that are zero-tilled, or with established tree crops or perennial forage as it may damage the root system. In addition to the method of fertilizer application, the time of fertilizer application is also essential in maximizing N use efficiency from urea-based fertilizers. In the northern Great Plain, spring-applied N fertilizers have been reported to produce greater grain yield than fall-applied N fertilizers (Nyborg and Malhi 1986; MAFRI 2007; Jaynes 2015). This is because of the shorter time interval between fertilizer application and crop N demand in the spring. Notwithstanding, most agronomists in Manitoba prefer fall N application to spring because it is cheaper, and it allows them to spread their spring workload (Amiro et al. 2017). As farm size increases, farmers will have to trade-off between agronomic and operational efficiencies. As such, broadcast instead of subsurface application and/or fall instead of spring application of urea-based fertilizers may be the efficient and/or desirable option for a farm operation.

To reduce ammonia volatilization from surface-applied urea-based fertilizers, farmers have the option to coat their urea with urease inhibitors such as N-(n-butyl) thiophosphoric triamide (NBPT). This urease inhibitor, NBPT, is known to effectively reduce ammonia volatilization under different soil and environmental conditions (Modolo et al. 2018; Sigurdarson et al. 2018). In addition to coating urea with NBPT, farmers may also use a nitrification inhibitor (NI) along with the NBPT to inhibit nitrification of ammonium formed from urea hydrolysis. In case a farmer prefers fall application to spring, he or she is advised to delay N application until mid-fall (early October) to late-fall (mid-October) when the soil temperature is below 5 °C at 10 cm depth (MAFRI 2007). The delay is supposed to reduce N losses before the soil freezes up by inhibiting microbial activities. However, recent studies in the northern Great Plain have shown that significant ammonia volatilization could occur from urea-based fertilizers that were not treated with NBPT in late fall even when the soil temperature was below 0 °C (Engel et al. 2017; Chapter 4). Also, the study of Chantigny et al. (2019) had shown that significant N transformation and losses could occur during winter months in the northern Great Plain. In addition to N losses, delaying N application until late fall may not be feasible due to snow on the Canadian prairies.

While NBPT has been shown to effectively reduce ammonia volatilization, its use with and without NI on grain yield and N uptake has produced conflicting results. Some studies (McKenzie et al.

2010; Grant 2014; Romero et al. 2017; Guardia et al. 2018) have found no significant difference in grain yield between spring-applied urea treated with and without NBPT while others such as Mohammed et al. (2016) found a significant increase in grain yield due to coating urea with NBPT. In addition to the conflicting results on the role of NBPT in increasing crop yield from springapplied N fertilizers, it is not clear if the reduced N losses via volatilization by NBPT can bridge the nitrogen use efficiency (NUE) gap between fall and spring surfaced-applied N fertilizers. If the reduced ammonia volatilization from fall-applied urea by NBPT produced greater yield than untreated urea or similar yield to spring-applied untreated urea, then this may encourage farmers to apply NBPT treated urea in the fall: a win-win situation for the farmers and the environment. Therefore, the objectives of this study were (i) to evaluate the effectiveness of NBPT with or without NI (3,4- dimethylpyrazole phosphate; DMPP) in increasing grain yield, N removal, and N uptake from fall and spring-applied urea-based fertilizers; (ii) to ascertain if the reduced N losses by inhibitors can bridge N use efficiency gap between fall and spring-applied urea-based fertilizers. We hypothesized that (i) the reduced N losses via ammonia volatilization by NBPT will result in an increase in grain yield, N removal, and N uptake; (ii) the application of NBPT and NI with fall-applied urea and UAN will produce similar grain yield, N removal, and N uptake as spring-applied untreated urea and UAN thereby allowing inhibitors to compensate for the reduced efficiency of fall-applied N fertilizers relative to spring-applied.

#### **5.3 Materials and Methods**

The site characteristics, land preparation, treatment types, and experiment design have been described in Chapter 4. However, they were repeated in this chapter as they appear in the submitted manuscript for publication.

# **5.3.1 Sites Characteristics**

We conducted field trials in 2016/2017 (first year) and 2017/2018 (second year) at two sites with contrasting soil types. The sites were (i) Carman, Manitoba (49° 29' 6" N, 98° 02' '2" W); (ii) Canada-Manitoba Crop Diversification Centre, Portage la Prairie, Manitoba (49° 57´ 9″ N, 98° 16´  $0^{"}$  W). The soil at Carman is a sandy loam mapped as an Orthic Black Chernozem in the Canadian soil classification system (Mills and Haluschak 1993). The soil at Portage la Prairie (herein referred to as Portage) is a clay loam mapped as a Gleyed Rego Black Chernozem in the Canadian soil classification system (Michalyna and Smith 1972). According to Agriculture and Agri-Food Canada (1998), the Black Chernozem is equivalent to Udic Boroll subgroup in the USDA classification system. Prior to establishing the study in the fall of 2016, soils were collected from 0 - 60 cm in a 15 cm increment at each site (Carman and Portage). The soils were ground and sieved (< 2 mm) to determine selected soil properties on 0 - 15 cm samples and residual nitrate-N on 0 - 60 cm (Table 5.1). The selected soil properties determined were soil organic matter (Walkley and Black 1934), pH (soil/water, 1:1), electrical conductivity, cation exchange capacity (Hendershot et al. 2008), Olsen P (Olsen et al. 1954), residual nitrate-N (Maynard et al. 2008), soil texture (Gee and Bauder 1986), and urease activity (Tabatabai and Bremner 1972).

Property <sup>a</sup>	Carman	Portage	
Soil series	Hibsin	Neurhorst	
Soil pH <sub>water</sub>	6.78	7.96	
Electrical conductivity (µS cm <sup>-1</sup> )	387	596	
Organic matter (g kg <sup>-1</sup> )	32	71	
Field capacity (m <sup>3</sup> m <sup>-3</sup> )	0.35	0.44	
CEC (cmol kg <sup>-1</sup> )	16	36	
Urease activity (mg NH4 <sup>+</sup> -N kg <sup>-1</sup> soil hr <sup>-1</sup> )	24	88	
Residual nitrate-N (kg ha <sup>-1</sup> )	77	66	
Bulk density (Mg m <sup>-3</sup> )	1.3	1.1	
Soil texture	Sandy loam	Clay loam	
Sand (g kg <sup>-1</sup> )	799	269	
Silt (g kg <sup>-1</sup> )	47	343	
Clay (g kg <sup>-1</sup> )	154	388	

Table 5.1 Physical and chemical properties of soil at Carman and Portage.

<sup>*a*</sup> Soil properties were determined on soil from 0 - 15 cm except for residual nitrate that was determined from 0 - 60 cm.

# **5.3.2 Land Preparation and Experimental Design**

In the fall of 2016 and 2017, the plot at Carman was tilled to bury residues and kill weeds whereas at Portage, tillage was carried out only in the fall of 2017 and not in fall of 2016 due to logistics such as equipment and labour availability. In the spring of 2017 and 2018, the plots were cultivated for seeding at both sites. The experimental design was a season by fertilizer treatment factorial layout arranged in a randomized complete block design with four replications. The plot size was 5 m by 3 m. The seasons were fall and spring. The fertilizer treatments consisted of two sources of N (urea and urea ammonium nitrate; UAN) and three sources of inhibitor formulations [ARM U (Active AgriScience Inc., BC), Agrotain Advanced (Koch Agronomic Services LLC, Wichita,

KS), and ARM U Advanced (Active AgriScience Inc., BC)]. ARM U and Agrotain Advanced are NBPT formulations containing 18% and 30% NBPT m/v, respectively, while ARM U Advanced is a double inhibitor (DI) containing 24% NBPT + 6% DMPP m/v. The treatments were surfaceapplied untreated urea (UR); urea treated with either ARM U (UR<sub>ARM</sub>), Agrotain Advanced (UR<sub>AG</sub>), or ARM U Advanced (UR<sub>DI</sub>); untreated UAN (UAN); UAN treated with either ARM U (UAN<sub>ARM</sub>) or ARM U Advanced (UAN<sub>DI</sub>); and a control with neither urea nor UAN. ARM U and Agrotain Advanced were used to coat urea at a rate of 2 L per 1000 kg urea and ARM U Advanced was used to coat urea at the rate of 1.5 L per 1000 kg urea. In the treatments containing UAN, ARM U and ARM U Advanced were mixed with UAN at the rate of 1 L per 1000 L UAN. Table 5.2 shows the details of the concentration of inhibitors applied with urea and UAN.

In the fall of the first year, fall plots received treatments at a rate of 100 kg N ha<sup>-1</sup>. In the spring of the first year, N rate was changed to 75 kg N ha<sup>-1</sup> due to the result from a greenhouse study in the winter of 2017 that showed the optimum rate for urea treated with ARM U to be 75 kg N ha<sup>-1</sup> for spring wheat (result not shown). To enable a comparison between fall and spring-applied untreated urea and urea treated with ARM U, we replaced the supposed spring-applied UR<sub>AG</sub> and control treatments with untreated urea and urea treated with ARM U applied at 100 kg N ha<sup>-1</sup>. In the second year of the study, all the seven N treatments listed above were applied at 75 kg N ha<sup>-1</sup> in the fall and in the spring. Table 5.2 provides details of the treatments applied in each year. Prior to treatment application in the spring of each year, the plots were seeded to spring wheat (*Triticum aestivum L.* "AC Brandon") and canola (*Brassica napus L.* "Invigor L233P") in the first and second year, respectively. Details of the treatment application and seeding dates at each site and year are shown in Table 5.3.

Season	Treatment <sup>a</sup>	Concentrations of NBPT/DMPP in urea and UAN <sup>b</sup>		First vear	Second year
		NBPT DMPP		N applic	cation rate
		mg kg <sup>-1</sup> urea o	or mg L <sup>-1</sup> UAN	k	g ha-1
Fall					
	Bare soil	0	0	0	0
	$UR_{(100)}$	0	0	100	NA
	UR <sub>ARM(100)</sub>	360	0	100	NA
	URAG(100)	600	0	100	NA
	UR <sub>DI(100)</sub>	360	90	100	NA
	UAN(100)	0	0	100	NA
	UANARM(100)	180	0	100	NA
	<b>UAN</b> <sub>DI(100)</sub>	240	60	100	NA
	UR(75)	0	0	NA	75
	UR <sub>ARM(75)</sub>	360	0	NA	75
	UR <sub>AG(75)</sub>	600	0	NA	75
	UR <sub>DI(75)</sub>	360	90	NA	75
	UAN(75)	0	0	NA	75
	UAN <sub>ARM(75)</sub>	180	0	NA	75
	UAN <sub>DI(75)</sub>	240	60	NA	75
Spring					
1 0	Bare soil	NA	NA	NA	0
	$UR_{(75)}$	0	0	75	75
	UR <sub>ARM(75)</sub>	360	0	75	75
	UR(100)	0	0	100	NA
	URARM(100)	360	0	100	NA
	UR <sub>AG(75)</sub>	NA	NA	NA	75
	UR <sub>DI(75)</sub>	360	90	75	75
	UAN(75)	0	0	75	75
	UANARM(75)	180	0	75	75
	UAN <sub>DI(75)</sub>	240	60	75	75

Table 5.2 Types and rates of treatment applied during the first (2016/2017 growing season) and second (2017/2018 growing season) years of study.

<sup>*a*</sup> UR, untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>DI</sub> are urea treated with ARM U, Agrotain Advanced, and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript; UAN, untreated urea ammonium nitrate; UAN<sub>ARM</sub> and UAN<sub>DI</sub> are UAN treated with ARM U and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript. ARM U and Agrotain Advanced are NBPT formulations while ARM U Advanced is a double inhibitor formulation (combined NBPT and DMPP). NA, treatment is not applicable.

<sup>*b*</sup> NBPT, N-(n-butyl) thiophosphoric triamide (urease inhibitor); DMPP, 3,4- dimethylpyrazole phosphate (nitrification inhibitor); mg kg<sup>-1</sup> urea is for the urea granule treatments while mg  $L^{-1}$  UAN is for the UAN solution treatments.

	Fall N application	Spring N application	Seeding	Harvest	Soil sampling
First year					
Carman	Oct. 20 2016	May 24 2017	May 15 2017	Aug. 14 2017	Sept. 18 2017
Portage	Oct. 20 2016	May 24 2017	May 12 2017	Aug. 28 2017	Sept. 27 2017
Second yes	ar				
Carman	Oct. 17 2017	May 16 2018	May 10 2018	Aug. 3 2018	Aug. 24 2018
Portage	Oct. 17 2017	May 16 2018	May 4 2018	Aug. 7 2018	Aug. 27 2018

Table 5.3 Dates of fertilizer application, seeding, harvest, and soil sampling in the first (2016/2017) and second year (2017/2018).

# 5.3.3 Agronomic Practices and Biomass Sampling

In the spring, each plot received 20 kg  $P_2O_5$  ha<sup>-1</sup> (13 kg  $P_2O_5$  ha<sup>-1</sup> was placed with the seed during seeding and 7 kg  $P_2O_5$  ha<sup>-1</sup> was broadcast on the soil surface after seeding) in form of triple superphosphate. Recommended herbicides, pesticides, and fungicides were applied to the plots at the preferred growing stages of the crops according to the Manitoba provincial guide for crop protection.

During the first year harvest, above-ground biomass (grain + straw) were taken from six contiguous rows of 1 m in length from two randomly selected locations within each plot (for a total area of  $2 \text{ m}^2$ ) and bagged for drying. In the second year, a sickle mower with a cutting length of 1.12 m was used to sample the above-ground biomass for an area of 6.72 m<sup>2</sup>. The total wet biomass collected within the 6.72 m<sup>2</sup> was weighed immediately and a subsample was weighed and

bagged for drying. The biomass samples were dried in a drying room set at 35 °C for at least two weeks. After drying, the biomass samples were weighed and threshed for grains with a Wintersteiger classic combine. The threshed grains were weighed. Grain and straw (total dry biomass minus grain) weights were scaled to Mg ha<sup>-1</sup> on a dry matter basis. Subsamples of straw and grains (except canola seed in the second year) were ground, digested for total N using wet oxidation method (Parkinson and Allen 1975). The concentration of N in the digested samples (grain and straw) was analyzed colorimetrically with Technicon auto-analyzer II (Pulse Instrumentation Ltd, Saskatoon, SK).

Grain N removal (kg ha<sup>-1</sup>) was calculated as the product of N concentrations in the grain and the grain yield. Nitrogen uptake (kg ha<sup>-1</sup>) was calculated as the sum of grain N removal and N in the straw (product of N concentrations in the straw and the straw biomass).

Nitrogen use efficiency of the treatments was calculated as agronomic efficiency (AE; kg grain kg<sup>-1</sup> N) and apparent recovery efficiency (ARE<sub>N</sub>) as described by Fageria and Baligar (2005):

$$AE = \frac{DMY_{g(N)} - DMY_{g(0)}}{N_{app}}$$
(5.1)

$$ARE_{N} = \frac{N_{up(N)} - N_{up(0)}}{N_{app}} \times 100$$
(5.2)

 $N_{up(N)}$  is the N uptake from N amended plots;  $N_{up(0)}$  is the N uptake from control plot with no N amendment;  $N_{app}$  is the amount of N applied as urea or UAN with and without inhibitors;  $DMY_{g(N)}$  is the grain dry matter yield from N amended plots;  $DMY_{g(0)}$  is the grain dry matter yield from the control plot.

## 5.3.4 Soil Sampling

Soil samples were collected from each plot at 0 - 15, 15 - 30, 30 - 45 and 45 - 60 cm depths in each year after harvest (Table 5.3). Fresh moist soil was extracted with 2 M KCl for nitrate concentration (Maynard et al. 2008) and analyzed using AQ2 Discrete Analyzer (SEAL Analytical Inc., WI). Gravimetric moisture content was also determined to correct for the moisture in the extracted soil. The nitrate concentrations were scaled up to kg ha<sup>-1</sup>. The nitrate in the four depths on each plot was summed as residual nitrate.

Temperature and precipitation data during the period of study were collected from Manitoba Agriculture weather stations that were located at about 200 m and 900 m from the study sites at Carman and Portage, respectively.

## **5.3.5 Statistical Analysis**

Statistical analysis was conducted with PROC GLIMMIX (SAS Institute, 2014; version 9.4). All data were analyzed separately by site and year except otherwise stated. In consistence with the experimental design, analysis of variance (ANOVA) was carried out in two steps: (i) comparison between the N amended plots versus control plot and (ii) comparison among the N amended plots without the control plot (Katanda et al. 2016). In the first year of the study, we used a one-way ANOVA to test for the significant effect of fertilizer treatments on grain yield, N removal, N uptake, and soil residual nitrate because of the differences in N rate and treatment types in the fall and spring. Since the types and N rate of the treatments were the same in the second year, we used a two-way ANOVA to determine the significant effects of fertilizer treatments and season of

application on grain yield, N removal, N uptake, and residual nitrate. Treatment and season were fixed effects while block (replicates) and its interaction with fixed effects were random effects. All data were normally distributed except for soil residual nitrate in the second year at Carman and a lognormal distribution was specified in its model. Also, PROC GLIMMIX was used to test the effect of season, treatments (urea and UAN treated with and without NBPT or DI) and their interaction on AE<sub>N</sub> and ARE<sub>N</sub>. In this model, UR<sub>ARM</sub> and UR<sub>AG</sub> were combined as UR<sub>NBPT</sub>. Treatments and seasons were fixed effects while block and year were random effects. We used Fisher's least significant difference (LSD) procedure for multiple means comparison even when the ANOVA was not significant (instead of doing repeated t-test between various pairs of treatments; SAS, 14.3 User's guide) to enable us to compare pairs of treatments that satisfy our research questions. CONTRAST in PROC GLIMMIX was used to compare groups of treatment combinations that satisfy our research question. Due to high variability among replicates, the mean comparison was deemed significant at a probability value of < 0.1.

### **5.4 Results**

# **5.4.1 Environmental Conditions**

According to the Manitoba Agriculture weather data, the climate normal mean annual temperature at Carman and Portage sites was 2.8 and 2.6 °C, respectively. Carman and Portage have climate normal annual precipitation of 521 and 513 mm, respectively, with only 284 and 279 mm of the precipitation occurring as rainfall during seeding to harvest of small grains (May to August), respectively. The two growing seasons of this study were relatively dry as Carman received 49 and 27% less rainfall than climate normal during May to August of 2017 and 2018, respectively,

while Portage received 55 and 28% less rainfall than climate normal during May to August of 2017 and 2018, respectively (Figure 5.1).



Figure 5.1. Daily precipitation and mean air temperature during the first (2016/2017) and second (2017/2018) years of study.

## 5.4.2 Grain Yield

In the first year of the study, ANOVA indicated that the differences in wheat grain yield were not significant among the N amended plots in Carman sandy loam (CSL) and Portage clay loam (PCL; Table 5.4). However, the difference in wheat grain yield between N amended plots and control plot with no N application was significant (Table 5.4). Wheat grain yields from treatments applied in the fall were not significantly different from corresponding treatments applied in the spring despite the lower N rates in the spring at both sites (Table 5.4). In both soils, fall-applied UR<sub>ARM</sub> had the greatest wheat grain yield (3.74 and 5.38 Mg ha<sup>-1</sup> in CSL and PCL, respectively) while the control with no N amendment had the smallest wheat grain yield (Table 5.4). Although ANOVA did not show any significant effect of treatments among the N amended plots on wheat grain yield in CSL, multiple means comparison showed that wheat grain yield from fall-applied URARM was significantly greater than that from fall-applied untreated urea (Table 5.4). The wheat grain yield from fall-applied UR<sub>ARM</sub> was not significantly different from wheat grain yield from springapplied untreated urea (at the same N rate of 100 kg N ha<sup>-1</sup>; Table 5.4). Also, in CSL, fall-applied untreated urea and spring-applied untreated urea (75 kg N ha<sup>-1</sup>) had the smallest wheat grain yield among the N amended plots with a pattern of increasing wheat grain yield with the addition of inhibitors. There was no significant effect of inhibitor on wheat grain yield in UAN treatments at Carman. In PCL, multiple means comparison did not show any significant difference between any pair of either urea or UAN treated with and without inhibitors (Table 5.4).

		Carr	Carman sandy loam		Portage clay loam		
		Grain yield	N removal	N uptake	Grain yield	N removal	N uptake
Season	Treatment <sup>a</sup>	Mg ha <sup>-1</sup>	——kg ha	1 <sup>-1</sup> ——	Mg ha <sup>-1</sup>	——kg ha <sup>-</sup>	1
	Control	2.24d	44.5e	57.5c	3.59d	77.6e	93.9b
Fall	UR(100)	2.82bcd	59.6cde	79.1bc	4.98abc	120.9abcd	160.0a
	UR <sub>ARM(100)</sub>	3.74a	78.3abcd	105.7ab	5.38a	137.5a	171.9a
	UR <sub>AG(100)</sub>	3.54abc	100.9a	134.8a	4.42c	104.2cd	142.6a
	UR <sub>DI(100)</sub>	3.20abc	63.8bcde	110.6ab	4.78abc	110.8bcd	157.2a
	UAN(100)	3.45abc	79.8abc	111.1ab	5.15ab	127.4abc	165.3a
	UANARM(100)	3.08abcd	65.0bcde	93.3bc	4.68abc	103.8cd	148.4a
	UAN <sub>DI(100)</sub>	3.66ab	78.7abcd	108.9ab	4.74abc	103.1d	167.8a
Spring	UR(75)	2.78cd	55.5de	78.4bc	5.04abc	111.6abcd	154.5a
	UR <sub>ARM(75)</sub>	3.48abc	71.3bcd	98.3ab	5.10abc	118.4abcd	165.9a
	UR(100)	3.65ab	83.4ab	112.7ab	5.01abc	128.5ab	167.1a
	URARM(100)	3.48abc	82.4abc	109.6ab	4.96abc	121.8abcd	179.8a
	UR <sub>DI(75)</sub>	3.57abc	76.1bcd	108.3ab	4.94abc	124.0abcd	178.2a
	UAN(75)	2.99abcd	72.9bcd	79.8bc	4.93abc	125.6abcd	181.7a
	UAN <sub>ARM(75)</sub>	3.20abc	75.0bcd	110.4ab	4.73abc	106.0bcd	140.2a
	UAN <sub>DI(75)</sub>	3.29abc	72.3bcd	105.2ab	4.47bc	121.1abcd	166.8a
Model effect	et <sup>b</sup> df			-Probabilit	y values —		
$\pm N$	1	0.0067	0.0061	0.0061	0.0001	0.0004	< 0.0001
+N	14	0.7455	0.2704	0.6728	0.7512	0.4168	0.8764
Contrast							
Fall vs sprin	ng	0.7887	0.7242	0.7563	0.9049	0.4278	0.3222
UR vs UR	N	0.1145	0.0776	0.0635	0.7192	0.9041	0.6225
UR <sub>s</sub> vs UR	FIN	0.4106	0.2058	0.1397	0.5662	0.7917	0.8468
UAN vs UA	AN <sub>IN</sub>	0.7738	0.7882	0.7665	0.1457	0.0483	0.2671
UANs vs UAN <sub>FIN</sub>		0.3926	0.9318	0.8101	0.5564	0.0795	0.2755

Table 5.4 Effects of treatments on grain yield, N removal, and N uptake of wheat in the first year (2016/2017).

<sup>*a*</sup> UR, untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>DI</sub> are urea treated with ARM U, Agrotain Advanced, and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript; UAN,

untreated urea ammonium nitrate;  $UAN_{ARM}$ , and  $UAN_{DI}$  are UAN treated with ARM U and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the subscript. ARM U and Agrotain Advanced are NBPT formulations while ARM U Advanced is a double inhibitor formulation (combined NBPT and nitrification inhibitor).

 $^{b} \pm N$ , model effect between N amended versus control treatment; +N, model effect among N amended treatment without control plot.

Means with different letters within a column in each season are significantly different at a probability level of < 0.1 LSD.

In the second year of the study, there was no significant season by treatment interactions on canola grain yield in either soil (Table 5.5). The effect of season on canola grain yield was significant in both soils. The average canola grain yield across treatments in the spring was greater than that in the fall by 15% and 12% in CSL and PCL, respectively (Table 5.5). In CSL, there was a significant effect of treatment on canola grain yield. The canola grain yield from either UAN<sub>ARM</sub> or UAN<sub>DI</sub> was significantly greater than the canola grain yield from untreated UAN (Table 5.5). Also, the average canola grain yield across the seasons was significantly greater from UR<sub>ARM</sub> than untreated urea in CSL (Table 5.5). There was a significant benefit of NBPT on the canola grain yield as the canola grain yield from fall-applied UAN<sub>ARM</sub> was significantly greater than canola grain yield from spring-applied untreated UAN (Figure 5.2). Unlike CSL, there was no significant effect of treatments on canola grain yield in PCL (Table 5.5). The addition of NBPT or DI did not have any significant benefit on canola grain yield in each season in PCL (Figure 5.2).

Contrast analysis showed that there was no significant difference in grain yield between untreated urea and urea treated with inhibitors (UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>DI</sub>) in each soil in each year (Table

		Carman sandy loam		Portage clay loam			
		Grain yield	N removal	N uptake	Grain yield	N removal	N uptake
		Mg ha <sup>-1</sup>	——kg ł	na <sup>-1</sup> ——	Mg ha <sup>-1</sup>	——kg ł	na <sup>-1</sup> ——
Treatment <sup>a</sup>	Control	0.88e	25.3d	30.4e	0.79b	20.8b	25.9c
	UR	1.53d	47.2c	57.2d	1.75a	56.7a	73.8ab
	UR <sub>ARM</sub>	1.70abc	56.3b	67.5c	1.82a	58.0a	73.8ab
	UR <sub>AG</sub>	1.61bcd	52.4bc	60.8cd	1.79a	55.7a	71.5b
	UR <sub>DI</sub>	1.58bcd	52.5bc	64.6cd	1.78a	56.2a	71.0b
	UAN	1.55cd	54.6b	68.6bc	1.99a	67.7a	89.4a
	UANARM	1.81a	65.0a	80.6a	1.78a	58.3a	75.3ab
	UAN <sub>DI</sub>	1.71ab	63.3a	76.2ab	1.77a	57.9a	73.8ab
Season (SS)	Fall	1.52b	50.9b	61.1b	1.69b	52.8b	66.5b
	Spring	1.75a	60.9a	74.8a	1.93a	64.5a	84.5a
Model effect <sup>b</sup>	df		]	Probability	values —		
$\pm N$	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
+N	6	0.0476	0.0009	0.0005	0.9166	0.7327	0.6201
SS	1	< 0.0001	< 0.0001	< 0.0001	0.0289	0.0055	0.0021
+N x SS	6	0.2570	0.1117	0.0440	0.6320	0.4466	0.6290
Contrast							
UR vs UR <sub>IN</sub>		0.2198	0.0639	0.0842	0.7801	0.994	0.8403
URs vs UR <sub>FIN</sub>		0.5467	0.7883	0.9121	0.1675	0.0740	0.0490
UAN vs UAN <sub>IN</sub>		0.0139	0.0100	0.0294	0.2269	0.1491	0.1045
UAN <sub>S</sub> vs UAN <sub>FIN</sub>		0.1216	0.2671	0.0672	0.1530	0.0761	0.0318

Table 5.5 Effects of season and treatments on grain yield, N removal, and N uptake of canola in the second year (2017/2018).

<sup>*a*</sup> UR, untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>DI</sub> are urea treated with ARM U, Agrotain Advanced, and ARM U Advanced, respectively applied at 75 kg ha<sup>-1</sup>; UAN, untreated urea ammonium nitrate; UAN<sub>ARM</sub>, and UAN<sub>DI</sub> are UAN treated with ARM U and ARM U Advanced, respectively applied at 75 kg N ha<sup>-1</sup>. ARM U and Agrotain Advanced are NBPT formulations while ARM U Advanced is a double inhibitor formulation (combined NBPT and nitrification inhibitor).

 $^{b}$  ±N, model effect between N amended versus control treatments; +N, model effect among N amended treatments without control plot.

Means with different letters within a column are significantly different at a probability level of < 0.1 LSD.

5.4 & 5.5). A similar observation was made between untreated UAN and UAN treated with inhibitors in PCL of both years and in CSL of the first year. Grain yield from spring-applied untreated urea or UAN was not significantly different from grain yield from fall-applied urea or UAN treated with inhibitors at each site in each year (Table 5.4 & 5.5).

# 5.4.3 Nitrogen Removal and Nitrogen Uptake

In the first year of the study, ANOVA did not show any significant effect of treatments among the N amended plots on wheat N removal and N uptake in both soils (Table 5.4). In CSL, fall-applied UR<sub>AG</sub> had the greatest wheat N removal while control with no N addition had the smallest wheat N removal. Wheat N removal from fall-applied UR<sub>AG</sub> (100.9 kg ha<sup>-1</sup>) was not significantly greater than wheat N removal from fall-applied UR<sub>ARM</sub> (78.3 kg ha<sup>-1</sup>) but was significantly greater than wheat N removal from fall-applied untreated urea (59.7 kg ha<sup>-1</sup>) and UR<sub>DI</sub> (63.8 kg ha<sup>-1</sup>). Interestingly in CSL, wheat N removal from fall-applied ut 75 kg N ha<sup>-1</sup> rate in the spring and similar to wheat N removal from untreated urea applied at 75 kg N ha<sup>-1</sup> rate in the spring (Table 5.4). Wheat N removal from fall-applied ut reatments was not significantly different from wheat N removal from fall-applied at 100 kg N ha<sup>-1</sup> rate in the spring (Table 5.4). Wheat N removal from fall-applied ut reatments was not significantly different from wheat N removal from fall-applied at 100 kg N ha<sup>-1</sup> rate in the spring (Table 5.4). Wheat N removal from fall-applied ut reatments was not significantly different from wheat N removal from the corresponding spring-applied treatments despite the lower N application rate in the



Figure 5.2. Effects of seasons and treatment on grain yield (A & D), grain N removal (B & E), and N uptake (C & F) of canola in the second year at Carman and Portage. Bars with different letters are significantly different at a probability level of < 0.1 LSD. Note: UR, untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>DI</sub> are urea treated with ARM U, Agrotain Advanced, and ARM U Advanced, respectively applied at 75 kg N ha<sup>-1</sup>; UAN, untreated urea

ammonium nitrate; UAN<sub>ARM</sub>, and UAN<sub>DI</sub> are UAN treated with ARM U and ARM U Advanced, respectively applied at 75 kg N ha<sup>-1</sup>. ARM U and Agrotain Advanced are NBPT formulations while ARM U Advanced is a double inhibitor formulation (combined NBPT and nitrification inhibitor).

spring. The pattern of wheat N uptake in CSL was similar to that of wheat N removal in the first year of the study. Fall-applied  $UR_{AG}$  had the greatest N uptake and control plot with no N added having the smallest wheat N uptake in CSL (Table 5.4). In PCL, wheat N removal from fall-applied  $UR_{ARM}$  (137.5 kg ha<sup>-1</sup>) was not significantly greater than wheat N removal from fall-applied untreated urea (120.9 kg ha<sup>-1</sup>) but was significantly greater than wheat N removal from fall-applied  $UR_{AG}$  (104.2 kg ha<sup>-1</sup>) and  $UR_{DI}$  (110.8 kg ha<sup>-1</sup>). There was no significant difference in wheat N removal among the spring-applied treatments irrespective of the rate or source of N in PCL (Table 5.4). Wheat N uptake for the N amended plots in PCL was also similar to the observed pattern of grain yield as wheat N uptake among the N amended plots was not significantly different from one another but significantly greater than wheat N uptake from the control (Table 5.4).

In the second year of the study, there were no significant season by treatment interactions on canola N removal at each site (Table 5.5). For the canola N uptake, a significant interaction between season and treatment was found in CSL but not in PCL (Table 5.5). At each site, there was a significant effect of season on canola N removal (Table 5.3). In CSL, average canola N removal was significantly greater in the spring than in the fall by 15%. The effect of season on canola N removal was prominent in the UAN treated with inhibitors than other treatments at CSL (Figure 5.2). Also, in CSL, there was a significant effect of treatments on canola N removal (Table 5.5). Average N removal from UAN<sub>ARM</sub> or UAN<sub>DI</sub> was significantly greater than canola N removal

from untreated UAN in CSL. Among the urea treated with inhibitors, only UR<sub>ARM</sub> had its average canola N removal to be greater than canola N removal from untreated urea (Table 5.5). The significant season by treatment interaction on canola N uptake in CSL was because canola N uptake from the spring-applied UAN (with and without inhibitors) was consistently greater than the corresponding N uptake from fall-applied UAN (with and without inhibitor), whereas no significant difference existed between fall and spring canola N uptake when urea granule was the source of N (Figure 5.2). In PCL, average canola N removal was significantly greater in the spring than in the fall by 14%. Unlike in CSL, there was no significant effect of treatment on canola N removal in PCL. In PCL, average canola N uptake was significantly greater in the spring than in the fall (Table 5.3). There was no significant effect of NBPT or DI with either urea or UAN on average canola N uptake in PCL (Table 5.5 & Figure 5.2).

In each year, contrast analysis showed that average N removal from urea treated with inhibitors was significantly greater than N removal from the untreated urea in CSL, but this trend was not observed in PCL (Table 5.4 & 5.5). Average N removal from fall-applied urea or UAN treated with inhibitor was not significantly different from N removal from spring-applied untreated urea or UAN, respectively, in CSL in each year. The N removal from fall-applied urea treated with inhibitors was not significantly different from N removal from spring-applied untreated urea in the first year in PCL but not in the second year. The N uptake between fall-applied urea or UAN treated with inhibitors and spring-applied untreated urea or UAN was not significantly different at both sites in the first year (Table 5.4). In the second year of the study, there was significantly greater N uptake in spring-applied urea or UAN than fall-applied urea or UAN treated with inhibitors, respectively in PCL.

# 5.4.4 Nitrogen Use Efficiency

There was no significant season by treatment interaction on  $AE_N$  at both sites (Table 5.6). While there was no significant effect of treatment on  $AE_N$ , there was a significant effect of season on  $AE_N$  at both sites. In CSL, average  $AE_N$  across treatment was significantly greater in the spring than in the fall by 32%. Contrast analysis showed that average  $AE_N$  in CSL was significantly greater from urea treated with inhibitors than untreated urea whereas there was no significant difference in  $AE_N$  between UAN treated with and without inhibitors (Table 5.6). Also,  $AE_N$  from spring-applied untreated urea or UAN was not significantly different from fall-applied urea or UAN treated with inhibitors in CSL. In PCL, average  $AE_N$  across treatment was significantly greater from UAN treated with inhibitors than untreated UAN whereas there was no significant difference in  $AE_N$  between urea treated with and without inhibitors (Table 5.6). Also,  $AE_N$  from spring-applied untreated urea or UAN was not significantly different from fall-applied urea or UAN treated with inhibitors in CSL. In PCL, average  $AE_N$  across treatment was significantly greater in the spring than in the fall by 27%. Unlike in CSL, average  $AE_N$  in PCL was significantly greater from UAN treated with inhibitors than untreated UAN whereas there was no significant difference in  $AE_N$  between urea treated with and without inhibitors (Table 5.6). Spring-applied untreated urea or UAN had significantly greater  $AE_N$  than fall-applied urea or UAN treated with inhibitors in PCL.

With the ARE<sub>N</sub>, there was no significant season by treatment interaction at each site (Table 5.6). While there was a significant effect of season on ARE<sub>N</sub> at both sites, significant effects of treatments existed only in CSL. Apparent recovery efficiency in CSL was significantly greater in the spring than in the fall by 39%. In CSL where there was a significant effect of treatments on ARE<sub>N</sub>, addition of either NBPT or DI increased ARE<sub>N</sub> of urea by 51 and 53%, respectively (Table 5.6).

		Carman san	dy loam	Portage clay loam	
		AE <sub>N</sub>	ARE <sub>N</sub>	AE <sub>N</sub>	<b>ARE</b> <sub>N</sub>
		kg grain kg <sup>-1</sup>	%	kg grain kg <sup>-1</sup>	%
Treatment <sup>a</sup> (TRT)	UR	8.5b	34.0b	14.1ab	67.6b
	UR <sub>NBPT</sub>	12.0a	51.4a	14.2ab	69.7b
	UR <sub>DI</sub>	11.2ab	51.9a	14.1ab	74.0ab
	UAN	9.8ab	54.8a	16.3a	88.7a
	<b>UAN</b> <sub>NBPT</sub>	11.6a	61.0a	13.1ab	62.6b
	UAN <sub>DI</sub>	12.4a	59.5a	12.3b	74.7ab
Season (SS)	Spring	12.4a	60.6a	15.7a	85.9a
	Fall	9.4b	43.7b	12.4b	59.9b
Model effect	df		— Probabil	ity values ——	
TRT	5	0.1915	0.0146	0.5768	0.322
SS	1	0.0054	0.0003	0.0063	< 0.0001
TRT x SS	5	0.8959	0.1672	0.787	0.6487
Contrast					
UR vs UR <sub>IN</sub>		0.0319	0.0063	0.9747	0.6617
URs vs UR <sub>FIN</sub>		0.8995	0.3108	0.042	0.054
UAN vs UAN <sub>IN</sub>		0.1927	0.4453	0.0589	0.0534
UAN <sub>S</sub> vs UAN <sub>FIN</sub>		0.7372	0.0871	0.0362	0.0036

Table 5.6 Effect of season and treatments on agronomic  $(AE_N)$  and apparent N recovery  $(ARE_N)$  efficiencies of urea-based fertilizers during the two years of study.

<sup>*a*</sup> UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT only (ARM U + Agrotain Advanced); UAN<sub>DI</sub>, UAN treated with double inhibitor (ARM U Advanced); UR<sub>IN</sub>, average of urea treated with inhibitors (ARM U, Agrotain Advanced, and ARM U Advanced); UR<sub>FIN</sub>, average of fall-applied urea treated with inhibitors; UR<sub>S</sub>, average of spring-applied untreated urea; UAN, untreated urea ammonium nitrate; UAN<sub>NBPT</sub>, and UAN<sub>DI</sub> are UAN treated with ARM U and ARM U Advanced, respectively; UAN<sub>IN</sub>, average of UAN treated with inhibitors (ARM U and ARM U Advanced); UAN<sub>FIN</sub>, average of fall-applied UAN treated with inhibitors; UAN<sub>S</sub>, spring-applied untreated urea untreated UAN.

Means with different letters within a column are significantly different a probability level of < 0.1 LSD.

Contrast analysis showed that ARE<sub>N</sub> from spring-applied untreated urea was not significantly

different from ARE<sub>N</sub> in fall-applied urea treated with inhibitors in CSL. In PCL, ARE<sub>N</sub> was

significantly greater in the spring than in the fall by 44%. Apparent recovery efficiency from spring-applied untreated urea was significantly greater than ARE<sub>N</sub> from urea treated with inhibitors at Portage (Table 5.6). Also, ARE<sub>N</sub> from spring-applied untreated UAN was significantly greater than ARE<sub>N</sub> from fall-applied untreated UAN in PCL.

#### **5.4.5 Residual Nitrate**

Analysis of variance did not show any significant effect of fertilizer treatment on residual nitrate in the first year in either CSL or PCL (Table 5.7). In CSL, the spring-applied untreated urea (applied at 75 kg N ha<sup>-1</sup>) and the control with no N application had the smallest residual nitrate (26.3 kg N ha<sup>-1</sup>). Multiple means comparisons revealed that there was a significant difference in residual nitrate between the two N rates of spring-applied untreated urea whereas the two N rates of spring-applied urea treated with ARM U were not significantly different from each other (Figure 5.3a). In PCL, multiple means comparisons did not show any significant differences in residual nitrate (Figure 5.3c).

In the second year, there was no significant season by treatment interaction effect on residual nitrate at each site (Table 5.7). The effect of season on residual nitrate was significant with average residual nitrate across the treatments being significantly greater in the spring-applied than in the fall-applied treatments (Table 5.7). In CSL, spring-applied UAN<sub>ARM</sub> had the greatest residual nitrate despite having the highest N uptake while the control had the smallest residual nitrate (Figure 5.3b). In PCL, there was no significant effect of N amendment on residual nitrate (Figure 5.3d).

	Fi	First year		nd year			
	<b>CSL</b> <sup>a</sup>	PCL	CSL	PCL			
Model effect <sup>b</sup>		Probability values					
$\pm N$	0.1441	0.4768	0.0002	0.0244			
+N	0.3724	0.9892	0.0147	0.7509			
Season <sup>c</sup> (SS)	0.3240	0.6570	0.0009	0.0013			
+N x SS	-	-	0.1481	0.9047			
Contrast <sup>d</sup>							
UR vs UR <sub>IN</sub>	0.4157	0.9936	0.4096	0.9402			
URs vs UR <sub>FIN</sub>	0.2854	0.8501	0.1156	0.1387			
UAN vs UAN <sub>IN</sub>	0.6617	0.8109	0.5448	0.3977			
UANs vs UAN <sub>FIN</sub>	0.3219	0.3351	0.2336	0.3833			

Table 5.7 Effect of treatment, season, and contrast analysis of treatment group means on residual nitrate in the first (2016/2017) and second year (2017/2018).

<sup>*a*</sup> CSL, Carman sandy loam; PCL, Portage clay loam.

 $^{b} \pm N$ , model effect between N amended versus control treatments; +N, model effect among N amended treatments without control plot.

<sup>c</sup> Season effect in the first year was a CONTRAST between fall versus spring N amended plot.

<sup>*d*</sup> UR, untreated urea; UR<sub>IN</sub>, average of urea treated with inhibitors (ARM U, Agrotain Advanced, and ARM U Advanced); UR<sub>FIN</sub>, average of fall-applied urea treated with inhibitors; UR<sub>S</sub>, spring-applied untreated urea; UAN, untreated UAN; UAN<sub>IN</sub>, average of UAN treated with inhibitors (ARM U and ARM U Advanced); UAN<sub>FIN</sub>, average of fall-applied UAN treated with inhibitors; UAN<sub>S</sub>, spring-applied untreated UAN.

Contrast analysis showed that the average residual nitrate from untreated urea or UAN was not

significantly different from urea or UAN treated with inhibitors at each site in each year (Table

5.7). Residual nitrate from spring-applied untreated urea or UAN was also not significantly

different from that in fall-applied urea or UAN treated with inhibitors at each site.



Figure 5.3. Effects of seasons and treatment on residual nitrate (0 - 60 cm) in the first (A & C) second (B & D) years.

Bars with different letters are significantly different at a probability level of < 0.1 LSD. UR, untreated urea; UR<sub>ARM</sub>, UR<sub>AG</sub>, and UR<sub>DI</sub> are urea treated with ARM U, Agrotain Advanced, and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated in the legend; UAN, untreated urea ammonium nitrate; UAN<sub>ARM</sub>, and UAN<sub>DI</sub> are UAN treated with ARM U and ARM U Advanced, respectively applied at the N rate (kg ha<sup>-1</sup>) indicated. ARM U and ARM U Advanced

are NBPT formulations while ARM U Advanced is a double inhibitor formulation (combined NBPT and nitrification inhibitor).

#### 5.5 Discussion

The two years of study were relatively dry years with smaller than the normal rainfall during the growing season. However, available residual soil water from the fall of 2016 provided sufficient moisture to the wheat crop in 2017 and ensured an average grain wheat yield of 4.1 Mg ha<sup>-1</sup>, which was the same as the Manitoba provincial wide highest record yield before 2017, but 25% more than the last 10-year average yield in the province of Manitoba (MASC 2018). In contrast, the average canola grain yield in 2018 (1.7 Mg ha<sup>-1</sup>) was 19% lower than the last 10-year average yield in the province of Manitoba because of the two back to back dry years (MASC 2019).

High variability amongst the four replicates hindered our ability to detect significant differences in some of the treatments even when the differences between treatments were relatively high. As reported by earlier studies (Tiessen et al. 2005; Jaynes 2015), we observed fall N application to be less efficient than spring N application in the second year. The lack of significant differences in grain yield between fall and spring application of the same treatment in the first year despite higher N application with the fall than spring treatments could be due to the relatively high residual nitrate at the start of the study (Table 5.1). The residual nitrate at the start of the study was sufficient to produce 2.2 and 1.9 Mg ha<sup>-1</sup> of wheat grain without N fertilization in CSL and PCL, respectively based on the Manitoba soil fertility guide (MAFRI 2007). This is supported by the amount of wheat grain produced in control plots relative to N fertilized plots.

The use of NBPT has been reported to reduce ammonia volatilization from surface-applied urea in all soils (Cantarella et al. 2018). However, the conserved N only translated into an increase in yield, N uptake, and NUE in only CSL but not in PCL. The lack or inconsistency of the effect of
NBPT (with or without NI) on the yield of small grain is common on the Canadian prairie as reported in previous studies of McKenzie et al. (2010), Grant (2014), and Perrott et al. (2018), where they found no effect of NBPT on grain yield from surface-applied urea. The lack of NBPT effect on yield and N uptake of small grains is because the N conserved with the use of NBPT is relatively small compared to the applied N fertilizers and soil N (residual nitrate + mineralization) to influence the yield of wheat and canola. Ammonia volatilization measurements in these two fields (from Chapter 4) following fertilizer treatment application showed an average of 7 kg N ha<sup>-</sup> <sup>1</sup> difference in N losses between urea-based fertilizers treated with and without inhibitors over the two growing seasons. A meta-analysis by Abalos et al. (2014) showed that yield response to the use of NBPT and NI occurs more consistently on crops with high than low to medium N requirements. Unlike in our study where we applied an average of 87.5 kg N ha<sup>-1</sup> over the two years of study, a study by Liu et al. (2019) that applied N rates that were 40 to 92% greater than our N rate to corn reported a significant increase in corn yield with NBPT at two sites over three years of study, because of the higher N requirement for the yield goal of corn than for wheat. Also, the lack of significant effect of inhibitor in PCL could be partly because of the amount of N supplied by the soil (mineralization) in PCL which was large enough to reduce N fertilizer response to grain yield as shown by the residual nitrate after harvest in each year (Figure 5.3). A review of literature on the use of NBPT with and without NI and its effect on yield by Li et al. (2018) and Rose et al. (2018) showed that the benefit from inhibitors is realized only when the quantity of soil N plus fertilizer N is below the optimum N rate for yield response.

Although average  $AE_N$  and  $ARE_N$  from spring-applied N fertilizer were greater than fall-applied N fertilizers, the lack of significant differences in  $AE_N$  and  $ARE_N$  between spring-applied untreated

urea/UAN and fall-applied urea/UAN treated with inhibitors supports our hypothesis in CSL but not in PCL. Relatively dry spring in the two years with the well-drained soil in CSL could have resulted in reduced N losses via denitrification in the spring after snowmelt from fall-applied treatments, allowing the inhibitors to bridge the N use efficiency gap between fall and springapplied fertilizer. In contrast, the poorly drained nature of the soil in PCL might have caused significant N losses via denitrification after snowmelt leading to a low AE<sub>N</sub> and ARE<sub>N</sub> from fallapplied urea/UAN treated with inhibitor than spring-applied untreated/UAN despite the conserved N by the inhibitors. Earlier studies in Manitoba had shown that the differences between fall versus spring application on N use efficiency were smaller on well-drained soils but greater on poorly drained soils and N losses via denitrification process has been suggested to account for this (Grant et al. 2001; Tiessen et al. 2005). Overall, while NBPT and NI did not consistently produce much of agronomic benefit, their use may protect the environment through reduced ammonia volatilization and provide farmers some flexibility in the timing of N application.

## **5.6 Conclusion**

This study confirmed previous observations that fall-applied N fertilizers are less efficient than spring-applied. Canola and wheat grain yields, N removal, and N uptake from urea and UAN treated with inhibitors were not consistently greater than grain yield, N removal and N uptake from untreated urea and UAN in this study. The use of inhibitors to bridge N use efficiency gap between fall and spring-applied urea-based fertilizers was observed in CSL only. The conserved N losses by the NBPT did not consistently show up in the soil. While NBPT may be efficient in reducing ammonia volatilization on the Canadian prairies, its use to increase grain yield, N removal, and N uptake relative to untreated urea and UAN may be soil specific. Also, the potential of NBPT with or without NI to bridge NUE gap between fall and spring-applied urea-based fertilizers is soil specific.

#### **5.6 References**

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#### **CHAPTER 6**

# 6 NITRIFICATION INHIBITOR REDUCES THE INHIBITORY EFFECT OF N-(n-BUTYL) THIOPHOSPHORIC TRIAMIDE (NBPT) ON THE HYDROLYSIS OF UREA

## 6.1 Abstract

The addition of nitrification inhibitor (NI) with a urease inhibitor, N-(n-butyl) thiophosphoric triamide (NBPT), has been reported to offset the reduction of ammonia volatilization by NBPT. An incubation study was conducted to investigate the interaction between NBPT and NI (3,4dimethylpyrazole phosphate) on hydrolysis of urea in five soils with a range of physico-chemical properties. Untreated urea (UR), NBPT treated urea (UR<sub>NBPT</sub>), or NBPT+NI treated urea (UR<sub>DI</sub>) were surface-applied (250 kg N ha<sup>-1</sup>) to each soil. The soils were incubated (21 °C) and destructively sampled nine times during a 22-day period. Urea hydrolysis rate  $(k; d^{-1})$  was measured by the disappearance of urea with time and modeled with a first-order kinetic. The urease inhibitor, NBPT, significantly reduced k in each soil, while the addition of a NI with NBPT significantly increased k when compared to NBPT alone. The value of k was in the order of UR  $(0.321) > UR_{DI}$  (0.183) > UR<sub>NBPT</sub> (0.151) across the five soils. We found that NI significantly reduced the half-life of urea by about 1 d when compared with NBPT alone. Principal component analysis showed that k did not depend on any of the soil properties, rather, it depends on the type of treatment. Net nitrification rate constant was significantly greater in UR than UR<sub>NBPT</sub> in loam and clay soils but not different in sandy loam soils. We conclude that the often-reported increase in ammonia volatilization with the double inhibitor relative to NBPT alone may not only be due to the persistence of ammonium but may also be due to an increased rate of urea hydrolysis in the presence of a NI.

## **6.2 Introduction**

Globally, urea accounts for more than 50% of nitrogen (N) fertilizers that are applied to agricultural and horticultural fields (IFA 2017). Its ease of handling, storage, application, and relative high N content are factors that encourage its high demand. When urea is applied to the soil, the presence of urease enzymes catalyzes its breakdown into ammonia and carbamate ion. The carbamate spontaneously breaks down to give a second ammonia and bicarbonate ion (Mazzei et al. 2019). This process induces an increase in soil pH around the urea granules thereby shifting the equilibrium between ammonium and ammonia toward ammonia. The ammonia produced has a high potential of being volatilized into the atmosphere with negative consequences to human health and the environment (Sheppard et al. 2010). In surface-applied urea, the N losses from urea via ammonia volatilization may be greater than 20% of applied N (Frame et al. 2012; Mira et al. 2017).

Several compounds or additives have been developed to reduce ammonia volatilization by slowing down the hydrolysis of urea. They include boric acid, N-(n-butyl) thiophosphoric triamide (NBPT), maleic-itaconic polymer, N-(n-propyl) thiophosphoric triamide, N-(2-nitrophenyl) phosphoric triamide, hydroquinone, benzoylthioureas (Cantarella et al. 2018; Modolo et al. 2018). Of these additives, NBPT is the most widely used urease inhibitor to reduce ammonia volatilization and it is effective even at low concentrations (Modolo et al. 2018). The inhibition of urea hydrolysis by NBPT has been thought to be as a result of its conversion to oxygen analogue, N-(n-butyl) phosphoric triamide (NBPTO), by the transformation of its P=S moiety to P=O moiety (Creason et al. 1990). However, a recent study has shown that in addition to NBPT being converted to NBPTO, NBPT may also be converted to N-(n-butyl) thiophosphoric diamide (NBPD) by

replacing one of its amide group by a hydroxyl group (Mazzei et al. 2019). The NBPTO and NBPD hydrolyze to diamido phosphoric acid and monoamido thiophosphoric acid, respectively. These then attack the urease enzyme at its two nickel ions active sites via two oxygen atoms and one NH<sub>2</sub> group (Mazzei et al. 2019). Field and greenhouse studies have shown that NBPT reduces ammonia volatilization from urea-based fertilizer by a global average of 52% through the slowing down of urea hydrolysis (Cantarella et al. 2018; Sigurdarson et al. 2018).

In addition to the use of NBPT, nitrification inhibitors (NI) such as 3,4- dimethylpyrazole phosphate (DMPP), nitrapyrin, and dicyandiamide are sometimes applied with urea to maximize agronomic efficiency and reduce undesirable products of nitrification such as nitrous oxide emission and nitrate leaching (Subbarao et al. 2006). Of these NI, DMPP has been reported to be the most effective at low concentrations and able to inhibit over a longer period of time than nitrapyrin, and dicyandiamide (Wissemeier et al. 2001; Zerulla et al. 2001; Guardia et al. 2018). The NI slows down the conversion of ammonium to nitrite by suppressing the activities of ammonia mono-oxygenase enzyme. This enables the applied N to persist longer in the ammonium form. The persistence of N in the ammonium form through the action of a NI has an unintended consequence of enabling N losses via ammonia volatilization when conditions are favourable (Soares et al. 2012; Pan et al. 2016).

While NBPT has been shown to effectively reduce ammonia volatilization from surface-applied urea, addition of NI with NBPT (as a double inhibitor, DI) to urea has been reported to offset the benefit of NBPT in reducing ammonia volatilization (Gioacchini et al. 2002; Zaman et al. 2008; Soares et al. 2012; Frame 2017). These studies have shown that even though ammonia

volatilization was lower from urea treated with DI than untreated urea, ammonia volatilization was greater from urea treated with DI than NBPT only. A similar observation was made in Chapter 4 in spring-applied urea. The study of Soares et al. (2012) found that while NBPT reduced ammonia volatilization from urea by an average of 66% in their two experiments, ammonia reduction from urea by DI was an average of 28%. The studies of Soares et al. (2012) and Frame (2017) showed that the potential to increase ammonia volatilization with DI relative to NBPT only increased as the concentration of NI in the DI increased. Greater ammonia volatilization from DI relative to NBPT has been assumed to be due to the persistence of ammonium by the NI thereby offsetting the benefit of NBPT to reduce ammonia volatilization. Despite this known consequence of DI in increasing ammonia volatilization relative to NBPT only, there is a dearth of information on the role of NI on the inhibition of urea hydrolysis by NBPT. This study was conducted to evaluate the interaction between NBPT and NI on the hydrolysis of urea. We hypothesized that the addition of NI with NBPT influences the inhibitory effect of NBPT on hydrolysis of urea.

## **6.3 Materials and Methods**

## 6.3.1 Soil and Inhibitor Treatments

Soil samples (0 – 15 cm) were collected from five locations in Manitoba, Canada for this study. The soils vary in physical and chemical properties (Table 6.1). The locations were Carman (CM; 49° 29' 6" N, 98° 02' 2" W), Carberry (CB; 49° 53' 7" N, 99° 22' 29" W), Deerwood herein in refer to as Dezwood (DZ; 49° 22' 1"N, 98° 23' 34" W), High Bluff (HB; 50° 01' 2" N, 98° 08' 9"W) and Beausejour (BJ; 50° 05' 13" N, 96° 29' 58" W). According to MAFRI (2010), CM, CB, DZ, and BJ soils belong to the Chernozemic soil order in the Canadian soil classification (an equivalent of

Mollisol in USDA classification system) while HB belongs to Regosolic soil order (an equivalent of Entisol in USDA classification system).

Prior to the start of the study, the soils were air-dried, sieved (< 2 mm) and characterized (Table 6.1) for soil texture (Gee and Bauder 1986), organic matter (Walkley and Black 1934), pH(soil/water, 1:2), electrical conductivity, cation exchange capacity (Hendershot et al. 2008), urease activity (Tabatabai and Bremner 1972), and available N (Maynard et al. 2008).

The inhibitor treatments used for the study were uncoated urea (UR), urea coated with NBPT (UR<sub>NBPT</sub>), urea coated with double inhibitor (UR<sub>DI</sub>). A control with no urea application was also included in the study. The UR<sub>NBPT</sub> (360 mg NBPT kg<sup>-1</sup> urea) was prepared by coating urea with an NBPT formulation known as ARM U formulation (18% NBPT m/v) while UR<sub>DI</sub> (360 mg NBPT + 90 mg DMPP kg<sup>-1</sup> urea) was prepared by coating urea with a DI formulation known as ARM U Advanced formulation (24% NBPT + 6% DMPP m/v). The rate of NBPT used was the optimum rate with the formulation for reducing ammonia volatilization from surface-applied urea in the previous study (Chapter 2).

## 6.3.2 Experimental Design and Incubation Setup

The experiment was set up as soil (5) by inhibitor treatment (4) by sampling time layouts (9), arranged in a complete randomized design with three replications. We weighed 25 g of each soil into a 30 mL volumetric cup, wetted the soil to 75% field capacity and covered the cups for 24

Table 6.1 Selected soil (0 - 15 cm) properties.

	Carman	Carberry	Dezwood	High Bluff	Beausejour
Soil classification	Orthic Black	Orthic Black	Orthic Dark Gray	Gleyed Cumulic	Gleyed Rego Black
	Chernozem	Chernozem	Chernozem	Regosol	Chernozem
Soil series	Hibsin	Fairland	Dezwood	High Bluff	Dencross
Soil pH <sub>water</sub>	5.51	6.65	6.62	7.46	7.76
Electrical conductivity ( $\mu$ S cm <sup>-1</sup> )	394	228	1853	899	1377
Organic matter (g kg <sup>-1</sup> )	27	33	34	45	88
Available N (mg kg <sup>-1</sup> )	31	15	186	58	22
Urease activity (mg NH4 <sup>+</sup> -N kg <sup>-1</sup> soil hr <sup>-1</sup> )	11	17	24	57	63
Cation exchange capacity (cmol kg <sup>-1</sup> )	16	14	23	28	47
Soil texture	Sandy loam	Sandy loam	Loam	Loam	Clay
Sand (g kg <sup>-1</sup> )	711	764	465	427	108
Silt (g kg <sup>-1</sup> )	123	128	318	325	322
Clay (g kg <sup>-1</sup> )	166	108	217	248	570

hours for soil water to equilibrate. After twenty-four, the cover was removed, and 50 mg (250 kg N ha<sup>-1</sup>) of each inhibitor treatment was applied to the center of each soil by sampling time layout. We arranged the soils on trays that contained water (to reduce evaporation rate from the samples) and placed in an incubator (Isotope Incubator, Model 304, Fisher Scientific) set at a temperature of 21 °C for 22 days. The cups were kept open in the chambers. Every two days during the experiment, we randomly weighed four cups of each soil for moisture loss. Water was added to the edge of each cup with a pipette to make the soil up to its initial moisture content.

## **6.3.4 Sampling and Laboratory Analysis**

On 0.5, 1, 2, 4, 7, 10, 14, 18, and 22 days after fertilization (d), we destructively sampled three replicates of each soil by inhibitor treatment (i.e 60 samples comprising five soils x four inhibitor treatments x three replicates). Each cup with the soil was put in a 1000 mL jar, and 250 mL of 1 M KCl containing 5 mg L<sup>-1</sup> phenylmercuric acetate was dispensed in the jar. The jars were placed on reciprocating shakers and shaken for one hour. After one hour, the samples were filtered with Watman #40 filter paper into 25 mL scintillating vials and the aliquots were stored in the refrigerator (4 °C). Within two days, the aliquots of the urea amended treatments were analyzed colorimetrically for urea-N (Mulvaney and Bremner, 1979). Ammonium-N and nitrate-N concentrations from the aliquots were also analyzed colorimetrically using AQ2 Discrete Analyzer (SEAL Analytical Inc. WI).

## **6.3.5 Calculations**

The urea-N measured at each sampling time was expressed as % of applied urea-N. Urea hydrolysis was measured by the disappearance of urea with time (equation 6.1).

$$U_{hyd} = U_0 - U_t \tag{6.1}$$

Where  $U_{hyd}$  is the hydrolyzed urea;  $U_0$  is the amount of urea applied at time zero (expressed as %);  $U_t$  is the amount of urea recovered (% of applied urea-N)

Net inorganic N concentration (ammonium or nitrate) in the soil was calculated as described by (Li et al. 2018; Wang et al. 2019):

$$NIN_{t} = IN_{t} - IN_{i}$$
(6.2)

Where NIN<sub>t</sub> is the net ammonium or nitrate concentrations (mg N kg<sup>-1</sup>) at time t;  $IN_t$  is the ammonium or nitrate concentrations (mg N kg<sup>-1</sup>) measured from the soil at time t;  $IN_i$  is the ammonium or nitrate concentrations (mg N kg<sup>-1</sup>) measured from the soil before the start of the study; t is time (d).

## 6.3.6 Kinetics and Statistical Analysis

All model fittings and statistical analyses were performed with SAS software (SAS Institute 2014; version 9.4). Different non-linear kinetic models such as first-order, first- and zero-order, firstplus linear-order, and hyperbolic models were tested to model urea hydrolysis rate constant (k; d<sup>-1</sup>) with PROC NLIN. Of these models, the first-order kinetic model (equation 6.2) best fits the data because it had the lowest Akaike's Information Criterion (AIC). The first-order kinetic model used to generate k is as follows:

$$U_{\rm hyd} = U_0 [1 - \exp(-kt)]$$
 (6.3)

Parameters are as defined above.

The generated *k*, the first-order rate constant, was used to calculate the half-life  $(t_{1/2})$  for the inhibitor treatments in each soil as:

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \tag{6.4}$$

For the net nitrate concentration which is also net nitrification, different sigmoid function models such as Gompertz, Richards, logistics and Weibull functions (Archontoulis and Miguez 2015) were used to model net nitrification rate constant of UR, UR<sub>NBPT</sub>, and UR<sub>DI</sub> with PROC NLIN in each soil. Unlike the urea hydrolysis rate constant where replicates were used in the model fitting, means of the replicates were used in these models due to the non-convergence of some replicates in the models. The logistics sigmoid function gave the best fit based on the AIC. The logistic function used is as follows:

$$NN = \frac{N_m}{\{1 + \exp[-k(t - t_m)]\}}$$
(6.5)

Where NN is net nitrification (mg N kg<sup>-1</sup>);  $N_m$  is the maximum NN (mg N kg<sup>-1</sup>); k is the nitrification rate constant (d<sup>-1</sup>); t is time (d);  $t_m$  (d) is the inflection point at which nitrification is maximized.

PROC GLIMMIX for repeated measure analysis was used to determine the effect of inhibitor treatment on urea-N recovered and net inorganic N with time. The repeated measured analysis was performed by soil in which inhibitor treatment and time are the main effects. In agreement with the experimental layout, the net inorganic N for repeated measure analysis was analyzed in a factorial + 1 control design [allowed for comparison (i) between the urea amended versus control treatments (ii) among the urea amended treatments]. Covariance structure with the lowest Akaike's Information Criterion that accounted for unequal repeated factor (time) interval was used in the model statement. The urea-N recovered and inorganic N concentrations best fit beta and normal distributions, respectively, as such their distribution was specified in the model.

Analysis of variance was conducted on the generated k and half-life with PROC GLIMMIX (gamma distribution) in which soils and inhibitor treatments were fixed effects. Mean comparison was deemed significant at a probability level of < 0.05 Fisher protected least significant difference (LSD). The goodness of fit for the model was tested with normalized root means square error and Nash Sutcliffe (Moriasi et al. 2007). Due to correlation among the soil properties with PROC CORR, we used principal component analysis (PCA) to determine the relationship between the soil properties and k as it can provide a summary of the specific soil properties that drive the hydrolysis rate while avoiding redundancy (Liu et al. 2011). Following PCA, stepwise regression was conducted for each inhibitor treatment to predict k with the soil variables. For the net nitrification, parameter estimates were considered significant if their 95% confidence interval did not overlap.

#### 6.4 Results and Discussion

### 6.4.1 Urea Recovery and Hydrolysis Rate Constant

There was a significant inhibitor treatment by time interaction on % of urea-N recovered in only two (CB and DZ) of the five soils (Table 6.2). The amount of urea-N recovered decreased with time due to the hydrolysis of urea (Figure 6.1). While there was a significant difference in the amount of urea-N remaining among the inhibitor treatments by 4 and 7 d in each soil, the amount of urea-N recovered among the inhibitor treatments was not significantly different from 10 d in BJ and CM soils, and from 14 d in CB and HB soils. Across the soils, the pattern of urea-N recovery was similar among the inhibitor treatments with UR having the lowest urea recovery in the first 10 to 14 d followed by UR<sub>DI</sub> (Figure 6.1). By 4 d, less than 40% of the applied urea-N was recovered from UR in each of the soils whereas between 40 and 74% of applied urea-N was recovered from UR<sub>NBPT</sub> and UR<sub>DI</sub> across the soils. Urea hydrolysis was near completion by 10 d in UR with at least 97% of the applied urea being hydrolyzed in all the soils. Urea-N recovery from each of the inhibitor treatments was least in CM soil which is a sandy loam with acidic pH and greatest in DZ soil which is loamy soil with neutral pH (Figure 6.1). While soils with acidic pH are reported to sometimes have a lower potential for ammonia volatilization from surfaceapplied urea than alkaline soils (as reported in Chapter 2), the low pH of the CM soil did not limit urea hydrolysis in this study. This is probably because it will require more consumption of  $H^+$  to drive the pH of acidic soil to alkaline pH that is favourable for ammonia volatilization since the relative quantity of ammonia and ammonium in soil is pH dependent. The least amount of urea-N recovered from urea treated with and without NBPT in CM (acidic) soil corroborated earlier studies where they recovered a lower amount of urea from sandy loam than clay soils (Suter et al.

2011) and lower recovery from acidic than alkaline soils (Engel et al. 2011).

Table 6.2. Effect of inhibitor treatment on urea-N recovered, net ammonium-N, and net nitrate-N concentrations.

		Carman	Carberry	Dezwood	High Bluff	Beausejour
	Model effect <sup>a</sup>	Probability values				
Urea-N rec	covered					
	Inhibitor treatment (I)	0.2195	0.0011	< 0.0001	0.0020	0.0004
	Time (T)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	ΙxΤ	0.3283	< 0.0001	< 0.0001	0.3616	0.0710
Net ammo	nium-N concentrations					
	±I	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Ι	0.4467	0.2081	0.4677	0.1146	0.0002
	Т	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	ΙxΤ	0.4042	0.0052	0.1845	0.5817	< 0.0001
Net nitrate-N concentrations						
	±Ι	< 0.0001	0.0088	< 0.0001	< 0.0001	< 0.0001
	Ι	0.2938	0.4852	0.2505	0.0966	0.0735
	Т	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	I x T	0.0923	0.1152	< 0.0001	0.0002	0.1662

 $^{a} \pm I$ , is the comparison between urea amended and control treatments. Probability values are significant at < 0.05.



Figure 6.1 Amount of urea-N recovered (% of applied urea-N) during the 28-d incubation period. Error bars are standard errors of the mean.

UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors). \* indicates days when urea-N recovered was significantly greater in UR<sub>NBPT</sub> than UR<sub>DI</sub> at a probability of < 0.05.

There was a significant soil by inhibitor treatment interaction on k (Table 6.3). The significant soil by inhibitor treatment interaction was because k was significantly smaller in UR<sub>NBPT</sub> than UR<sub>DI</sub> in all the soils except in CM soil (Figure 6.2). In CM soil, k was not significantly different between UR<sub>NBPT</sub> and UR<sub>DI</sub> (Figure 6.2). Expectedly, k was greatest in UR among the inhibitor treatment in each of the soils. Dezwood soil, with neutral pH, had the smallest k with each of the three inhibitor treatments, while CM soil had the greatest k with each of the three inhibitor treatments (Figure 6.2). The greatest k in the CM soil may be in part due to its texture and pH. The sandy loam texture of CM soil would enable greater diffusion of urea and the ammonium produced in the soil since diffusion is dependent on soil pore sizes and tortuosity (Neira et al. 2015). With the exclusion of CM soil, k increased as the pH increased from neutral to alkaline. This is similar to the results from a previous study that showed an increase in k as the pH of a soil was adjusted from acidic to alkalinity with potassium hydroxide solution (Cabrera 1991). Averaged across the soils, the k was in the order of UR > UR<sub>DI</sub> > UR<sub>NBT</sub> (Table 6.3).

The addition of NBPT reduced k by 30% to 65% across soils. The addition of NBPT had little effect on k in the acidic soil compared to soils with neutral or alkaline pH. This is similar to results from previous studies that found the inhibition of urea hydrolysis by NBPT to be lower in acidic than alkaline soils (Suter et al. 2011; Fan et al. 2018). An NBPT degradation study by Engel et al. (2015) showed that the degradation of NBPT was faster in acidic than in alkaline soils; hence the greater k for UR<sub>NBPT</sub> and UR<sub>DI</sub> in acidic soil than alkaline soil. The addition of NI with NBPT significantly reduced the inhibitory effect of NBPT on urea hydrolysis by an average of 21% across soils. The increased rate of urea hydrolysis with DI relative to NBPT only, partly explains the

Model effect	k	t <sub>1/2</sub>	
	(d <sup>-1</sup> )	(d)	
Inhibitor treatment <sup>a</sup> (I)			
UR	0.321a	2.2c	
UR <sub>DI</sub>	0.183b	3.8b	
UR <sub>NBPT</sub>	0.151c	4.6a	
Soil (S)			
Carman	0.278a	2.5e	
Carberry	0.183d	3.8b	
Dezwood	0.145e	4.8a	
High Bluff	0.211c	3.3c	
Beausejour	0.245b	2.8d	
	—— Probability values—		
Ι	< 0.0001	< 0.0001	
S	< 0.0001	< 0.0001	
I x S	< 0.0001	< 0.0001	

Table 6.3 Effect of inhibitor treatment and soil on urea hydrolysis rate constant (*k*) and half-life  $(t_{1/2})$ .

<sup>*a*</sup> UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors).

Means with different letters within a column are significantly different at Probability values of < 0.05 Fisher protected LSD.

reason for the greater ammonia volatilization from  $UR_{DI}$  than  $UR_{NBPT}$  measured in previous studies (Soares et al. 2012; Frame 2017).

The half-life of urea hydrolysis showed significant soil by inhibitor treatment interaction (Table 6.3). At the rate of urea hydrolysis, it will take an average of 2.2 d for 50% of untreated urea to hydrolyze, 3.8 d for 50% of UR<sub>DI</sub> to hydrolyze and 4.6 d for 50% of UR<sub>NBPT</sub> to hydrolyze. While the addition of NBPT extended the half-life of urea in soils, the addition NI with NBPT reduced the half-life of urea relative to NBPT only. The half-life of urea with and without inhibitor

corroborate the commonly observed pattern of ammonia volatilization in urea with untreated urea having most of the ammonia volatilization within the first 7 d when conditions are favorable whereas peak ammonia volatilization from urea treated with NBPT is usually delayed to after 7 d (Soares et al. 2012; Viero et al. 2014).



Figure 6.2 Effect of soil and inhibitor treatment on urea hydrolysis first-order kinetics rate constant. Error bars are standard errors of the mean.

UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors).

Bars with different letters are significantly different at a probability level < 0.05 Fisher protected LSD.

The normalized root means square error showed that the deviation between the observed urea hydrolysis and the estimated urea hydrolysis with the first-order *k* ranged from 2.8 to 15.0% across soil by inhibitor treatment. Similarly, the Nash–Sutcliffe model efficiency ranged from 95.4 to 99.6% in all the soil by inhibitor treatment. The first-order kinetics model has been reported to efficiently predict urea hydrolysis in soil under various conditions (Rodriguez et al. 2005; Lei et al. 2018).

The PCA showed that only two principal components (PC 1 and PC 2) accounted for 88% of the total variance (Figure 6.3). The clustering of the variables showed that k is independent of the selected soil properties (Figure 6.3). Instead, k was influenced by the type of inhibitor treatments. The lack of dependency of k on soil pH as shown in Figure 6.3a contrasts with the study by Cabrera (1991) where k increased with an increase in pH. The significant relationship between k and pH in the study of Cabrera (1991) maybe because the pH adjustment was made on a single soil type while all other parameters remained unchanged. Even when soils were collected from the same location, but different profiles and pH was adjusted to be similar, as was carried out by Fisher et al. (2016), the k was significantly different between the profiles. In addition, the component scores plot (Figure 6.3b) showed that the five soils clustered into three groups along PC 1 that accounted for most of the variance: (i) CM and CB soils (ii) DZ and HB soils (iii) BJ soil. This grouping followed the pattern of their soil texture, pH, organic matter, and urease activity (Table 6.1). The implication of this is that the hydrolysis of urea may be soil and inhibitor treatment specific with interactions between various soil variables and the inhibitor treatment. When the regression was performed by inhibitor treatment, k for each inhibitor treatment was influenced by a different



Figure 6.3 Component pattern (a) and component scores (b) of the principal component analysis of soil properties. FC, field capacity; OM, organic matter; H\_conc, hydrogen ion concentration; k is urea hydrolysis rate constant; urease, urease activity.

Inhibitor	$\mathbb{R}^2$	<b>RMSE</b> <sup>b</sup>	Soil parameters	Parameter	Probability
treatment <sup>a</sup>				coefficient	values
UR	0.400	0.042	H <sup>+</sup> concentration	27333	0.0220
			Organic matter	0.0120	0.0510
UR <sub>DI</sub>	0.959	0.01	H <sup>+</sup> concentration	41626	< 0.0001
			Urease activity	0.0031	< 0.0001
			Silt	-0.0033	0.0001
UR <sub>NBPT</sub>	0.946	0.014	H <sup>+</sup> concentration	54123	< 0.0001
			Organic matter	0.0405	0.0006
			Urease activity	0.0011	0.0105
			Clay	-0.0045	0.0022

Table 6.4 Parameter estimates for soil properties influencing hydrolysis rate constant by inhibitor treatment.

<sup>*a*</sup> UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors).

<sup>b</sup> RMSE, model root means square error.

Probability values are significant at < 0.05.

combination of soil properties (Table 6.4). While *k* in UR was influenced by two soil variables (H<sup>+</sup> concentrations and organic matter), *k* in UR<sub>NBPT</sub> was influenced by four soil variables (H<sup>+</sup> concentrations, clay, urease activity, and organic matter; Table 6.4). A study that measured ammonia volatilization from urea treated with and without NBPT or maleic-itaconic copolymer (urease and nitrification inhibitor) in 79 soils under the same conditions found that the relationship between ammonia volatilization and soil variables is not only soil specific but also treatment specific (Sunderlage and Cook 2018).

## 6.4.2 Net Inorganic N Concentrations

When the urea amended treatments were compared to the control without urea addition, net ammonium concentration in soil was significantly greater in the urea amended treatments than the control (Table 6.2). The ANOVA with repeated measure analysis showed that there was a significant inhibitor treatment by time interaction on ammonium concentrations in CB and BJ soils but not in CM, DZ, and HB soils (Table 6.2). Significant inhibitor by time interaction in CB soil was because net ammonium concentration in UR was significantly greater than net ammonium concentration in  $UR_{DI}$  and  $UR_{NBPT}$  on 4 d whereas net ammonium concentration was significantly greater in  $UR_{DI}$  and  $UR_{NBPT}$  than UR on 10 and 14 d (Figure 6.4). In BJ soils, net ammonium concentration was significantly greater in UR than  $UR_{DI}$  and  $UR_{NBPT}$  than UR on 10 and 14 d (Figure 6.4). In BJ soils, net ammonium concentration was significantly greater in UR than  $UR_{DI}$  and  $UR_{NBPT}$  than UR on 18 d. Net ammonium concentrations from urea amended treatments in each of the soils were very small on 0.5 and 1 d and not significantly different from the net ammonium in the control. The lack of significant difference in net ammonium concentration between the urea amended treatments and control during these first two sampling periods was an indication that it takes 1 to 2 d for a remarkable amount of ammonium to be measured following the application of urea (Tian et al. 2015).

This pattern of net ammonium production is similar to the trend of ammonia volatilization that usually requires at least two days for ammonia losses from surface-applied urea to be greater than control or soil background ammonia losses (Tian et al. 2015; Frame 2017). Across soils, net ammonium concentrations in the inhibitor treatments were relatively greater in CM soil than in the other four soils possibly because of the greater k in the CM soil (Figure 6.4). We found that net ammonium concentrations in each soil from UR was similar to net ammonium concentrations from UR<sub>DI</sub> and UR<sub>NBPT</sub> during most of the sampling periods (Figure 6.4). The similarity of net ammonium measured in the soils between UR and urea treated with the inhibitor was possibly due



Figure 6.4 Net ammonium concentration in each soil. Error bars are standard errors of the mean. UR, untreated urea;  $UR_{NBPT}$ , urea treated with NBPT;  $UR_{DI}$ , urea treated with double inhibitor (combined NBPT and nitrification inhibitors).

to ammonia volatilization of the ammonium produced from UR with the fastest urea hydrolysis rate as well as the nitrification of the ammonium produced. This is similar to the observation made by Ni et al. (2018) where ammonium concentration from urea treated with urease and/or NI did not differ from untreated urea. The later decrease in ammonium concentration from all the treatments was an indication of the reduction of the substrate (urea) and continuous nitrification.

There was a significant inhibitor treatment by time interaction on net nitrate concentrations in DZ and HB soils but not in CM, CB, and BJ soils (Table 6.2). The pattern of net nitrate concentrations followed a sigmoid curve with a very small quantity of nitrate accumulation in the early days and accumulation increased with time (Figure 6.5). In each soil, the net nitrate concentrations were not significantly different among the inhibitor treatments in the first 4 d (Figure 6.5). Also, the net nitrate concentrations from the urea amended soil were not significantly different from the control with no urea application because of the time required for urea hydrolysis to produce ammonium, the substrate for nitrification. Sandy loam soils (CM and CB) had the smallest net nitrate concentrations while the clay soil (BJ) had the greatest net nitrate concentrations during the sampling periods. The greater net ammonium in the sandy loam soils, especially CM, did not translate to greater nitrate accumulation when compared to the loam and clay soils. A study by Goos and Guertal (2019) had reported lower nitrate accumulation from urea treated with and without inhibitors as the sand content of soil increases. In addition, net nitrate concentrations were positively correlated to soil pH and organic matter when substrate (ammonium) is not a limiting factor as nitrification process increases with an increase in pH from acidity to alkalinity (Ste-marie and Pare 1999; Subbarao et al. 2006; Yao et al. 2011; Hanan et al. 2016). The acidic pH of CM soil could have limited nitrification whereas the alkaline pH in BJ soil provided an optimum condition for nitrification. The use of NI such as DMPP and dicyandiamide allowed applied N to



Figure 6.5 Net nitrate concentration in each soil. Error bars are standard errors of the mean. UR and  $UR_{(p)}$  are measured and predicted untreated urea, respectively;  $UR_{NBPT}$  and  $UR_{NBPT(p)}$  are measured and predicted urea treated with NBPT, respectively;  $UR_{DI}$  and  $UR_{DI(p)}$  are measured and predicted urea treated with double inhibitor (combined NBPT and nitrification inhibitors), respectively.

persist longer in the ammonium form by inhibiting the oxidation of ammonia, thereby reducing

nitrate accumulation (Subbarao et al. 2006).

The logistic function used to model net nitrification showed that there was no significant difference in the maximum net nitrification ( $N_m$ ) and the rate of net nitrification among the inhibitor treatments in each sandy loam soil (CM and CB soils; Table 6.5). In contrast, the rate and maximum net nitrification in each loamy soil (DZ and HB soils) were significantly greater in UR

Soil	Inhibitor treatments <sup>a</sup>	Parameter estimates <sup>b</sup>			
		$k_n (d^{-1})$	N <sub>m</sub> (mg kg <sup>-1</sup> )	t <sub>m</sub> (d)	
Carman	UR	0.248a	272a	14.5a	
	UR <sub>DI</sub>	0.274a	285a	13.5a	
	UR <sub>NBPT</sub>	0.274a	281a	13.3a	
Carberry	UR	0.311a	231a	12.9a	
	UR <sub>DI</sub>	0.435a	239a	12.9a	
	UR <sub>NBPT</sub>	0.344a	260a	14.7a	
Dezwood	UR	0.600a	334b	6.0b	
	UR <sub>DI</sub>	0.349ab	534a	11.5a	
	UR <sub>NBPT</sub>	0.232b	548a	12.0a	
High Bluff	UR	0.497a	407b	8.3b	
	UR <sub>DI</sub>	0.310ab	507ab	12.1a	
	UR <sub>NBPT</sub>	0.243b	679a	13.8a	
Beausejour	UR	0.391b	775a	9.3a	
	UR <sub>DI</sub>	0.454ab	736ab	9.5a	
	UR <sub>NBPT</sub>	0.665a	626b	8.2b	

Table 6.5 Logistic model parameters for net nitrification in each soil by the inhibitor treatment.

<sup>*a*</sup> UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors).

 $^{b}$  k<sub>n</sub>, net nitrification constant; N<sub>m</sub>, maximum net nitrification; t<sub>m</sub>, inflection point at which net nitrification is maximized. Parameters comparison was performed by soil and they were considered significantly different if their 95% confidence interval did not overlap. Parameter estimates with different letters in a column within each soil are significantly different.

Note: Carman, Carberry, Dezwood, High Bluff, and Beausejour are denoted as CM, CB, DZ, HB, and BJ soils, respectively in the manuscript.

than  $UR_{NBPT}$  (Table 6.5). This inconsistency was because the quantity of substrate (ammonium) differed among the inhibitor treatments. Although lower net nitrification rate in  $UR_{NBPT}$  than  $UR_{DI}$  of loamy soils was not significant, the lower net nitrification rate in  $UR_{NBPT}$  than  $UR_{DI}$  coincided with the delay in substrate production for nitrification as a result of lower urea hydrolysis rate constants. On average, BJ soil had the shortest time to reach maximum net nitrification, while CM soil had the longest time to reach maximum net nitrification, which is an indication that nitrification increases as the soil pH increases (Subbarao et al. 2006; Hanan et al. 2016).

## 6.5 Conclusion

The use of NI with NBPT has been reported to reduce the efficacy of NBPT to reduce ammonia volatilization from surface-applied urea. We found that while NBPT significantly reduced the hydrolysis of urea in each of the soils, the addition of NI with NBPT significantly reduced the ability of NBPT to inhibit urea hydrolysis. The greater urea hydrolysis rate with double inhibitor than NBPT only is the cause of the often-reported lower reduction of ammonia volatilization from urea with double inhibitor than NBPT only. We conclude that the often-reported increase in ammonia volatilization from UR<sub>DI</sub> relative to UR<sub>NBPT</sub> is not only due to the persistence of ammonium by NI but also due to a greater urea hydrolysis rate from UR<sub>DI</sub> relative to UR<sub>NBPT</sub>. Future studies may investigate how nitrification inhibitors affect the persistence of NBPT in soil.

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#### CHAPTER 7

# 7 KINETICS AND THERMODYNAMICS OF UREA HYDROLYSIS IN THE PRESENCE OF UREASE AND NITRIFICATION INHIBITORS

### 7.1 Abstract

Urease inhibitor [N-(n-butyl) thiophosphoric triamide (NBPT)] and nitrification inhibitor [3,4dimethylpyrazole phosphate (NI)] have been used to reduce nitrogen (N) losses from urea-based fertilizers. This study evaluated the effect of temperature, NBPT, and NI on kinetics and thermodynamics properties of urea hydrolysis in six soils. Soils were amended (250 kg N ha<sup>-1</sup>) with untreated urea (UR), NBPT treated urea (UR<sub>NBPT</sub>), or NBPT+NI treated urea (UR<sub>DI</sub>); incubated at 5, 15, or 25 °C and destructively sampled eight times during an 18-day period. We measured urea hydrolysis rate by the disappearance of urea with time and determined the rate constant  $(k; d^{-1})$  assuming a first-order kinetics. Our results showed that k significantly increased with temperature in the order of 0.07 at 5 °C, 0.12 at 15 °C, and 0.20 at 25 °C across soils and inhibitor treatments, with a  $Q_{10}$  of approximately 2. Also, k significantly declined in the order of UR (0.19) > UR<sub>DI</sub> (0.11) > UR<sub>NBPT</sub> (0.08) across soils and temperatures. While the urease inhibitor, NBPT, significantly reduced k, the addition of a NI with NBPT significantly increased k compared to NBPT alone by 23, 27 and 35% at 5, 15, and 25 °C, respectively, across soils. Thermodynamics parameters showed that urea hydrolysis was nonspontaneous, and enthalpy and entropy changes were not significantly different among the inhibitor treatments in five of the six soils. We conclude that the often-reported greater ammonia volatilization from UR<sub>DI</sub> than UR<sub>NBPT</sub> may not only be due to the persistence of ammonium in the presence of NI but also because NI increased the rate of urea hydrolysis when added with NBPT.

### 7.2 Introduction

In agricultural and horticultural production, urea accounts for more than half of the global source of nitrogen (N) fertilizers. When urea is applied to soils, it hydrolyzes to ammonia with the aid of the ubiquitous urease enzyme. The hydrolysis of applied urea occurs in two stages (Zambelli et al. 2011). The first stage is the breaking down of urea by urease enzyme into ammonia and carbamate ions. The second stage is the rapid decomposition of the carbamate ion into another molecule ammonia and bicarbonate. The rate of urea hydrolysis increases with an increase in temperature as a result of an increase in urease activity (Cartes et al. 2009; Lei et al. 2018a). The overall urea hydrolysis process results in an increase in soil pH around the urea granules, thereby subjecting the ammonium produced to volatilization into the atmosphere in the form of ammonia (Overrein and Moe 1967). The magnitude of ammonia volatilization from urea may be greater than 15% of applied urea-N when urea is surface-applied without incorporation irrespective of the soil temperature (Mariano et al. 2019). The volatilized ammonia may be deposited on the soil surface with a potential to cause soil acidification or N enrichment of N limited ecosystem; or combined with acidic gases in the atmosphere to form particulate matters that are detrimental to human health (Aneja et al. 2008; Sheppard et al. 2010). In addition, ammonia volatilization from urea fertilizers is an agronomic loss to farmers as a result of reduced N use efficiency of urea fertilizers.

The ammonia formed during urea hydrolysis that is not volatilized may subsequently be converted to nitrate by a process known as nitrification or be taken up by crop or becomes immobilized by soil microorganism. The nitrification process is a sequential transformation of ammonium into nitrite and then nitrate by microorganisms (Sahrawat 2008). While ammonium and nitrate are both desirable by plants for uptake, continuous accumulation of nitrate in soil poses an environmental challenge of nitrate leaching to the groundwater in the event of a large amount of rainfall (Zaman et al. 2008). Also, unintended nitrous oxide emission to the atmosphere during the nitrification of ammonium and denitrification of nitrate makes the pathway of the ammonium to nitrate undesirable (Wrage et al. 2001).

The use of urease inhibitor especially N-(n-butyl) thiophosphoric triamide (NBPT), has been reported to effectively reduce ammonia volatilization by a global average of 52% from surfaceapplied urea (Silva et al. 2017; Cantarella et al. 2018). The reduction of ammonia volatilization by NBPT is due to inhibition of urea hydrolysis through the reduction of urease activity (Christianson et al. 1993). To inhibit urease activity, NBPT is converted to N-(n-butyl) phosphoric triamide (NBPTO) or N-(n-butyl) thiophosphoric diamide (NBPD) (Creason et al. 1990; Mazzei et al. 2019). The NBPTO or NBPD hydrolyzes to diamido phosphoric acid or monoamido thiophosphoric acid, respectively which then blocks the two-nickel ion active sites of the urease enzymes; thereby preventing contact between the urease enzyme and urea (Mazzei et al. 2019). Although the rate of urea hydrolysis is very slow at temperatures  $\leq 5$  °C, studies have shown that ammonia volatilization was still significantly greater from untreated urea than NBPT treated urea in cold soils in Montana, USA (Engel et al. 2017). Similar observation was made under late-fall temperatures (< 5 °C) on the Canadian prairies (Chapter 4). With the use of a nitrification inhibitor (NI), the activity ammonia-oxidizing organism that oxidizes ammonia to nitrite is inhibited (Subbarao et al. 2006). This allows applied N to persist longer in the ammonium form in the soil. Common NI includes dicyandiamide (DCD), nitrapyrin, and 3,4- dimethyl pyrazole phosphate

(DMPP). The NBPT and NI are usually applied with N to maximize agronomic return while safeguarding the environment.

Several studies have reported that the addition of NI with NBPT (double inhibitor, DI) on urea often interfere with the effectiveness of NBPT to reduce ammonia volatilization (Gioacchini et al. 2002; Zaman et al. 2008; Soares et al. 2012; Frame 2017; Mariano et al. 2019). A two-year study on efficacy of NBPT with and without NI to reduce ammonia volatilization showed that the addition of NI with NBPT lowered the reduction of ammonia volatilization with NBPT by 27% on spring-applied urea across two sites (Chapter 4). Other studies have found that the potential to increase ammonia volatilization from double inhibitor treated urea (UR<sub>DI</sub>) relative to NBPT treated urea (UR<sub>NBPT</sub>) increased as the concentration of the NI increased (Soares et al. 2012; Frame 2017). The greater ammonia volatilization from UR<sub>DI</sub> than UR<sub>NBPT</sub> has been attributed to the persistence of ammonium in the presence NI. However, a recent incubation study (conducted at 21 °C) clearly showed that the rate of urea hydrolysis was significantly greater in UR<sub>DI</sub> than UR<sub>NBPT</sub> from four of five soils used in the study (Chapter 6). Previous studies have shown that the rate of urea hydrolysis with and without NBPT increased as the temperature increased (Suter et al. 2011; Engel et al. 2013). Nevertheless, there is a dearth of information on the coupled effect of temperature and urease inhibitor, NBPT, with and without NI on the hydrolysis of urea. Also, there is little information in the literature on the thermodynamic parameters such as activation energy  $(E_a)$ , Gibb's free energy ( $\Delta G$ ), enthalpy change ( $\Delta H$ ), entropy change ( $\Delta S$ ) of urea hydrolysis, particularly urea treated with NBPT or DI. The objective of our study was to evaluate the interactive effect of temperature, urease inhibitor (NBPT), and NI (DMPP) on the kinetics and thermodynamics parameters of urea hydrolysis.

### 7.3 Materials and Methods

#### 7.3.1 Soil Characteristics

This study was conducted with soils (0 - 15 cm) collected from six different sites in Manitoba, Canada. The location of the sites were Carman (CM; 49° 29' 6" N, 98° 02' 2" W), Carberry (CB; 49° 53' 7" N, 99° 22' 29" W), Deerwood herein in refer to as Dezwood (DZ; 49° 22' 1"N, 98° 23' 34" W), High Bluff (HB; 50° 01' 2" N, 98° 08' 9"W), Portage la Prairie (PP; 49° 57′ 9″ N, 98° 16′ 0″ W), and Beausejour (BJ; 50° 05' 13" N, 96° 29' 58" W). The soils were air-dried, ground, and passed through a 2 mm sieve. Subsamples of the soils were collected to determine urease activity (Tabatabai and Bremner 1972), soil texture (Gee and Bauder 1986), electrical conductivity, pH (soil/water, 1:2), cation exchange capacity (Hendershot et al. 2008), organic matter (Walkley and Black 1934), and available N (Maynard et al. 2008) (Table 7.1).

### 7.3.2 Experimental Design and Treatment Applications

The experiment design was a randomized complete block design with a split-plot layout. The splitplot layout consisted of temperature as the main plot and factorial combination of soils by inhibitor treatments by sampling time as the subplot. The temperatures (replicated three times) were 5, 15, and 25 °C; soils were CM, CB, DZ, HB, PP, and BJ; inhibitor treatments were untreated urea (UR), NBPT treated urea (UR<sub>NBPT</sub>), and NBPT+NI (double inhibitor) treated urea (UR<sub>DI</sub>). We prepared UR<sub>NBPT</sub> (360 mg NBPT kg<sup>-1</sup> urea) by coating urea with ARM U formulation (18% NBPT m/v) and UR<sub>DI</sub> (360 mg NBPT + 90 mg DMPP kg<sup>-1</sup> urea) by coating urea with ARM U Advanced formulation (24% NBPT + 6% DMPP m/v). Our sampling times were 0.5, 1, 2, 4, 7, 10, 14, and Table 7.1 Selected soil (0 - 15 cm) properties.

	Carman	Carberry	Dezwood	High Bluff	Beausejour	Portage
Soil classification <sup>a</sup>	Orthic Blac	ck Chernozem	Orthic Dark Gray	Gleyed Cumulic	Gleyed Rego Black	
			Chernozem	Regosol	Chern	ozem
Soil series	Hibsin	Fairland	Dezwood	High Bluff	Dencross	Neurhorst
Soil pH <sub>water</sub>	5.51	6.65	6.62	7.46	7.76	7.96
Electrical conductivity ( $\mu$ S cm <sup>-1</sup> )	394	228	1853	899	1377	596
Organic matter (g kg <sup>-1</sup> )	27	33	34	45	88	71
Available N (mg kg <sup>-1</sup> )	31	15	186	58	22	82
Urease activity (mg NH4 <sup>+</sup> -N kg <sup>-1</sup> soil hr <sup>-1</sup> )	11	17	24	57	63	88
Cation exchange capacity (cmol kg <sup>-1</sup> )	16	14	23	28	47	36
Soil texture	Sandy loam	Sandy loam	Loam	Loam	Clay	Clay loam
Sand (g kg <sup>-1</sup> )	711	764	465	427	108	269
Silt (g kg <sup>-1</sup> )	123	128	318	325	322	343
Clay (g kg <sup>-1</sup> )	166	108	217	248	570	388

<sup>*a*</sup> Soil classification is according to MAFRI (2010)

18 days after fertilization (d). Due to a large number of the experimental units, replicates of each experimental unit were blocked with time.

Twenty-five grams of each air-dried soil (< 2 mm) was weighed into a 30 mL cup. The soils were wetted to 75% field capacity, covered, and left for 24 hours at room temperature to allow soil and water to equilibrate. After 24 hours, we applied 50 mg of inhibitor treatment to the centre of the soil surface. The cups were arranged on a tray containing water and set in an incubator at a temperature of 5, 15, or 25 °C. Water on the tray helped to reduce the rate of evaporation from the soil surface and kept the incubator relatively humid. Each incubator contained soil (6) by inhibitor treatment (3) by sampling time (8) cups. Every two days, three random cups of each soil by treatment by temperature were weighed to determine moisture loss. The difference in mass (as a result of moisture loss) was adjusted by adding de-ionized water to the edge of the cups with a pipette.

### 7.3.3 Soil Sampling and Analysis

At each sampling time, a set of samples (6 soils x 3 inhibitor treatments x 3 temperatures for a total of 54 samples) was destructively sampled for extraction and analysis. Soils in each cup were quantitatively transferred into a 1 L jar containing 250 mL of 1 M KCl-phenyl mercuric acetate and placed on a reciprocating shaker for 60 minutes. After 60 minutes, the samples were filtered (Watman #40) into a 25 mL scintillating vials and refrigerated. The filtrate was analyzed colorimetrically for urea-N (Mulvaney and Bremner 1979). Ammonium and nitrate concentrations from the filtrate were analyzed with AQ2 Discrete Analyzer (SEAL Analytical Inc. WI).

The urea-N measured in each soil was expressed as a percentage of applied urea-N. The hydrolyzed urea was calculated as the disappearance of urea-N with time (Eq. 1):

$$\mathbf{U}_{\text{hvd}} = \mathbf{U}_0 - \mathbf{U}_{\text{t}} \tag{7.1}$$

Where  $U_{hyd}$  is the hydrolyzed urea-N;  $U_0$  is the amount of urea-N applied;  $U_t$  is the amount of urea-N recovered (% of applied urea-N) at time t; t is time or day after the start of the incubation (d).

Net inorganic N concentration (ammonium + nitrate) in each soil was calculated (Li et al. 2018; Wang et al. 2019) as:

$$NIN_{t} = IN_{t} - IN_{i}$$
(7.2)

Where NIN<sub>t</sub> is the net inorganic N concentrations (mg N kg<sup>-1</sup>) at time t;  $IN_t$  is the inorganic N concentrations (mg N kg<sup>-1</sup>) measured from the soil at time t of the experiment;  $IN_i$  is the inorganic N concentrations (mg N kg<sup>-1</sup>) measured from the soil before the start of the study.

## 7.3.4 Kinetics, Thermodynamics, and Statistical Analysis

We performed all model fittings and statistical analyses with SAS software (SAS Institute 2014; version 9.4). All model fittings were performed by replicates for each soil x inhibitor treatment x temperature experimental unit. We fitted different kinetic equations (first- and zero-order, first- order, first- plus linear-order, and hyperbolic models) with PROC NLIN to generate urea hydrolysis rate constant (*k*) and found the first-order kinetic model to best fit the data based on the lowest Akaike's Information Criterion (Archontoulis and Miguez 2015). The first-order kinetic equation used was as follows:

$$U_{\rm hvd} = U_0 [1 - \exp(-kt)]$$
(7.3)

Parameters are as defined above.

The *k*, the first-order kinetic constant, determined from Eq. 7.3 was used to calculate half-life  $(t_{1/2})$  and  $Q_{10}$  as follows:

$$t_{1/2} = \frac{\ln 2}{k} \tag{7.4}$$

$$Q_{10} = {\binom{k_b}{k_a}}^{[10/(T_b - T_a)]}$$
(7.5)

Where  $k_a$  and  $k_b$  are first-order kinetic rate constants at 5 and 15 °C, respectively, 15 and 25 °C, respectively, or 5 and 25 °C, respectively;  $T_a$  and  $T_b$  are incubation temperatures at 5 and 15 °C, respectively, 15 and 25 °C, respectively, or 5 and 25 °C, respectively.

The *k* dependence on temperature was used to determine the thermodynamic parameters of urea treated with and without inhibitors in soils. The thermodynamic parameters determined were activation energy (E<sub>a</sub>), Gibb's free energy ( $\Delta$ G), enthalpy change ( $\Delta$ H), and entropy change ( $\Delta$ S). The E<sub>a</sub> (KJ mol<sup>-1</sup>) for each soil by inhibitor treatment was determined with PROC NLIN using the Arrhenius equation (Eq. 7.6)

$$k = A e^{-E_a/RT} \tag{7.6}$$

Where T is temperature in Kelvin (K); R is the gas constant (8.314 J mol<sup>-1</sup> K<sup>-1</sup>); A is a preexponential factor. Also, the  $\Delta G$  (KJ mol<sup>-1</sup>) for each soil by inhibitor treatment was determined with PROC NLIN using the Van't Hoff equation (Eq. 7.7).

$$K_{\rho} = e^{-\Delta G/RT} \tag{7.7}$$

Where  $K_e$  is the equilibrium constant. Since urea hydrolysis is not a chemical equilibrium reaction, the absolute reaction-rate or transition-state theory of the relationship between *k* and  $K_e$  (Glasstone et al. 1941; Kumar and Wagenet 1984; Lei et al. 2018b) was used to re-write the Van't Hoff equations as follows:

$$K_e = \frac{N_o kh}{nRT} \tag{7.8}$$

Where  $N_o$  is Avogadro's constant; h is Plank's constant (6.6261 x  $10^{-34}$  J s); n is the number of moles. But  $N_o$  and R are related via Boltzman constant (k<sub>b</sub>; 1.3806 x  $10^{-23}$  J K<sup>-1</sup>) as shown in Eq. 9.

$$nRT = N_0 k_b T$$
(7.9)

Then,

$$k = \left(\frac{k_{\rm b}T}{\rm h}\right) e^{-\Delta G/RT} \tag{7.10}$$

To determine  $\Delta H$  and  $\Delta S$ , the  $\Delta G$  for each soil and inhibitor treatment at each temperature was calculated using Eq. 7.10 and linear regression with PROC REG was used to estimate  $\Delta H$  (intercept) and  $\Delta S$  (slope) using their relationship in Eq. 7.11.

Analysis of variance (ANOVA) with repeated measure analysis in PROC GLIMMIX was used to determine the effect of temperature and inhibitor treatment on urea-N recovered and NIN with time for each soil. In this model, temperature and inhibitor treatment were fixed effects, replicate was a random effect and time was the repeated factor. A covariance structure with the lowest AIC was used in the model statement. We used a three-way ANOVA in PROC GLIMMIX to determine the effect of temperature, soil, inhibitor treatment, and their interactions on the *k* and  $t_{1/2}$  generated using a gamma distribution. Temperature, soil, and inhibitor treatment were fixed effects while replicate and its interaction with fixed effects were random effects. Similarly, PROC GLIMMIX was used to compare the  $Q_{10}$ ,  $E_a$ ,  $\Delta G$ ,  $\Delta H$ , and  $\Delta S$  for the inhibitor treatments and soil. We used the SLICE statement in PROC GLIMMIX to request for mean separation by soil in all the GLIMMIX procedures. Means comparison was performed at a probability level of < 0.05 Fisher's protected least significant difference (LSD).

### 7.4 Results and Discussion

### 7.4.1 Effect of Inhibitor Treatment and Temperature on Urea-N Recovery

There was no significant temperature by inhibitor treatment by time interaction in the amount of urea-N recovered in all soils except the neutral pH soils (CB and DZ Table 7.2). There was a significant inhibitor treatment by time interaction in the amount of urea-N recovered in all the soils except CM soil because the amount of urea-N recovered across the three temperatures at a given time was lower than the preceding time in each of the inhibitor treatment (Table 7.2; Figure 7.1-

7.3). Overall, the amount of urea-N recovered decreased with an increase in time across inhibitor treatments by temperatures for each soil (Figure 7.1-7.3).

Table 7.2	Effect	of	temperature,	inhibitor	treatment,	and	time	on	urea-N	recovered	and	net
inorganic I	N conce	ntra	ations in each	soil.								

Model effect	Carman	Carberry	Dezwood	High Bluff	Beausejour	Portage	
	Probability values <sup>a</sup>						
Urea-N recovered							
Temperature (T)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	
Inhibitor treatments (I)	0.0016	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	
Time (t)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	
ТхІ	0.4043	0.1645	0.0510	0.7154	0.8917	0.9937	
T x t	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.3743	0.7865	
I x t	0.8154	< 0.0001	< 0.0001	0.0054	0.0013	0.0171	
T x I x t	0.7089	0.0003	0.0480	0.1966	0.6501	0.9350	
Net inorganic N concentrations							
Т	< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0001	< 0.0001	
Ι	0.5516	0.2142	0.0287	0.0682	0.0045	0.3463	
t	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	
ΤxΙ	0.9730	0.3918	0.1479	0.8792	0.8273	0.9425	
T x t	0.1641	0.1862	0.5028	0.1248	0.2193	0.9289	
I x t	0.9990	0.1861	0.9402	0.8810	0.8370	0.7592	
T x I x t	1.0000	0.5719	0.9588	0.9548	0.9997	0.9998	

<sup>*a*</sup> Probability values are significant at < 0.05.



Figure 7.1 Urea-N recovered (% of applied urea-N) in soils during an 18-d incubation period at 5  $^{\circ}$ C. Error bars are standard errors of the mean.

UR, untreated urea;  $UR_{NBPT}$ , urea treated with NBPT;  $UR_{DI}$ , urea treated with double inhibitor (combined NBPT and nitrification inhibitors).



Figure 7.2 Urea-N recovered (% of applied urea-N) in soils during an 18-d incubation period at 15 °C. Error bars are standard errors of the mean.

UR, untreated urea;  $UR_{NBPT}$ , urea treated with NBPT;  $UR_{DI}$ , urea treated with double inhibitor (combined NBPT and nitrification inhibitors).



Figure 7.3 Urea-N recovered (% of applied urea-N) in soils during an 18-d incubation period at 25 °C. Error bars are standard errors of the mean.

UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors).

The trend of urea-N recovery among the inhibitor treatments was similar at each temperature for each soil. The amount of urea-N recovered with time was lowest in UR and greatest in UR<sub>NBPT</sub> at each temperature for each soil. Also, the amount of urea-N recovered decreased with an increase in temperature for each inhibitor treatment. For example, less than 20% of applied urea-N in UR was recovered on 4 d in all soils (except DZ soil) at 25 °C whereas at least 40% of the applied urea-N was recovered in all the soils at 5 or 15 °C on 4 d. Low urea-N recovery with an increase in temperature in our study was because of the increase in urease activity at high temperatures as reported in the study of Xu et al. (1993). As the temperature increased from 5 to 25 °C, we found that the ability of NBPT to increase urea-N recovery was least effective in CM soil (Figure 7.1-7.3). As such, urea hydrolysis in CM soil was almost completed in all the inhibitor treatments by 10 d at 25 °C (Figure 7.3). The low effectiveness of NBPT at 25 °C in CM soil relative to other soils was because the efficacy of NBPT is lower in acidic than alkaline soils (Hendrickson and Douglass 1993) coupled with the increase in urea hydrolysis as a result of increased temperature. Similarly, results from a previous study that compared urea-N recovery at different soil pH (5.4, 7.8, and 8.1) found that urea was completely hydrolyzed in NBPT treated urea at 15 and 25 °C in acidic soil by 7 d when less than 40% of the applied urea-N had hydrolyzed in the alkaline soils (Suter et al. 2011).

### 7.4.2 Kinetics and Thermodynamics of Urea Hydrolysis

There was no significant temperature by inhibitor treatment by soil interaction on k (Table 7.3). Also, there was neither significant effect of temperature by soil interaction nor temperature by inhibitor treatment interaction on k (Table 7.3). Irrespective of the soil or inhibitor treatment, k

Model effect	k	t <sub>1/2</sub>
	d <sup>-1</sup>	d
Temperature (T)		
5 °C	0.07c	10.0a
15 °C	0.12b	5.7b
25 °C	0.20a	3.5c
Inhibitor treatment <sup>a</sup>	(I)	
UR	0.19a	3.8c
UR <sub>DI</sub>	0.11b	6.5b
UR <sub>NBPT</sub>	0.08c	8.3c
Soil (S)		
Carman	0.15a	4.8c
Carberry	0.10b	6.8b
Dezwood	0.08c	9.0a
High Bluff	0.13a	5.5c
Beausejour	0.14a	5.1c
Portage	0.14a	4.9c
	Probabilit	y values
Т	< 0.0001	< 0.0001
Ι	< 0.0001	< 0.0001
S	<0.0001	< 0.0001
ТхІ	0.3286	0.2589
T x S	0.4876	0.5041
I x S	< 0.0001	< 0.0001
T x I x S	0.2925	0.2342

Table 7.3 Effect of temperature, inhibitor treatment, and soil on urea hydrolysis rate constant (*k*) and half-life  $(t_{1/2})$ .

<sup>*a*</sup> UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors).

Means with different letters within a column are significantly different at probability values of < 0.05 Fisher protected LSD.

significantly increased with an increase in temperature. The significant increase in k with an increase in temperature was an indication that the activities of urease in soils increase with an

increase in temperature as reported in (Lei et al. 2018a). There was a significant effect of inhibitor treatment by soil interaction on k (Table 7.3). The significant inhibitor treatment by soil interaction was because when averaged across the three temperatures, k was significantly greater in UR<sub>NBPT</sub> than UR<sub>DI</sub> in each of the soils except CM soil. The lack of significant difference in k between UR<sub>NBPT</sub> and UR<sub>DI</sub> in CM soils corroborated our previous study that compared the k between UR<sub>NBPT</sub> than UR<sub>DI</sub> in all the soils used in this experiment (except PP) at 21 °C (Chapter 6).

In each of the soils, the pattern of k at each temperature was similar (Figure 7.4). Overall, k was in the significant order of  $UR > UR_{DI} > UR_{NBPT}$  across soil-temperature (Table 7.3). In CM soil, there was no significant effect of NBPT on k at 25 °C possibly due to the rapid decomposition of NBPT in acidic soil compared to alkaline soil coupled with faster urea hydrolysis at high temperature (Engel et al. 2015; Suter et al. 2011). This suggests that acidic soils may require a higher concentration of NBPT than alkaline soils to effectively inhibit urea hydrolysis as the persistence of NBPT in soils is proportional to its concentration (Hendrickson and Douglass 1993). However, studies have shown that an increase in NBPT concentration may not necessarily result in a significant decrease in ammonia volatilization (Watson et al. 1994; Frame et al. 2012). In each soil, there was approximately a two-fold increase in k with a 10 °C rise in temperature ( $Q_{10}$ ~2). There was no significant difference in  $Q_{10}$  between any pair of increasing temperature from 5 to 15 °C, 15 to 25 °C, or 5 to 25 °C for each inhibitor treatment in each soil (results not shown). A previous study had reported that the rate of urea hydrolysis in soil increased by a factor of approximately two for every 10 °C rise in temperature up to an optimum temperature of 45 °C (Garcia et al. 2014).





UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors).

Bars with different letters within each soil are significantly different at P < 0.05 Fisher protected LSD.



Figure 7.5 Percentage reduction of urea hydrolysis rate by NBPT relative to untreated urea at 5, 15, and 25 °C across soils. Error bars are standard errors of the mean. UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors).

Bars with different letters are significantly different at a probability level of < 0.05 Fisher protected LSD.

The addition of NI with NBPT reduced the inhibitory effect of NBPT by 23, 27, and 35% at 5, 15, and 25 °C, respectively, across soils. The percentage inhibition of *k* by NBPT was not significantly dependent on temperature in UR<sub>NBPT</sub> but significantly dependent on temperature in UR<sub>DI</sub> across soils (Figure 7.5). Also, while NBPT increased the half-life of urea from 3.8 to 8.3 d, NI reduced the half-life of NBPT treated urea by approximately two days across soil-temperature (Table 7.3).

The  $E_a$ , an indicator of the energy barrier that must be overcome for hydrolysis of urea to occur, ranged from 20 - 54 kJ mol<sup>-1</sup> (Table 7.4). While there was no significant effect of NBPT on  $E_a$  in CM, HB, and PP, there was a significant effect of NBPT on  $E_a$  in CB, DZ, and BJ soils. The values of  $E_a$  in our soils were within the range of 20 - 80 KJ mol<sup>-1</sup> reported in the literature (Gould et al. 1973; Kumar and Wagenet 1984; Moyo et al. 1989; Marshall et al. 1990; Lei et al. 2018b). There was a significant effect of inhibitor treatment on  $\Delta G$  of urea hydrolysis in each soil (Table 7.4). Except in CM soils where  $\Delta G$  was not significantly different between UR and UR<sub>DI</sub>,  $\Delta G$ significantly increased in the order of UR<sub>NBPT</sub> > UR<sub>DI</sub> > UR in each soil (Table 7.4).

We found that  $\Delta H$  and  $\Delta S$  for each soil were not significantly different among the inhibitor treatments except in DZ soil where UR had the smallest  $\Delta H$  and  $\Delta S$  among the inhibitor treatments (Table 7.4). The lack of significant difference in  $\Delta H$  and  $\Delta S$  between untreated urea and urea treated with inhibitor (UR<sub>DI</sub> and UR<sub>NBPT</sub>) corroborated the study of Juan et al. (2010) that reported that the use of NBPT had a greater impact on kinetics than thermodynamics of urea hydrolysis. Even when untreated urea with different application rates was used, Lei et al. (2018b) found that the interaction between urea application rates and temperature was significant on the kinetics of urea hydrolysis but not its thermodynamics parameters. As suggested by Moyo et al. (1989), the wide variations or differences in thermodynamic parameters among and within soils were due to other soil factors such as urea application rate, treatment type, and moisture that interacted with temperature. The values of  $\Delta G$  and  $\Delta H$  being > 0 and  $\Delta S$  being < 0 showed that the hydrolysis of urea in soil was endothermic and nonspontaneous. The lack of spontaneity of urea hydrolysis corroborated results from an earlier study that found  $\Delta G$  and  $\Delta H$  of different rates of untreated urea to be > 0 (Lei et al. 2018b).

Soil	Inhibitor treatment <sup>a</sup>	Ea	ΔG	$\Delta H$	$\Delta S$
			—kJ mol <sup>-1</sup> –		J mol <sup>-1</sup> K <sup>-1</sup>
Carman	UR	48.9a	75.5b	41.1a	-116.4a
	UR <sub>DI</sub>	54.4a	75.8b	51.6a	-81.4a
	UR <sub>NBPT</sub>	53.8a	76.2a	48.8a	-92.6a
Carberry	UR	26.6b	75.7c	25.9a	-168a
	$UR_{DI}$	45a	77.4b	43.7a	-113.5a
	UR <sub>NBPT</sub>	40.1a	78.1a	37.9a	-135.4a
Dezwood	UR	20.6c	76.5c	19.4b	-192.7b
	UR <sub>DI</sub>	50.5a	77.9b	44.8a	-111.8a
	UR <sub>NBPT</sub>	36.9b	79.0a	37.8ab	-138.7ab
High Bluff	UR	35.2a	75.7c	31.8a	-148.1a
	UR <sub>DI</sub>	29.7a	76.8b	29.6a	-159.3a
	UR <sub>NBPT</sub>	32.3a	77.5a	29.2a	-163.2a
Beausejour	UR	39.8a	75.4c	32.9a	-143.8a
	UR <sub>DI</sub>	32ab	76.7b	28.7a	-162.1a
	UR <sub>NBPT</sub>	25.1b	77.4a	24.1a	-179.9a
Portage	UR	29.8a	75.6c	25.9a	-168.1a
	UR <sub>DI</sub>	27.3a	76.6b	26.8a	-168.4a
	UR <sub>NBPT</sub>	29.1a	77.2a	26.3a	-172.1a

Table 7.4 Activation energy (Ea), Gibb's free energy ( $\Delta G$ ), Enthalpy ( $\Delta H$ ), and Entropy ( $\Delta S$ ) of the inhibitor treatments in each soil.

<sup>*a*</sup> UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors).

Means with the same letters within a column for each soil are not significantly different at a probability value of < 0.05 Fisher protected LSD.

### 7.4.3 Net Inorganic N Concentrations

In the repeated measure ANOVA for net inorganic N concentrations, neither the three-way nor two-way interaction was significant for each soil (Table 7.2). However, there were significant effects of temperature and time in each soil. The net inorganic N concentrations in each inhibitor treatment across soils increased with an increase in temperature and/or time (Figure 7.6-7.8). The increase in net inorganic N concentrations with an increase in temperature indicated the dependence of urea hydrolysis and nitrification products (ammonium and nitrate, respectively) on temperature (Cartes et al. 2009). The increase in net inorganic N concentrations was positively correlated ( $R^2 = 0.80$ ) to the hydrolysis of urea across soils by inhibitor treatments by temperatures. The greater proportion of the net inorganic N concentrations during the sampling periods was in ammonium form. Also, the difference in net inorganic N concentrations among the inhibitor treatments was mainly associated with the ammonium concentration fraction as nitrate concentrations were very similar among treatments in each soil. At 25 °C, CM soil had the greatest net inorganic N concentrations among soils probably due to its greatest urea hydrolysis rate. The rapid urea hydrolysis in UR than UR<sub>DI</sub> or UR<sub>NBPT</sub> consistently resulted in an earlier peak of net inorganic N concentrations across soils and temperatures in the UR treatment. A steady increase in net inorganic N concentration and decrease in urea-N recovered at 5 °C confirmed results from previous studies which showed that N transformation could occur at temperatures typical of the fall season (Clark et al. 2009; Chantigny et al. 2019). The implication of this for Canadian prairie farmers is that N losses such as ammonia volatilization could occur from surface-applied urea when the temperature is  $\leq 5 \, {}^{\circ}$ C (Chapter 4).





UR, untreated urea;  $UR_{NBPT}$ , urea treated with NBPT;  $UR_{DI}$ , urea treated with double inhibitor (combined NBPT and nitrification inhibitors).



Figure 7.7 Net inorganic N (ammonium + nitrate) concentrations during an 18-d incubation period at 15  $^{\circ}$ C. Error bars are standard errors of the mean.

UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors).



Figure 7.8 Net inorganic N (ammonium + nitrate) concentrations during an 18-d incubation period at 25 °C. Error bars are standard errors of the mean.

UR, untreated urea; UR<sub>NBPT</sub>, urea treated with NBPT; UR<sub>DI</sub>, urea treated with double inhibitor (combined NBPT and nitrification inhibitors).

### 7.5 Conclusion

Our study demonstrated that urease inhibitor, NBPT, could reduce hydrolysis of urea at temperatures of 5, 15, and 25 °C across soils. The effectiveness of NBPT was greater in neutral to alkaline than in acidic pH. Our study showed that the addition of NI significantly reduced the inhibitory effect of NBPT on urea hydrolysis across soils. We found that the percent inhibition of urea hydrolysis by NBPT only was independent of temperature but percentage inhibition by DI decreased as the temperature increased. Also, we found that the thermodynamic parameters of urea treated with and without NBPT or DI was nonspontaneous. The often-reported greater ammonia volatilization from UR<sub>DI</sub> than UR<sub>NBPT</sub> may not only be due to the persistence of ammonium in the presence of NI but also because NI increased the rate of urea hydrolysis when added with NBPT.

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## **8 OVERALL SYNTHESIS**

## 8.1 Findings and Implication of the Research

The need to increase food production and sustain the ever-growing world population has resulted in a continuous application of plant essential elements in the form of fertilizers. Nitrogen (N) is one of the plant essential elements that is often applied to soil to optimize crop growth. Globally, urea-based fertilizers account for more than one-half of the source of N fertilizers due to their ease of storage, handling, and applications (IFA 2017). However, losses due to ammonia volatilization in addition to other losses such as nitrate leaching, dinitrogen gas emission, and nitrous oxide emission are major factors that limit the N use efficiency of urea-based fertilizers (Aneja et al. 2008). Urease inhibitors especially N-(n-butyl) thiophosphoric triamide (NBPT) has been reported to reduce ammonia volatilization from surface-applied urea-based fertilizers (Chien et al. 2009). However, the stability and longevity of commercially available NBPT formulations in reducing ammonia volatilization are influenced by the solvent and other additives used in the formulation (Canterella et al. 2018). Nitrification inhibitors (NI) can also be combined with urease inhibitor (i.e double inhibitor, DI) to reduce other forms of N losses such as nitrate leaching and nitrous oxide emission. The overall purpose of the research was to evaluate the efficacy of urease inhibitor with and without nitrification inhibitor in improving the efficiency of urea-based fertilizers.

A relatively new NBPT formulation, ARM U (Active AgriScience Inc., BC) that contained a polymer which allows for low NBPT application rate was tested (Chapter 2) for its efficacy in comparison with two other NBPT formulations (Agrotain Advanced, Koch Agronomic Services LLC, KS), Arborite, Weyerhaeuser Co., WA) in greenhouse and field studies. The studies showed

that ARM U applied at a rate of 360 mg NBPT kg<sup>-1</sup> urea reduced ammonia volatilization from granular urea by an average of 85% in the greenhouse and field studies and by 46% from UAN solutions. This study showed that the effectiveness of ARM U in reducing ammonia volatilization from urea-based fertilizers was not significantly different from that with Agrotain Advanced (600 mg NBPT kg<sup>-1</sup> urea) and Arborite (480 mg NBPT kg<sup>-1</sup> urea. ARM U) despite the lower NBPT concentration per kg urea applied with ARM U.

It is known that fall-applied N fertilizer is less efficient than spring-applied N fertilizer in Manitoba (MAFRI 2007). As such, late-fall application of N fertilizers when soil temperature at 10 cm is < 5 °C is usually recommended to farmers who prefer to apply their N fertilizer in the fall instead of the spring (MAFRI 2007). The advice assumed that N losses such as ammonia volatilization will be reduced due to low late-fall temperature with the potential to improve efficiency of fall N application. The study reported in Chapter 4 showed that a significant amount of ammonia could be lost from urea-based fertilizers applied in the advised late-fall period even when soil and air temperatures were below 3 °C. The potential for ammonia volatilization from surface-applied ureabased fertilizers in the late-fall was not different from that in the spring despite the differences in soil and air temperatures. The use of NBPT or DI reduced ammonia volatilization from urea-based fertilizers in fall and spring applications by 65 and 45%, respectively. As a result, the use of inhibitors should not be limited to spring N application but should also be used with fall N application when considering surface application of urea-based fertilizers especially in zero till, forage, or tree crop field. Grain yield from canola and wheat in the two years of study (Chapter 5) showed that the efficiency of urea-based fertilizers across sites and years was significantly lower with fall N application than spring application at each site. The conserved N by the inhibitors with

fall application showed up in the crop yield and N removal only at Carman site with sandy loam soil but not at Portage site with clay loam soil. Hence, agronomic and apparent nitrogen use efficiencies were not significantly different between fall-applied urea or UAN treated with inhibitors and spring-applied urea or UAN without inhibitors at Carman but not at Portage. This study showed that the effectiveness of the inhibitors to increase yield or bridge N use efficiency gap between fall and spring-applied urea-based fertilizers is soil or site specific.

One of the reasons why there was no benefit of inhibitor at Portage was because the soil organic matter was greater in Portage clay loam (71 g kg<sup>-1</sup>) than in Carman sandy loam (32 g kg<sup>-1</sup>). Following mineralization of the soil organic matter, the greater soil organic matter in Portage clay loam could provide the deficit N needed by the crop to optimize yield. As such, farmers need to know the potential of their soils to supply N which may then be used to adjust N application rate in order optimize the N saved by the inhibitors. Also, the lack of a consistent response to NBPT on grain yield in this study and as reported in other previous studies (McKenzie et al. 2010; Grant 2014) on the Canadian prairie may be due to the inherent high fertility level of Canadian prairie soils to supply N coupled with the relatively low N requirement of canola and wheat compared to corn which requires about twice as much as N application rate than wheat. A meta-analysis by Abalos et al. (2014) showed that yield response to NBPT increased as N application rates increases as the N application rates increase thereby resulting in yield response to N saved by NBPT at high N application rates in corn (Li et al. 2018).

The lack of consistent increase in yield by the inhibitors will impact its adoption by the farmers since it is an additional input cost despite its environmental benefit. In Manitoba, the addition of NBPT with and without NI in Manitoba has increased the cost per kg N in urea by 22 to 35% from 2014 – 2018 (John Heard, Manitoba crop nutrient specialist, personal communication, July 06, 2020). As such, only an increase in yield can justify the additional increase in cost from the farmers' perspectives. Although there is scanty of literature on the net returns of the use of urea treated with inhibitors over conventional urea on the Canadian prairie, a study on the profitability of enhanced urea fertilizer in the United States showed that the use of NBPT increase net returns by \$283 ha<sup>-1</sup> in a corn production (Zhou et al. 2018). A survey of Manitoba agronomists showed that as much as 62% of the agronomists will recommend or use these inhibitors only if the price difference between fertilizer treated with inhibitor and conventional fertilizers is less than 10% (Amiro et al. 2017).

The results in Chapter 4 showed that the addition of NI with NBPT has the potential of lowering the efficacy of NBPT in reducing ammonia volatilization from granular urea. The results were similar to some other previous observations (Gioacchini et al. 2002; Soares et al. 2012; Frame 2017). These previous studies have attributed lower ammonia volatilization reduction with DI than NBPT only to be due to the persistence of ammonium by the NI. The results in Chapters 6 and 7 clearly showed that urea hydrolysis rate was greater in DI treated urea than NBPT treated urea across six soils in four temperatures (5, 15, 21 and 25 °C) used in these studies. The inhibitory effect of NBPT to reduce urea hydrolysis rate was not dependent on temperature but when double inhibitor was used the inhibitory effect of NBPT was reduced as the temperature increased. The reduction of urea hydrolysis rate by NBPT was least effective in acidic soil, especially at 25 °C.

The implication of this study is that the observed increase in ammonia volatilization from DI treated urea relative to NBPT treated urea was not only due to the persistence of ammonium in soil but also by a greater urea hydrolysis rate with DI than NBPT only.

## 8.2 Recommendations

The use of a urease inhibitor on urea-based fertilizers is an additional cost to food production. Minimizing the concentration of NBPT to be used in commercial NBPT formulations will reduce the cost of producing NBPT formulations by manufacturers which may also be transferred to the farmers. However, the efficacy of ARM U formulation in reducing ammonia volatilization and increasing crop yield at this low NBPT concentration was only tested at the study sites in this research. There is a need for testing the formulations over a wider range of soil and environmental conditions that appropriately describe where the products will be used. Furthermore, future studies may consider investigating the use of this formulation on other pathways of N losses such as nitrate leaching and nitrous oxide plus dinitrogen gas emissions. An N rate response curve for crop yield is necessary to be developed for urea-based fertilizers treated with the inhibitors over a wide range of soils and environments.

Also, future studies may investigate the implication of NI on the rate of NBPT degradation in soils as a mechanism to explain the greater urea hydrolysis rate in urea treated with double than urea treated with NBPT only. The studies conducted in this report did not evaluate the effect of the interaction between urease and nitrification inhibitors on soil microbial dynamics. The dynamics of urease enzyme in the presence of double inhibitor versus NBPT only may provide information on the observed differences in urea hydrolysis rates. There is also a need to investigate the impact of acidic pH on the degradation of NBPT in soils as the effectiveness of NBPT in lowering the rate of urea hydrolysis was reduced on acidic soil.

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