Ammonia Volatilization, Urea Hydrolysis, and Urease Inhibition with the Application of Granular Urea in Agroecosystems

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Abstract

Synthetic nitrogen (N) fertilizers play a key role in human nutrition and crop production. The most widely used N source globally is urea; however, N loss via ammonia volatilization can be great in agricultural systems where urea is surface-applied. The objectives of the experiments reported in this dissertation were: 1) evaluate the performance of a new laboratory ammonia volatilization measurement system for measuring ammonia volatilization from coated granular urea; 2) determine if urease can be extracted from corn and soybean residues; 3) determine if differences in urease activity are present in corn and soybean residues; and 4) evaluate N content and yield of corn treated with surface-applied coated urea fertilizers. The laboratory ammonia volatilization system had a system recovery efficiency (SRE) of 97% of the applied N and the lowest variation in mg N captured in the acid traps when the air flow rate was 1.00 L min^{-1}, at 26^{o}C, and an acid trap volume of 100 ml 0.02M phosphoric acid. Ammonia volatilization was greatest from 12-24 h after N application with a total of 17% of the applied N being lost during that period. The urease inhibitor N-(n-butyl) thiophosphoic triamide (NBPT) was the most effective ammonia volatilization control treatment and reduced ammonia losses 30-40% compared to urea in the laboratory trials. Urease was extracted from soybean residue and retained activity during extraction; however, urease from corn residue could not be identified in extracts. The agronomic field trials indicated that NBPT increased N concentration in corn ear leaves; however the effect on corn grain yield was masked by environmental conditions. The data from this study suggests that ammonia volatilization from granular urea can be effectively controlled using NBPT, and corn tissue N content in the field indicates that NBPT allows for

more N to be utilized by the plant. The urease extraction showed that there may be differences in urease activity in different crop residues. Further research is needed to determine if varying levels of volatilization control are needed for urea applied to different crop residues in no-till systems.

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Attributions

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Chapter 3: In Vitro Evaluation of Coatings to Control Ammonia Volatilization from Surface-applied Urea

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1. Introduction

2. Design and Validation of a Laboratory System for Measurement of Volatilized Ammonia

Preface

The mission of the soil fertility research group at Virginia Tech has been to understand and improve the efficiency of cropping systems. One focus of the group is improved nitrogen use efficiency in non-legume systems. Nitrogen loss via ammonia volatilization has been the focus of many studies within the group and the development of the ammonia volatilization system was done in collaboration with Timothy Woodward, Garnett Whitehurst and Brooks Whitehurst. The system has allowed the group to test many coated materials on granular urea as well as ammonia volatilization from urea ammonia nitrate solutions. The laboratory trials were then transposed to field agronomic studies to evaluate agronomic impact of the coated granular urea on corn grain yields and corn ear leaf nitrogen concentration.

The second mission of the soil fertility group is to be innovative in some aspect of agronomy. The extraction, identification, and activity analysis of urease from crop residues in no-till cropping systems will allow for the further understanding of agronomic systems, but also incorporates biochemistry with agronomic studies. The study was done in collaboration with Devin Ridgely (Ph.D student, Biological Systems Engineering (BSE)), Dr. Justin Barone (Associate Professor, BSE), Kathryn Gaasch, Dr. Chao Shang (Crop and Soil Environmental Sciences). The expertise of these scientists was instrumental in solving and understanding the complex problems associated with extracting, identifying, and determining the activity of urease in corn and soybean residues.

1 Introduction

Nitrogen (N) is found ubiquitously on earth, varying in forms such as amino acids to the most abundant gas, N_2, in the atmosphere. Despite its abundance in the atmosphere, N is the most limiting nutrient in non-legume agricultural cropping systems. Starting in the early 1960's grain production and the global community went through a period known as the "Green Revolution." The Green Revolution marked the beginning of use of improved genetics, synthetic pesticides, and synthetic N fertilizers. Synthetic N fertilizers are manufactured by reacting N_2 gas in the atmosphere with hydrogen gas, H_2, under extreme temperatures and pressure, during the Haber-Bosch process. The resulting product is ammonia gas, NH_3, and can be used as a fertilizer or further reacted to produce N fertilizers such as urea, ammonium nitrate, and ammonium sulfate.

Close to 40% of the increase in grain production over the past 60 years is attributed to synthetic N fertilizer (Brown, 1999; Mosier et al., 2004; Smil, 2002). In the early part of the 21st century, 50% of the total N used for global crop production could be traced to synthetic N fertilizer (Mosier et al., 2004). Worldwide demand for synthetic N fertilizer is expected to increase from approximately 128 Tg in 2007/2008 to 139 Tg in 2011/2012 (FAO, 2008). Though the total demand for N is increasing around the world, distribution is not uniform. Developed or developing areas such as the U.S., Europe, China, and India use the vast majority of the N supply, whereas places such as the sub-Sahara in Africa apply little fertilizer N. This is largely due to the cost per unit of N, being cheaper in the U.S. and China and more expensive in less developed areas due to transportation and infrastructure limitations.

The nitrogen use efficiency (NUE) of an agronomic system is a measure how efficiently N is being utilized by the crop of interest and is calculated using Eq. 1.1:

Eq.1.1: Calculation of nitrogen use efficiency (NUE)

$$NUE = \frac{[(total\ crop\ N\ removed) - (N\ coming\ from\ the\ soil + N\ deposited\ in\ the\ rainfall)]}{(fertilizer\ N\ applied\ to\ cereals)}$$

(Raun and Johnson, 1999)

NUE typically falls short of 50% worldwide with some production systems as low as 20% (IFA/FAO, 2001; Mosier et al., 2004; Raun and Johnson, 1999; Smil, 2002). Raun and Johnson (1999) estimated that every 1% increase in NUE would save 490,000 Mg of N worldwide keeping yields constant. The annual value of that savings would be approximately $234,660,000 globally. By increasing NUE in agroecosystems less N is needed to optimize crop yields, profitability of cropping systems increases, and nutrient loading in the environment decreases.

Nitrogen fertilizer that is not used by the crop can potentially be lost from the agroecosystem. Nitrogen loss mechanisms include denitrification, leaching, ammonia volatilization, runoff and erosion. The absolute percentages for individual N loss mechanisms are difficult to estimate for agricultural production systems due to the effects of climate, soil, and N fertilization sources. Gaseous losses of N, ammonia volatilization and denitrification, are the major sources of loss for many production systems (IFA/FAO, 2001).

Among the gaseous loss mechanisms ammonia volatilization is the greatest (Mosier et al., 2004; Peoples et al., 2004). Of the total N applied as fertilizer, model estimates are that about fourteen percent is lost via volatilization, and almost eight percent of the N applied as manure is estimated to be volatilized as ammonia (IFA/FAO, 2001; Peoples et al., 2004). In most parts of the world urea serves as the principal N source; the exception being in Europe where nitrate fertilizers dominate (Mosier et al., 2004). High N analysis (46% N), ease of handling, and its relative low cost have made urea the fertilizer of choice in many production

systems (Kiss and Simihaian, 2002). The use of urea as the dominant N source around the globe makes ammonia volatilization the loss mechanism of greatest importance with respect to applied fertilizer. Primarily surface applied in granular form in many regions, urea must have enough moisture to dissolve and then undergoes a hydrolysis reaction in the soil to be converted to ammonium (Eq. 1.2)

Eq.1.2: Urea Hydrolysis

$$H_2N-CO-NH_2+H_2O \xrightarrow{Urease} H_2N-COO^- + NH_4^+$$

$$H_2N-COO^- + H_2O \rightarrow HCO_3^- + NH_3$$

(Krajewska, 2009; Ciurli, 1999)

The urea hydrolysis reaction is catalyzed by urease (urea amidohydrolases E.C.3.5.1.5) to form ammonia and bicarbonate (Ciurli et al., 1999; Krajewska, 2009) shown in Eq. 1.2. Ureases are found in many organisms ranging from bacteria, plants, and as an extracellular enzyme in soil. Urease increases the hydrolysis reaction rate to as much as 10^{14} times faster than the uncatalyzed reaction (Krajewska, 2009). The function of urease varies depending on the species, but in plants ureases are involved in urea metabolism as well as metabolism of N containing molecules within plant cells (Krajewska, 2009). Though ureases serve different metabolic functions for individual species, the amino acid sequences of ureases from different species exhibit high levels of homology (Krajewska, 2009). Plant urease subunits consist of single-chain polypeptides as compared to bacterial urease subunits consisting of two to three polypeptides (Balasubramanian and Pannuraj, 2010). Though there are differences in the subunits of plant and bacterial ureases, the amino acid sequences are homologous (Balasubramanian and Pannuraj, 2010). The subunits of plant ureases have molecular weights circa 90 kiloDaltons (atomic mass unit = mass of one proton) (kDa) and

research has shown that urease extracted from living tissue have different activities among species (Krajewska, 2009; Balasubramanian and Pannuraj, 2010).

Urease in soil environments is found in two distinct pools: the first being intracellular urease of living microorganisms and the latter being extracellular urease released from microbes and plant cells upon death (Mobley and Hausinger, 1989). Extracellular soil urease is bound to humic substances and adsorbed on clay minerals which is responsible for the observed increased stability of the enzyme in soils (Mobley and Hausinger, 1989). The rapid hydrolysis of urea to bicarbonate and ammonia results in an increase in pH around the dissolved zone of urea which favors volatilization of ammonia at the soil surface (Bremner, 1995; Ciurli, 1999).

Many studies have been conducted to find ways of controlling ammonia volatilization from surface-applied urea and urea-derivative fertilizers. The studies range from using tillage as a volatility control measure to using heavy metals as inhibitors of urease. These studies have typically found that urease activity is not consistent throughout different soil types (i.e. changes in texture, organic carbon, pH and residue cover) (Antisari et al., 1996; Beri et al., 1978; Beyrouty et al., 1988; Broadbent et al., 1985; Watson, 2005). The most successful management practice found to date for controlling volatilization from surface applied urea is applying the urea right before a rainfall event of at least 14.6 mm so that the fertilizer is dissolved and moved below the soil surface (Holcomb and Horneck, 2011). However, since weather is unpredictable and the crop needs the nutrients at specific growth stages, growers must fertilize at certain points during the growing season, with or without dependable rainfall.

One of the most effective ammonia volatilization controls for urea involves inhibiting urease in agroecosytems by applying the chemical N-(n-butyl) thiophosphoric triamide (NBPT) with urea-based N fertilizer (Watson, 2005). An enzyme inhibitor decreases the

efficiency of the enzyme thus slowing the chemical reaction (Chang, 2000). However, NBPT is not the true urease inhibitor as studies have shown that once in the soil environment NBPT is degraded to N-(n-butyl) phosphoric triamide (NBPTO) (Christianson et al., 1990; Creason et al., 1990; McCarty and Bremner, 1989). In the soil system NBPT undergoes an oxidative desulfuration reaction to form NBPTO (Creason et al., 1990). By replacing the sulfur atom in the structure with an oxygen atom, the structure more closely imitates the electron configuration of urea allowing greater inhibition of soil urease. Upon transformation in the soil NBPTO is the actual competitive inhibitor that reduces urease activity thereby reducing ammonia volatilization.

Urea treated with NBPT has been extensively studied in soil and on urease from ureolytic microorganisms to ascertain the percent inhibition. Turner et al. (2010) reported a reduction in N loss through ammonia volatilization from 9.5% of applied N from uncoated urea to 1.0% of applied N. Essentially no ammonia was reported in flood waters when urea was treated with NBPT (Byrnes and Amberger, 1989). Rawluk et al. (2001) reported a reduction in ammonia loss of 75-85% when NBPT was added to urea. The data set for controlling N loss in Virginia and North Carolina cropping systems with NBPT is limited and needs to be expanded as urea containing fertilizers are major N sources in this region.

Until recently, Agrotain® International has been the only supplier of coated urea with NBPT and the source of the inhibitor for nearly all research on ammonia volatilization from urea fertilizers. Recently a NBPT coated urea fertilizer has been developed by Weyerhaeuser Inc. (Seattle, WA) and Whitehurst and Associates (New Bern, NC). The binding agent for the new coated urea fertilizer has yet to be evaluated against the industry standard. This new product has the capacity to coat urea with physical coatings in conjunction with the urease inhibitor NBPT. Being able to physically coat the urea will allow for the application of minor and micro-nutrients to crops with one spreader pass across the field. Weyerhaeuser has

utilized this technology for the past decade on loblolly pine stands across the southeast and sees the potential for the technology in agronomic crop production (Personal communication, Robert Campbell, 2010).

The overall objectives of this research was to conduct field and laboratory trials that describe ammonia volatilization from urea fertilizers with and without volatility control measures in cropping systems representing those found in Virginia and North Carolina. The specific objectives of the research were: 1) test and evaluate the design and performance of a new laboratory ammonia volatilization measurement system; 2) determine if urease can be extracted from corn and soybean residues; 3) determine if differences in urease activity are present in corn and soybean residues; and 4) evaluate N content and yield of corn treated with surface-applied coated urea fertilizers.

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2 Design and Validation of a Laboratory System for Measurement of Volatilized Ammonia

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2.1 Abstract

The design of laboratory systems for studying ammonia (NH_3) released from fertilizers varies widely, and few designs have been tested to determine the accuracy and precision in measuring ammonia loss. A standard volatilization system design is needed for reliable and comparable studies of ammonia volatilization from nitrogen fertilizer. The objectives of this study are: (i) to describe the design of a system capable of controlling air flow rate and temperature for laboratory measurement of NH_3 volatilized from N fertilizers; and (ii) assess the system's efficiency and variation in recovering NH_3 lost from NH_4Cl applied to an alkaline sand media. The system is comprised of individual chambers for soil and fertilizer, where temperature can be varied from room temperature to ~32ºC; humidity is maintained near saturation, air flow rate can be varied, and acid traps are used to capture volatilized NH_3. Two initial trials (I and II) were conducted at an N rate of 90 kg N ha^{-1} using air flow rates of 2.00 and 1.00 L min^{-1} and trapping acid volumes of 50 and 100 ml, respectively. A third trial was conducted at 30^{o}C. A fourth trial (IV) was performed using a range of N application rates (25 to 250 kg N ha^{-1}). The system recovered 89.3% to 97.1% of the N applied over all four trials and provided accurate and repeatable results under the conditions tested. Rapid, precise comparisons of NH_3 volatilization losses from N fertilizers under laboratory conditions can be made with this system.

2.2 Introduction

Volatilization of NH_3 from N fertilizers is one of many processes where N is lost from the soil environment. Volatilization of NH_3 after fertilizer application did not receive substantial attention as a possible source for N loss until the 1950's (Freney et al., 1983). NH_3 volatilization reduces N-use efficiency (defined as N yield per unit of applied N) and creates uncertainty in the management of N at the farm level. The study of NH_3 volatilization

requires equipment and practices that limit direct and indirect influences of factors that affect the volatilization of NH_3. Developing models to accurately predict NH_3 volatilization amounts in cropping systems, pastures or forests is difficult given the complexity of these biological systems.

Methods of measuring NH_3 volatilization from N sources, organic amendments or inorganic fertilizers, can be divided into two general classes: i) in-situ and ii) in-lab or controlled environment experiments. All in-situ NH_3 volatilization methods are disadvantaged by the inability to control climatic factors (e.g. rainfall timing and amount, humidity, and temperature) that directly influence NH_3 volatilization rates. These studies typically monitor these factors, but are unable to replicate exactly the factors across multiple studies or treatments unless all are performed during the same period and on the same site. Also, seasonal fluctuations limit the period in which reliable in-situ studies can be performed.

In-lab controlled studies can maintain environmental factors affecting NH_3 volatilization across multiple treatments and multiple treatment periods. Also, in-lab studies can be performed year-round with limited time needed for set-up between treatment periods. Reduced sample sizes allow for a larger number of treatments and replications. Results obtained from well-designed systems can provide reliable comparisons between treatments.

The primary method to measure NH_3 volatilization in laboratory studies utilizes a closed chamber, containing soil and N fertilizer amendments to be evaluated, and forced air flow across the treatment surface with an acid trap to capture the volatilized NH_3 (Hargrove and Kissel, 1979; Terman, 1979; Shi et al., 2001; Kissel et al., 2004; Portejoie et al., 2004; Todd et al., 2006, Cole et al., 2005; Miles et al., 2008; Ndegwa et al., 2009). Many in-lab systems have incorporated humidification features, temperature controls, acid traps to scrub NH_3 from air being drawn into the system, and air flow controls. The design of in-lab systems

is inconsistent, but performance of several laboratory systems used to measure ammonia volatilization have been studied and NH_3 recovery values have varied from 72.9 % to 103% with varying levels of consistency in the measurements (Table 2.1). Currently, in-lab volatilization systems are primarily being used to measure NH_3 released from manures (Cole et al., 2005; Miles et al., 2008; Portejoie, 2004; Shi et al., 2001; and Todd et al., 2006). Inorganic fertilizer studies using in-lab systems are less common, but are increasingly being performed (Knight, 2007; Rochette et al., 2009; and Susilawati et al., 2009). The detailed construction and operation of in-lab systems vary among studies, and again, few experiments have examined factors affecting NH_3 recovery within the specific system (Miles et al., 2008 and Ndegwa et al., 2009). These factors include: air flow rate, trapping acid (source, concentration, and volume), temperature and humidity conditions. In order to ascertain the validity of data from controlled environment systems, newly designed in-lab systems need to be tested to determine the system's NH_3 recovery efficiency within an estimated range of potential NH_3 volatilization rates.

The objectives of this study were to: i) describe a controlled-environment volatilization system used for laboratory studies of NH_3 volatilization; and ii) assess the system's efficiency and variation in recovering NH_3 lost from NH_4Cl applied to an alkaline sand media.

2.3 Materials and Methods

2.3.1 System Design

The NH_3 volatility measurement system as tested consists of three temperature-controlled enclosed cabinets that house six chambers each to which treatments are applied to soil or other media. Humidified (near 100%) air is passed through each chamber above the soil media of each chamber at a constant flow rate and temperature. Air exiting the chamber is collected by acid traps to recover ammonia lost by volatilization. Soil temperature within

each chamber is monitored using thermocouples to ensure accurate and even temperatures within and across each chamber (Brooks Whitehurst Associates Inc., New Bern, NC). A materials list and prices are in Table 2.2 and the schematic for the system is shown in Fig. 2.1.

The enclosed cabinets are constructed using 9.5 mm thick plywood with the internal dimensions of 61 cm in width, 61 cm in length, and 46 cm in depth. The lid of the cabinets lifts off and was constructed to overhang the outside edge of the cabinet. The lid has a rim that fits securely within the internal dimensions and was insulated using 13 mm thick Styrofoam® sheath insulation (R value = 3). The temperature within the cabinets is controlled using a 1/32 Din programmable controller (CN7533, Omega Engineering Inc., Stamford, CN). The air temperature sensor (RTD-805, Omega Engineering Inc., Stamford, CN) used by the temperature controller is mounted on the opposite side of the cabinet from the heating element. The heating element was fabricated by attaching a silicone rubber heat strip (2.5 cm x 20 cm, 80 W total,SRFG-108/10-P, Omega Engineering Inc., Stamford CN) to an angular aluminum strip mounted on a rectangular block (4.1 cm x 4.1 cmx 20 cm long - 90°). An electric fan (2000 rpm, 120x120x25mm, 115 V, 60 Hz, 0.10 A) (BT12025B1L, CoolerGuys.com, Kirkland, WA) was placed above the heating element to disperse heat and circulate air inside the cabinet. A wooden baffle (19 mm wide, 30.8 cm long, 28 cm tall) with ends that angle (20-30^{o}) towards the chambers (14 cm long outside and 13cm long inside) (Fig. 2.1). A raised platform for the chambers are used to insure uniform heat dispersal around the chambers (Fig. 2.1).The air supply to the entire system is provided by a oil-less linear air pump (DDL80-101, Gast Manufacturing Inc., Benton Harbor, MI) that pressurizes and fills a supply tank. This pump has a max power output of 93 W, air flow rates range from 111-120 L min^{-1} from 50-60 Hz, and maximum pressure of 0.48 bars. The lines carrying the air from pump to the supply block are 16 mm OD and 13 mm ID vinyl lines. The air

pressure is monitored and regulated for the system at the supply tank. Air exiting the supply tank is supplied to three distribution manifolds via the same size vinyl line entering the supply tank. All fittings and connections for the 16 mm vinyl lines are secured by plastic hose clamps (SNP-2, Cole-Parmer Instrument Co. 95613-03) Each distribution manifold is used to supply air to six flow meters (0.4-5.0 L min^{-1} flow meters - EW22461-50, Cole-Parmer Instrument Co., Vernon Hills, IL). Air exiting the distribution manifold flows through 6.4 mm OD and 4.3 mm ID vinyl hose. This size vinyl hose is what carries the air throughout the rest of the system. All fittings and connections for this size vinyl hose are secured with SNP-1 plastic hose clamps (Cole-Parmer Instrument Co. 06832-01). Air from the flow meters is then sparged through air stones (Coarse Bubbles, Kordon LLC., Hayward, CA) to reduce the bubble size inside a set of closed cylinders (external humistats) containing water purified by reverse osmosis (RO). This step saturates the air prior to entering the temperature controlled cabinet. Air exiting the external humistats is sparged through a second set of air stones inside a second set of closed cylinders containing RO water (internal humistats) within the controlled-temperature cabinets. This ensures no decrease in humidity due to the change in temperature between outside and inside the cabinet. Also, the second humidification step ensures the air temperature is brought to the temperature within the cabinets. The system was designed to increase the humidity of the air entering the chambers to a level that approaches 100% humidity and levels cannot be varied with the current system. Air from the internal humistats is routed into the individual chambers and then into an acid trap specific to each chamber. Since this is a laboratory system and based on the assumption that ambient NH_3 concentrations are below our capacity to detect, the humistats are for humidity purposes only and do not act as NH_3 scrubbers.

The chambers consist of threaded 100 mm by 150mm beakers (21650 B, Kimble Chase Life Science and Research Products LLC, Vineland, N.J.) and three-hole plastic caps

(21650 C3, Kimble Chase Life Science and Research Products LLC, Vineland, N.J.). Two 9.5 mm holes with threaded fittings (threaded on top surface) are used in the cap for the air entry and exit ports, and a third 3.2 mm threaded hole is used to position the thermocouple into the soil media. The air entry port uses a portion of a small PVC pipe, same size as the incoming vinyl tubing, allowing the air to enter 6.4 mm above the soil surface. The air exit port extends 6.4 mm below the bottom face of the lid. The design allows for the air to move across the soil surface collecting ammonia, before being forced up exiting the chamber. Once filled the head space above the sand is 0.527 ± 0.005 L, and at an air flow rate of 1.00 L min^{-1} the air within each chamber is replaced every 32s.

Temperature data from the soil media chambers were collected by type T thermocouples (TT36-18U-6-SB, Omega Engineering Inc., Stamford, CN) inserted through a 1/8 hole in the cap of each chamber. Analog signals from the thermocouples were converted to digital temperature readings using a 22 bit A/D converter (DAQ-56, Omega Engineering Inc., Stamford, CN). Temperature data from type T thermocouples and temperature control equipment were calibrated against a NIST Traceable™ digital thermistor (90080-12, Cole-Parmer Instrument Co., Vernon Hills, IL). Software provided with the A/D converter enables temperature data storage on the linked computer.

The acid traps were 125 ml plastic bottles (PCR8DB, Specialty Bottles, Seattle, WA) filled with 50 or 100 ml of acid solution. An air stone is used in each acid trap to reduce the bubble size passing through the 0.02 M phosphoric acid solution.

2.3.2 System Validation

To validate the laboratory system, four loss-and-recovery trials (I, II, III and IV) were conducted to determine the recovery efficiency and variability of NH_3 volatilization between each individual chamber. We used a similar method outlined by Cabrera et al. (2001) in that

we applied ammonium chloride (NH_4Cl) to an alkaline sand media. The sand media was a mixture of $CaCO_3$ and sand, purchased at a local hardware store. Trials I and II tested the accuracy and precision of the system to measure NH_3 loss at different air flow rates and acid trap volumes at a standard N rate of 20 mg N per chamber (90 kg N ha^{-1}) and a temperature of 26ºC. Nitrogen rates were based on a weight basis, using 2.245 million kg soil per ha furrow slice, then calculating the 90 kg N ha^{-1} rate. Nitrogen was applied as an ammonium chloride solution to deliver the required N rate by dripping five milliliters of solution on the surface of the sand-$CaCO_3$ mixture. Trial III evaluated accuracy and precision of the system at 30 ºC. Trial IV tested the system over multiple N rates using the most accurate and precise air flow and acid trap volumes determined from trials I and II.

The duration of trial I was 3 wk (504 h). We limited the run length of trials II, III, and IV to 2 wk as volatilization losses were minimal after 2 wk. The air flow rates were 2.00 L min^{-1} and 1.00 L min^{-1} for trials I and II, respectively. The volume of acid in the acid traps for trials I and II were 50 and 100 ml, respectively. Trial II air flow rate and acid volume were found to produce more precise NH_3 loss measurements; therefore, an air flow rate of 1.00 L min^{-1} and an acid volume of 100 ml were used for trial III, at a temperature of 30ºC, and trial IV with multiple N rates. N rates for trial IV were 0, 25, 50, 150, 200, and 250 kg N ha^{-1} replicated three times. The temperature during trials I, II and IV was 26.0±0.5ºC and 30.0±0.3^{o}C for trial III.

The set-up of the system prior to commencing a trial consisted of making the alkaline sand media by adding calcium carbonate ($CaCO_3$) to oven dried (60°C) sand at a rate of 12.5 g $CaCO_3$ kg^{-1} dry sand. The mixture was brought to 20% moisture and oven dried overnight at 60ºC. The wetting-drying step was repeated three times to ensure the pH of the mixture equilibrated (pH 7.8-8.1). Five-hundred grams of the oven dried mixture were placed into each chamber and the chambers placed inside the cabinets. Air flow was then calibrated using

an NIST Traceable™ digital flow meter (FMA 1700/1800, Omega Engineering Inc., Stamford, CN) at the inlet to each chamber. The external and internal humistats were filled with 900 and 450 ml of reverse osmosis (RO) purified water, respectively. The internal humistats were positioned in the cabinet and the cabinet temperature controls were set to the desired temperature. The water in the internal humistats and the sand-$CaCO_3$ mixture was allowed to come to temperature overnight (~12 h).

The following day RO water was added to the sand-$CaCO_3$ mixture to attain 10% moisture content. The acid trap bottles for the initial sampling interval were filled to the desired volume with 0.02 M phosphoric acid and placed in position. A 5 ml NH_4Cl solution at an N concentration to deliver the desired N rate was surface applied onto the sand-$CaCO_3$ mixture. All air connections were secured and the pump was engaged to commence the trial.

Trapping acid was changed with fresh acid at intervals of 1, 3, 6, 9, 12, 24, 48, 96, 144, 192, 240, 288, 336, 388, 432, and 504 h after starting the trial (trials II, III and IV ended at h 336). The replaced acid was then weighed and analyzed for NH_3 concentration colormetrically with a Lachat QuickChem Automated Ion Analyzer (Lachat Instruments, Loveland, CO).

Once the trial concluded a 5 g sample of dry sand-$CaCO_3$ mixture from each chamber was extracted using 2 M KCl and analyzed colormetrically for NH_3 and nitrate (NO_3) with a Lachat QuickChem Automated Ion Analyzer. Moisture content of the sand-$CaCO_3$ mixture was determined and then used to calculate how much of the mixture was needed to attain a five gram sample of dry sand-$CaCO_3$ mixture.

2.3.3 Statistics

Using PROC MEANS for SAS 9.2 (SAS Institute, Cary, NC) mean N captured and standard deviation, for each sampling interval as well as cumulative N captured were

computed for each trial. System recovery efficiency (SRE) and acid trap efficiency (ATE) were calculated for all trials using the following formulas:

Eq. 2.1: System Recovery Efficiency

$$\frac{acidtrapN + \mathrm{Re}sidualN}{Nadded + \mathrm{Pr}iorN} * 100 = SRE(\%)$$

Where:

acidtrapN = cumulative mg N captured in acid traps

ResidualN = total mg N extracted in 2M KCl from the sand-$CaCO_3$ mixture after completion of the trial

Nadded = mg N added to each chamber

Prior N = total mg N extracted in 2 M KCl from the sand-$CaCO_3$ mixture with no N added

SRE = System Recovery Efficiency

Eq. 2.2: Volatilization Loss

$$(Nadded + \mathrm{Pr}iorN) - \mathrm{Re}sidualN = Volatiliza\,tionLoss(VL)$$

Where:

PriorN = N in media prior to addition of fertilizer N

Residual N = NO_3-N + NH_4-N in the media at the end of the trial

VL = *Volatilization Loss*

Eq. 2.3: Acid Trap Efficiency

$$\frac{acidtrapN}{VL} \times 100 = ATE(\%)$$

Where:

ATE = Acid trap efficiency

Two samples of the sand-$CaCO_3$ mixtures with no N applied were taken from two separately mixed batches and used to calculate *PriorN* for trials I and II. Both batches were mixed at the same rates and with the same sources of sand, $CaCO_3$, and water. No detectable NO_3-N or NH_3-N was present in those samples using the 2 M KCl extraction method and colorimetric analysis.

Analysis of variance was conducted for SRE and ATE using PROC MIXED in SAS 9.2 to determine if acid trapping efficiency changed with N rate in trial IV.

2.4 Results and Discussion

2.4.1 Trials I, II, and III

Mean volatilization loss, acid trap N recovered, ATE, total N recovered from both the trapping acid and the sand-$CaCO_3$ mixture after completion of the trial (system recovery), and SRE for trials I , II, and III are shown in table 2.3. Volatilization loss would be a parameter of interest during the study of simulated production systems using this laboratory system. The sand and NH_4Cl system evaluated does not replicate agricultural environments and was only intended to serve as a means to validate the laboratory system. The mean amount of N recovered by the trapping acid was 18.7±0.4 mg N. The mean total amount of system recovery was 18.8±0.3 mg N. SRE was 94.1±1.5% and ATE was 94.0±1.6%. Mean acid trap N recovery was 16.8±0.4 mg N. The mean system recovery was 19.4±0.3 mg N.

ATE and SRE were 96.7±1.8% and 97.1±1.6%, respectively. These values are similar or exceed the recovery efficiencies shown by Cabrera et al. (2001). The standard deviations of these values indicate that even relatively small, i.e. 1 mg N loss during a two week period, treatment differences in future studies should be readily detectable. Upon raising the temperature to 30^{o}C the efficiency of the system decreased in trial III (Table 2.3). However, recovery efficiencies were greater than 90% for trial III, and the variation between chambers was similar to trial II (Table 2.3).

Mean cumulative N captured by the acid traps is shown in Fig. 2.2 for trials I, II, and III. Between flow rates, temperatures, and acid trap volumes, the amount of nitrogen trapped is very similar at the 336 h sampling interval. The standard deviations for total acid trapped at various times in trials I, II, and III are shown in Fig. 2.3. Variation in cumulative N captured by the acid traps increases from 1 to 96 h then decreases for the duration of the first three trials. The maximum variation observed at 96 h can be attributed to the longer sampling interval (48 h compared to 24h), resulting in larger amounts of NH_3 collected in the acid traps. Trial II exhibited less variation in terms of N capture than trial I, which indicates that by decreasing flow rate to 1.00 L min^{-1} and increasing the volume of trapping acid to 100 ml the system's variation is decreased. Ndegwa et al. (2009) also found that as the air flow rate decreased, the efficiency of the acid trap increased. Since the acid trap container was kept constant during trials I and II, doubling the volume of acid resulted in an increase in depth of the acid trap. Ndegwa et al. (2009) also found that deeper acid traps increased the efficiency of the trap. Because air flow rate was decreased and acid depth increased from trial I to trial II neither factor can solely be identified as responsible for the increase in acid trap and system efficiency, however both factors may have contributed to the increased recovery observed in trial II.

Trial III showed that similar cumulative amounts of NH_3 were captured when compared to trials I and II (Fig. 2.2). Based on the results from trials I and II, the same flow rate and acid trap volume were used in trial III. The variation in values was similar between trials II and III (Fig 2.3). Increasing the temperature to 30^{o}C had little effect on variation among chambers within the system. This is evidence that the system will produce repeatable measurements up to 30^{o}C. Measurements above 30^{o}C were not obtainable due to the heating strip capacity.

From the results of trials I and II, 100 ml of trapping acid and 1.00 L min^{-1} air flow rate were chosen to evaluate the system over multiple nitrogen rates (trial IV), since variation between chambers was decreased and SRE and ATE were highest in trial II Table 3).

2.4.2 Trial IV (Multiple N Rates)

Six N rates were applied randomly to the soil chambers within each cabinet to evaluate the efficiency of the system over a wide range of N application rates. Equations 1, 2 and 3 were used to calculate SRE and ATE for each N application rate and mean separations can be found in table 4, respectively. Percentages for the 0 kg N ha^{-1} are not given because no measureable N was found in the acid traps at each sampling interval or in the sand after the trial was completed. The SRE decreased as the N rate increased with the 25 kg N ha^{-1} rate having the highest SRE, 95.7±1.5% and 250 kg N ha^{-1} rate having the lowest with 89.3±2.3%. Standard deviations ranged from 1.5 to 3.0% (Table 2.4).

The ATE for all N rates applied in trial IV decreased with increasing N application rates (Table 2.4). The highest ATE was 95.3±1.2% for the 25 kg N ha^{-1} rate and was significantly higher than all other treatments. When N rates increased to 250 kg N ha^{-1} the ATE dropped to 84.7±2.3%, and was significantly lower than all treatments, except the 200 kg N ha^{-1} rate. The decrease in ATE with increasing N rates agrees with data by Ndgewa et

al. (2009), who also found that the efficiency of the acid traps decreases as the amount of ammonia to be trapped increases. System recovery efficiency decreased as ATE decreased which is expected since both SRE and ATE are calculated using N captured in the acid traps as a variable.

2.5 Conclusions

The need to evaluate a system that will be used in quantitatively measuring a parameter of interest is essential in producing results that are precise and accurate. The laboratory ammonia volatilization system described was able to detect and capture over 90% of the ammonia volatized and maintain variation within individual chambers which will allow for reliable comparisons of fertilizer or simulated production systems in future trials. Decreasing the air flow rate to each chamber and increasing trapping acid volumes between trial I and II resulted in the reduction of variation between chambers and increased ATE and SRE. We believe this is due to the increased time during the gas-liquid interface in the acid traps, which allows for increased conversion of NH_3 to ammonium (NH_4) in the acid. This increased reaction time may also allow the NH_3 to be captured by individual traps more consistently across all chambers. Since the system performed best at an air flow rate of 1.00 L min^{-1} and with 100 ml of trapping acid this configuration was used for trial III. The relatively low SD values (1.2 to 3.5 %) for all N application rates indicate that relative comparisons between treatments can be made with confidence.

Increasing the temperature to 30^{o}C decreased the SRE and ATE slightly compared to that of trials I and II, but was still above 90% at this temperature. The variation remained similar to that in trial II showing that repeatability did not decrease as temperature increased. With the high recovery efficiencies and low variation this system will be able to operate at temperatures up to 30^{o}C in order to simulate different environments in future studies.

SRE and ATE decreased as N rates increased from 0 to 250 kg N ha^{-1}. This range covered N fertilization rates that are routinely used in commercial agricultural production systems. The reduced SRE and ATE rates at higher N rates can be attributed to the total amount of NH_3 volatilized per unit of time. As NH_3 volatilized per unit time increases, the amount of NH_3 within each air bubble increases, and since the time for the reaction to take place remains constant (constant depth of acid and air flow rate) a lower percentage of NH_3 is successfully captured in the trap. Greater acid trap volumes need to be tested to potentially increase SRE for higher N rates, i.e. 200 to 250 kg N ha^{-1}.

2.6 References

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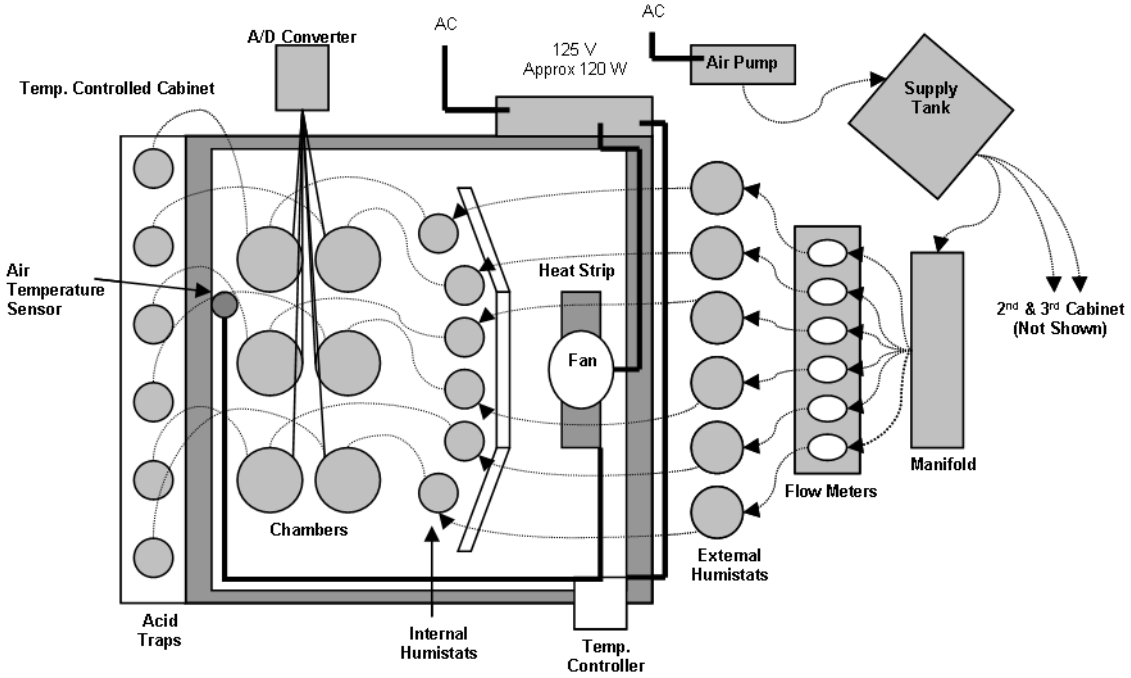

Fig. 2.1: General schematic of the system used for in-lab ammonia volatilization studies as tested

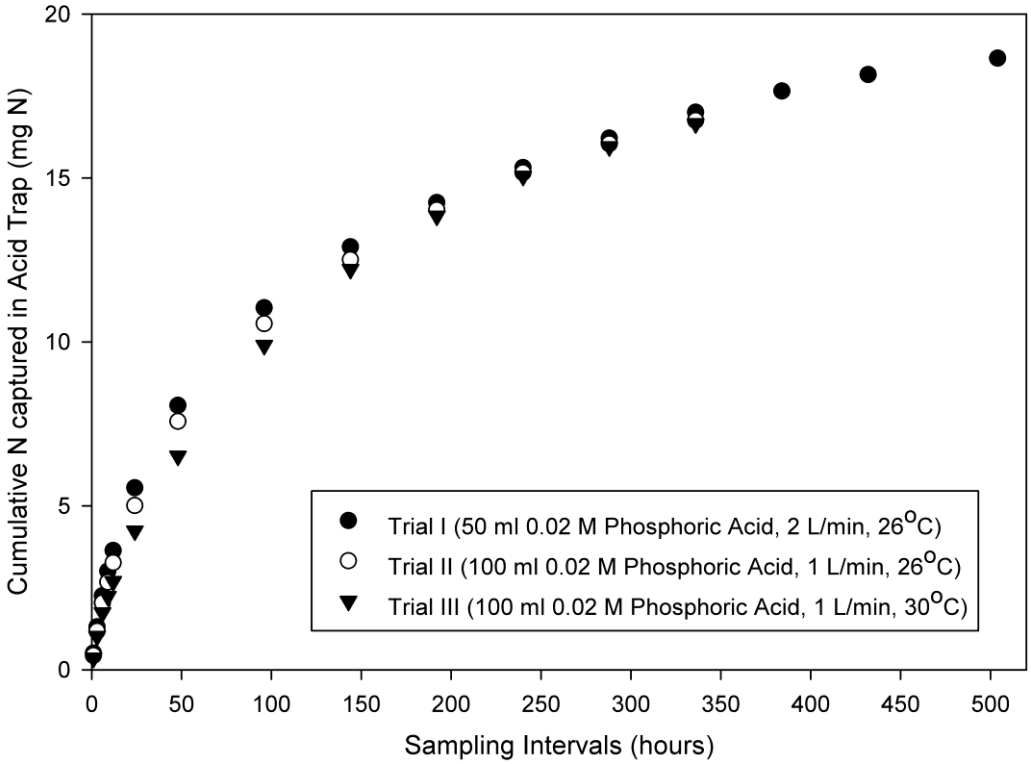

Fig. 2.2:Cumulative N captured in acid traps during trials I ,II, and III

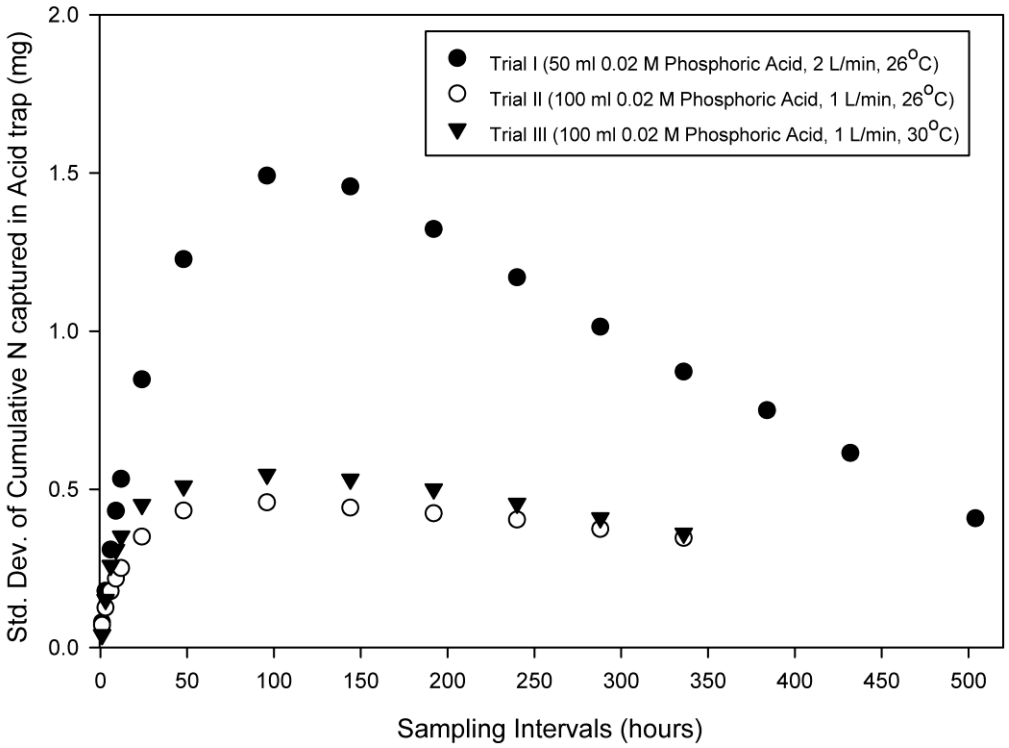

Fig. 2.3: Standard Deviation for cumulative N captured for trials I, II, and III

Table 2.1: Comparison of system efficiencies and variation for laboratory systems measuring NH3 volatilization.

Study	N Source	Trapping Acid	System Efficiency[†]	System Variation
		molarity (volume)	%	
O'Halloran (1993)	Liquid Hog Manure	0.32M H_3BO_3 (50&100mL)	82.1-83.5[¶¶]	NR[¶]
		or		
		0.9M H_2SO_4 (50mL)	94.9[¶¶]	NR
Kissel et al. (2004)	Granular Urea	0.05M H_2SO_4 (50mL)	72.9-100.6[¶¶]	NR
Todd et al. (2006)	Cattle Manure	0.9M H_2SO_4 (100mL)	NR	2.67[‡] mg
Cole et al. (2005)	Cattle Manure	0.9M H_2SO_4 (100mL)	NR	1.45[‡] mg
Miles et al. (2008)	Poultry Litter	H_3BO_3 (60mL)	NR	2.6-6.3%[§]
Ndegwa et al. (2009)	NH_4Cl	0.2M H_2SO_4 (150&470mL)	93.5-94.8	NR
Torello et al. (1983)	$(NH_4)_2SO_4$	1.5M H_3BO_3	95.1-103.0	NR

[†]System efficiency is accountable N post trial divided by applied or previously present N.

‡Standard error of the mean NH_3 recovered in the acid traps

§Standard deviation of the mean NH_3 recovered in acid traps

¶ Data not reported in article

¶¶ Calculated from data provided in article for comparison reasons

Table 2.2: Detailed materials list with appropriate costs for volatilization system

Section	Description	Quantity	Approx. Cost[a]	Total Cost[a]
Air Supply	Air Pump	1	530	
	Supply Tank	1	100	
	Distribution Manifold	3	45	
	Flow Meter	18	1440	
	External Humistat	18	500	
	Internal Humistat	18	200	
	Tubing and Connections		150	2965
Cabinet Structure	61 cm x 61 cm Paneling	15	165	
	Lid Panel	3	25	
	Insulation	3	55	
	Baffles	3	20	
	Chamber Cradle	3	130	
	Misc. Lumber and Hardware		50	445
Power Supply	Flanged Inlet	3	70	
	Red Indicator Lamp	3	15	
	Enclosure	3	130	
	Green Indicator Lamp	3	20	
	Switch	3	25	
	Terminal Strips	6	40	
	Wiring		10	
	Power Cord (2.44 m)	3	15	325
Temp. Control	Controller	3	270	
	Air Sensor	3	260	
	Heat Strip (20 cm)	3	55	
	Fan	3	55	
	Wiring and Connections		10	650
Temp. Data Collection	A/D Converter	3	3750	
	Thermocouples	18	510	4260
Chamber	Three Hole Cap	18	3630	
	Beaker	18	840	
	Fittings		30	4500
Calibration	Traceable™ Thermocouple	1	590	
	Flow Meter	1	900	
	Drying Tube	1 pk	30	
	Fittings		50	1570
[a]US Currency			Total	14715

Table 2.3: Volatilization N loss, acid trap N recovery, system N recovery and calculated efficiencies for trialsI, II, and III with a 90 kg N ha^{-1} surface application

	Trial		
	I‡	**II¶**	**III¶**
Mean†	26°C		30°C
Volatilization Loss (mg N)	19.8±0.2§	17.4±0.3	18.1±0.3
Acid Trap N Recovery (mg N)	18.7±0.4	16.8±0.4	16.7±0.4
System Recovery (mg N)	18.8±0.3	19.4±0.3	18.6±0.2
SRE (%)	94.1±1.5	97.1±1.6	93.0±1.2
ATE (%)	94.0±1.6	96.7±1.8	92.2±1.3

†Averaged across all chambers per trial
‡2.00 L min^{-1} air rate and 50 mL trapping acid
¶1.00 L min^{-1} air rate and 100 mL trapping acid
§ mg N ± standard deviation (SD)

Table 2.4: Mean system recovery efficiencies (SRE) and standard deviation (SD) for all N rates over two weeks for trial IV at 1 L min air flow and 100 ml of 0.02 M phosphoric acid trap volume.

N Rate	Total Acid Trap N	SRE	SD	ATE	SD
$kg \cdot ha^{-1}$	mg	%			
0	0 f*§	-	-	-	-
25	4.75 e	95.7 a¶	1.5	95.3 a¶¶	1.2
50	8.48 d	93.0 b	3	91.7 b	3.5
150	22.55 c	91.0 bc	2	88.0 c	3
200	26.02 b	90.7 bc	1.1	86.0 cd	1.7
250	32.18 a	89.3 c	2.3	84.7 d	2.3

*Significant at the 0.05 probability level.
§ **$LSD_{0.05}$ = 2.49 mg N**
¶**$LSD_{0.05}$ = 2.4%**
¶¶ **$LSD_{0.05}$ = 3.1%**

3 In Vitro Evaluation of Coatings to Control Ammonia Volatilization from Surface-applied Urea

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3.1 Abstract

Ammonia volatilization from surface-applied nitrogen (N) fertilizers containing urea can be substantial if environmental conditions are favorable. Physical and chemical coating urea has been effective in preventing ammonia volatilization losses. The objectives of this study were to quantify the in vitro N loss from surface-applied urea; and measure the rate and total N volatilization loss from urea coated with calcium sulfate, potassium sulfate, alone and in combination with the urease inhibitor, N-(n-butyl) thiophosphoric triamide (NBPT). Six trials, each lasting fourteen days, were conducted using a laboratory system at 26^{o}C, 1.00 L min^{-1}air flow, an N rate equal to 44.6 mg N kg^{-1}air-dried soil, and 100 ml of 0.02M phosphoric acid to recover volatilized ammonia. Cumulative ammonia loss from urea ranged from 33.9- 37.2% of the applied N in all trials. Initial ammonia volatilization losses were delayed by the addition of calcium and potassium sulfate coatings alone. The inhibitor NBPT reduced cumulative ammonia losses to 17.9-24.7% of applied N and delayed ammonia volatilization for 96 hours after N application when applied at the 0.08% w/w application rate over trials I-IV. During trials V and VI, applying NBPT at 0.02% w/w reduced cumulative ammonia volatilization from 35.6% and 35.1% to 25.4% and 24.1% respectively. No difference in cumulative ammonia loss was observed above NBPT rates of 0.04% in both trials. The inhibitor NBPT had the greatest influence on ammonia volatilization losses in these studies and further research needs to be conducted to determine optimum levels of the urease inhibitor in field situations.

3.2 Introduction

Urea fertilizer supplies N to a majority of agricultural systems worldwide. With the increasing need for more efficient nutrient management strategies, there has been extensive research conducted to reduce the inefficiencies associated with urea. The major inefficiency

of urea arises from volatilization of ammonia (NH_3) during the hydrolysis of urea by urease, an enzyme found ubiquitously throughout agricultural systems (Krajewska, 2009). Urease catalyzes the reaction that converts urea to carbamate and ammonium. The carbamate molecule decomposes into bicarbonate and ammonium (Ciurli et al., 1999). The bicarbonate ion increases the soil pH converting ammonium to ammonia (Ciurli et al., 1999; Kissel et al., 2008; Krajewska, 2009). Once urea is converted to ammonia, N is lost through volatilization if left on the soil surface. Nitrogen losses from urea through volatilization can be as great as 70% of the applied fertilizer N, with losses of 35% to 50% of applied fertilizer N in laboratory and field studies commonly reported under favorable environmental conditions (Antisari et al., 1996; Bayrakli, 1990; Beyrouty et al., 1988; Carmona et al., 1990; Christianson et al., 1990; Christianson et al., 1994). Ammonia volatilization loss of N contributes to the reduction of nitrogen use efficiency (NUE) of agronomic systems which have been estimated to average 33% worldwide (Raun and Johnson, 1999).

Urea hydrolysis raises soil pH adjacent to urea granules, inhibiting nitrification, resulting in excess ammonia and conditions favoring ammonia volatilization. Surface application of urea without a means to control the hydrolysis reaction will result in ammonia on the soil surface in this high pH zone. Urease activity varies with soil characteristics such as soil texture, moisture, temperature, crop residue, and organic carbon (Antisari et al., 1996; Bremner and Douglas, 1973; Carmona et al., 1990; Krajewska, 2009; Rawluk et al., 2001; Sanz-Cobena et al., 2008). Soil urease is unique compared to ureases derived from living organisms in that it is more stable and persistent in the soil environment. The enzyme urease accumulates in soil systems and can be found adsorbed to soil mineral particles and humic substances (Kiss and Simihaian, 2002). This adsorption inhibits the ability of microorganisms

to metabolize urease allowing for excess amounts to build within the soil with the result that more than enough urease to rapidly hydrolyze fertilizer urea exists in most soils.

Multiple control measures can be implemented to prevent N loss as ammonia volatilization from agricultural systems. Growers typically try to apply urea-based N fertilizers prior to a rainfall event to ensure movement of urea into the soil profile, or they incorporate urea into the soil. Both management strategies are extremely effective in preventing ammonia volatilization from urea. However, many times growers do not have the luxury of waiting for rain or irrigation to apply nutrients because they must ensure that nutrients are applied so that plant growth is not limited by N. In order to do this, farmers may apply urea when rain is not in the immediate future, which can potentially lead to volatilization losses of N. Alternative approaches have incorporated urea fertilizers using banded applications next to the row while others used chemicals that inhibit soil urease creating a time after urea application in which volatilization losses are low with the anticipation that rain will occur to move the urea into the soil.

Many studies have been conducted to find the most effective urease inhibitor but few have produced commercially viable options for controlling ammonia volatilization (Kiss and Simihaian, 2002). The urease inhibitor, N-(n-butyl) thiophosphoric triamide (NBPT), has been found to be the most effective in controlling ammonia volatilization (Byrnes and Amberger, 1988; Chai and Bremner, 1987; Watson, 2005). NBPT is degraded in soil to N-(n-butyl) phosphoric triamide (NBPTO) which is a competitive inhibitor of urease. Competitive inhibitors of enyzmes bind directly to the active site of the enzyme, thus competing with the substrate (urea in the case of urea N fertilizers) at the enzyme active site. During field and laboratory studies NBPT has successfully decreased urea hydrolysis in varying soil types and tillage systems. Carmona et al. (1990) reported a reduction in N losses through ammonia volatilization from 52% in uncoated urea to only 14.7% using 0.05% NBPT on a weight

basis. Byrnes and Amberger (1989) showed similar results with the N loss of 73.4% in a simulated flooded soil using untreated urea with only 34.7% loss of applied N when treated with NBPT. Antisari et al. (1996) found that the magnitude of reduction in ammonia volatilization varied from soil to soil and concluded different rates of NBPT may be needed for different soil types. This supports the evidence of changing urease activity with changing soil characteristics previously mentioned.

Until recently, Agrotain® (Koch Agronomic Services, Wichita, KS) was the only commercially available source of NBPT being widely marketed. Weyerhaeuser Co. (Federal Way, WA) and Brooks Whitehurst Associates Inc. (New Bern, N.C.) have developed a different solvent system (Trade Name Arborite® Ag) to adhere NBPT to the surface of urea granules. The NBPT solvent system developed by Weyerhaeuser and Brooks Whitehurst Associates Inc. can also be used as a binder to attach physical coatings (i.e. calcium sulfate and potassium sulfate) to the surface of urea granules alone, or in combination with the urease inhibitor, NBPT. This technology has the potential to supply various nutrients and adjust nutrient blends for selected regions or cropping systems because the coatings are applied at the fertilizer blend plant prior to application.

The objectives of this experiment were: 1) quantify ammonia volatilization losses from surface applied urea under controlled laboratory conditions favoring volatilization; 2) measure the rate and total N volatilization loss from uncoated urea and urea coated with calcium sulfate, potassium sulfate, and with or without NBPT incorporated into the sulfur-coatings and 3) measure the effect of NBPT application rate upon ammonia volatilization losses from granular urea.

3.3 Material and Methods

3.3.1 General

The study consisted of six trials to quantify the reduction, if any, in ammonia volatilization from physical and chemical coatings applied to granular urea. Each trial was conducted in the laboratory using the ammonia volatilization system described by Woodward et al. (2011). The ammonia volatilization measurement system consisted of three temperature controlled cabinets, each containing six individual soil chambers. Each soil chamber was filled with 500 grams of air-dried soil limed to a pH of 6.5-6.7. During all trials, air flow rates through the soil chambers were controlled at 1.00 L min^{-1} and temperatures were maintained at 26 °C. The headspace in the chambers during the trials was on average 0.43 L resulting in 2.33 exchange volumes per minute. Moisture laden air was used to sweep ammonia vapors from the soil chambers (Woodward et. al 2011).

Nitrogen was applied at the rate of 44.6 mg N kg^{-1} air dry soil, N application rate approximating 100 kg N ha^{-1}, for all fertilizer treatments. All urea fertilizers were sieved to pass an 8 mesh sieve and retained on a 10 mesh sieve. This ensured uniformity of granule size and more accurate treatment weights. Five to six granules were equally spaced on the surface of the soil in each chamber to achieve the desired N rate.

The soil used was the A horizon from a Fine-loamy, mixed, active, mesic Ultic Hapludalf (Wheeling silt loam). Soil was air-dried and passed through a 2 mm sieve. Physical and chemical characteristics of the soil are in table 3.1. Before each trial the soil was limed with calcium oxide (CaO) at a rate of 0.7 g CaO kg^{-1} air dried soil to adjust the pH to 6.5-6.7 (Table 3.1). After liming, the soil was weighed for each individual chamber and 65 ml of deionized water were added to the soil in each chamber and thoroughly mixed to ensure consistent moisture throughout the soil within the chamber. The addition of water raised the soil moisture content to approximately two-thirds of field capacity (~14%) (Table 3.1). This

ensured adequate soil moisture to dissolve urea granules and provide conditions for volatilization. Once the moisture equilibrated throughout the soil, the chamber was placed inside the specified temperature controlled cabinet for twenty-four hours to equilibrate the moistened soil to 26 °C. After equilibration, the fertilizer granules were placed on the moistened soil surface of each chamber.

Acid traps containing 100 ml of 0.02M ortho-phosphoric acid captured volatilized NH_3 in the air stream flowing over the soil surface, and acid traps were changed at 1, 3, 6, 9, 12, 24, 48, 72, 96, 120, 144, 168, 216, 264, 312, and 336 hours (two weeks) after initiation of each trial. Acid traps were weighed and total volume of solution calculated using the density of 0.02M phosphoric acid at 25 ^{o}C. Ammonical N concentration in the acid traps was determined colorimetrically using a Lachat QuickChem Automated Ion Analyzer (Lachat Instruments, Loveland, CO). Ammonia captured from soil without N fertilization was averaged and subtracted from other acid trap totals at each sampling interval for individual trials. Ammonia levels in the acid traps from soil checks ranged from 0.05-0.25 mg N captured over the duration of a two week trial, and these levels did not fluctuate from trial to trial.

Upon completion of each trial the soil from each chamber was immediately air-dried in a greenhouse for 24 h. Air-dried soil was extracted with 2M KCl to determine ammonium and nitrate concentrations. Concentrations of ammonium and nitrate were determined colormetrically using the Lachat QuickChem Automated Ion Analyzer (Lachat Instruments, Loveland, CO). The soil nitrate, soil ammonium, and the ammonia in the acid traps were used to calculate the mass balance system N recovery (Woodward et al, 2011). The average percent of urea-N hydrolyzed over all chambers during each trial in this study ranged from 96.3-99.7% of the applied N with average standard deviations ranging from 2.2-3.1% of

applied N. The recovery of applied N over all chambers indicated that urea hydrolysis was nearly complete over the two week trials.

All trials were analyzed as randomized complete block designs with repeated measures. Blocks were individual cabinets with treatments randomized among each chamber within each cabinet, totaling three replications for each treatment. Each trial was analyzed separately using analysis of variance (ANOVA) with the Proc Mixed model in SAS (SAS Institute, 2009). Least significant difference (LSD) mean separation was used to determine treatment differences in cumulative N captured in acid traps and N captured at each sampling interval.

3.3.2 Trials I and II

Trials I and II consisted of four different coating treatments on granular urea, urea, and a "check" Wheeling silt loam to which no N was applied, totaling six treatments (Table 3.2). The urea treatment in trials I and II served as the positive control treatment. The urease inhibitor, N-(n-butyl) thiophosphoric triamide (NBPT) was applied to the granular urea using two different solvent systems to bind the molecules of NBPT to the granule. The inhibitor was applied at the North American Industry standard rate of 0.08% w/w. The first method to bind the inhibitor to urea is the commercially available formulation, Agrotain® (Agrotain-coated urea). The second method is a new solvent system technology under the trade name, Arborite® Ag. Using Arborite® Ag two physical coatings were applied in conjunction with NBPT to urea. Calcium sulfate (-325 mesh) ($CaSO_4$-coated+NBPT) and potassium sulfate (-50 mesh) (K_2SO_4-coated+NBPT) were applied in a powder form to urea resulting in fertilizers with the analyses of 39.9-0-0-3.0S and 36.6-0-7.4-3.2S, respectively. All coatings were prepared by Brooks Whitehurst Associates Inc. laboratories, developer of the Arborite® Ag technology.

3.3.3 Trials III and IV

Trials III and IV differentiate the impact of the physical coatings from the urease inhibitor, NBPT. Treatments in trial III were non-coated urea, Agrotain®-coated urea, Arborite® Ag urea, and the check. The last two treatments in this trial are the $CaSO_4$-coated urea, with and without the NBPT. In trial IV the first four treatments remain the same as in trial III; however the last two treatments were K_2SO_4-coated urea, with and without NBPT. The calcium and potassium sulfate coated urea without NBPT used the same solvent system of Arborite® Ag but without NBPT.

3.3.4 Trials V and VI

Trial V and VI assessed the application rate of NBPT applied to the urea granule. Six rates of NBPT, on a percent by weight basis, were used during this trial being 0.00, 0.02, 0.04, 0.06, 0.08, and 0.1%. The Arborite® Ag binder was used as the carrier to coat the urea granules with the different concentrations of NBPT during this trial.

3.4 Results and Discussion

3.4.1 Trials I and II

Trials I and II were conducted to compare urea to coated urea containing NBPT from Agrotain® and Arborite® Ag as well as physically coated urea with Arborite® Ag. Ammonia losses were negligible from N treatments for one to six hours after N application (Fig. 3.1). At 9 h after fertilizer application ammonia volatilization from urea was greater than the coated treatments in trials I (0.65%) and II (0.58%).This loss was not significant for trial I due to slightly larger variation, standard error of 0.28%, compared to a standard error for trial II of 0.18%. From 12 to 72 h ammonia volatilization at each sampling interval was significantly greater from urea than the coated urea products for both trials I and trial II. The

greatest loss of N from urea came between 12 and 24h after application with losses of 17.2 % (1.4 % of applied N h^{-1}) and 17.6 % (1.5% of applied N h^{-1}) of applied N for trials I and II, respectively. The ammonia loss from 12 to 24 h equaled 46.7 and 51.9% of the total ammonia loss during the two week period for trials I and II respectively. Ammonia loss, within sampling intervals, from urea decreased from 24 to 336 h after urea application and was significantly lower than the coated treatments from 96 to 264 h in both trials I and II.

Cumulative ammonia volatilization N losses from urea plateau beyond 96 h after application, and cumulative N loss was 36.8 and 33.9 % of applied N for trials I and II, respectively (Figs. 3.1 and 3.2). Cumulative N loss for non-coated urea was significantly higher than all NBPT-coated materials for trials I and II, but none of the coating treatments differed in cumulative N loss (Figs. 3.1 and 3.2). The coated materials slowed ammonia volatilization losses out to 96 h after N application (Figs. 3.1 and 3.2). For the inhibitor only coatings, Agrotain® and Arborite® Ag, cumulative ammonia loss as well as ammonia loss at each sampling interval did not differ for trial I (Fig. 3.1). Ammonia losses from Agrotain® and Arborite® Ag were greatest from 96 to 120 h with a maximum ammonia loss rate of 0.20% and 0.19% of the applied N h^{-1} during trial I. Losses during trial II for Arborite® Ag were greatest from 120 to 144 h (0.16% of applied N h^{-1}) whereas Agrotain® did not reach a maximum ammonia loss rate until 168 h (0.15% of applied N h^{-1}) (Fig. 2). Agrotain® had significantly lower ammonia loss at the 120 h (2.70% of applied N) and 144 h (3.31% of applied N) sampling intervals than Arborite® Ag (3.46% of applied N at 120 h and 3.93% of applied N at 144 h) during trial II. These two sampling intervals during trial II were the only sampling intervals throughout trials I-IV where Agrotain® and Arborite® Ag were found to be significantly different (Figs. 3.1-3.4). In all trials, cumulative N loss at the end of the two week sampling period from Agrotain® and Arborite® Ag were similar.

The $CaSO_4$-coated+NBPT and K_2SO_4-coated+NBPT reached a maximum ammonia loss rate from 120 to 144 h with maximum ammonia loss rates of 0.17% and 0.16% of applied N h^{-1} during trial I. The $CaSO_4$-coated+NBPT treatment lost significantly lower amounts of ammonia at the 96 h (2.42% of applied N) than Arborite® Ag (3.28% of applied N) as well as being significantly lower at 120 h (3.86% of applied N) than Agrotain® (4.67% of applied N) and Arborite® AG (4.79% of applied N) during trial I. The K_2SO_4-coated+NBPT treatment lost significantly less ammonia at 96 (2.01% of applied N) and 120h (3.18% of applied N) than Arborite® AG (3.28% of applied N at 96h and 4.79% of applied N at 120 h) and Agrotain® (2.99% at 96h of applied N and 4.67% of applied N at 120 h), during trial I. The $CaSO_4$-coated+NBPT and K_2SO_4-coated+NBPT treatments were not statistically different during any sampling interval during trial I.

During trial II, the $CaSO_4$-coated+NBPT treatment reached a maximum ammonia loss rate from 120 to 144 h with an ammonia loss rate of 0.15% of the applied N h^{-1}, while the K_2SO_4-coated+NBPT treatment reached a maximum ammonia loss rate of 0.16% from 144 to 168 h. However, the $CaSO_4$-coated+NBPT treatment was only significantly different from Arborite® Ag during the 216 h sampling interval with ammonia losses of 3.77% and 3.22% of applied N, respectively. The K_2SO_4-coated+NBPT treatment had significantly lower ammonia loss (2.69% of applied N) than Arborite® AG (3.46% of applied N) at 120 h , but ammonia losses for K_2SO_4-coated+NBPT (4.57% of applied N) were significantly higher than Arborite® AG (3.22% of applied N) at 216 h .The physically coated treatments did not differ from Agrotain at any sampling interval during trial II.

Cumulative N loss did not differ between $CaSO_4$-coated+NBPT, K_2SO_4-coated+NBPT, Agrotain® and Arborite® Ag (Figs. 3.1 and 3.2). Cumulative N loss for $CaSO_4$-coated+NBPT, K_2SO_4-coated+NBPT, Agrotain® and Arborite® Ag ranged from 22.1-

24.8 % and 17.9-20.8% for trials I and II, respectively. Physically coating the urea granule did not significantly decrease the amount of ammonia loss beyond that of the inhibitor, NBPT alone over the two week trial period.

3.4.2 Trials III and IV

Trial III was designed to determine the effect on ammonia volatilization when urea was coated with $CaSO_4$ alone compared to products utilizing the inhibitor NBPT alone, or in combination with the $CaSO_4$ coating. Ammonia volatilization from non-coated urea was similar to trials I and II with the peak ammonia loss occurring at the 24 h sampling interval, 17.8% of applied N. When $CaSO_4$ was applied to urea granules, ammonia volatilization was delayed 24 h and the maximum ammonia loss rate was 0.74% of applied N h^{-1} with a total of 8.93% of the applied N being lost by the 24 h sampling interval. The hourly ammonia loss rate with CaSO4-coated urea reached a maximum during the same sampling interval as urea alone (1.5% of applied N h^{-1}) but the physical coating reduced the ammonia loss rate by 50% during the sampling interval. Ammonia loss from CaSO4-coated urea was 12.13, 4.87, and 2.48% of applied N at 48, 72, and 96 h respectively. Ammonia losses for CaSO4-coated urea were significantly higher than urea (6.08, 2.71, and 1.68% of the applied N) over the same sampling intervals. Ammonia losses were the same for CaSO4-coated urea and urea alone beyond 96 h after N application. The higher losses of N from CaSO4-coated urea resulted in cumulative losses that were the same as urea at the conclusion of trial IV (Fig. 3.3)

The $CaSO_4$-coated+NBPT treatment significantly reduced ammonia losses during the 96, 120, and 144 h sampling intervals with (1.34, 2.27, 3.29% of the applied N) compared to Arborite® Ag (2.14, 3.55, and 4.55% of the applied N). $CaSO_4$-coated+NBPT (2.27, 3.29, and 3.49% of the applied N) significantly reduced ammonia losses compared to Agrotain® during the 120, 144, and 168 h sampling intervals (3.32, 4.39, and 4.61% of the applied N). The

maximum ammonia loss rates for the coatings containing NBPT occurred at 144 h for Arborite® Ag (0.19% of the applied N h^{-1}) and 168 h for Agrotain® (0.19% of the applied N h^{-1}) and $CaSO_4$-coated+NBPT (0.15% of the applied N h^{-1}). Coatings containing NBPT continued to volatilize significantly higher amounts of ammonia within sampling intervals until 264 h (11 days) after N application. The higher levels of ammonia loss at later sampling intervals indicated that urea hydrolysis was still occurring at 264 h when NBPT was used. The higher losses of ammonia were due to the delayed urea hydrolysis with NBPT indicated by lower ammonia loss from 0 to 96 h and low concentrations of urea left at the soil surface due to the rapid hydrolysis of urea without NBPT. However, ammonia loss was the same among all treatments within the sampling intervals from 312 to 336 h. All coatings that received NBPT had significantly lower cumulative N loss than urea applied without NBPT (Fig. 3). When NBPT was applied with and without the $CaSO_4$ coating cumulative ammonia volatilization losses were 24.6, 23.3, and 19.4% for Agrotain®, Arborite® Ag and $CaSO_4$-coated+NBPT respectively (Fig. 3.3).

Trial IV was identical in treatment design as trial three with K_2SO_4 replacing $CaSO_4$ as the physical coating. Non-coated urea released ammonia within 9 h after N application and reached a maximum rate of ammonia volatilization 24 h after application with 17.8% (1.5% of the applied N h^{-1}) of the N being lost from 12 to 24 h (Fig. 3.4). When urea was coated with K_2SO_4, ammonia volatilization was delayed until 24 h with 0.7% of the applied N lost. Ammonia loss from the K_2SO_4- coated urea was 3.31% of the applied N at 48 h and was significantly lower than 7.98% loss from urea. Ammonia loss from K_2SO_4- coated urea was significantly higher than from urea at 72 to 216 h. However, the maximum ammonia loss rate of 1.4% of the applied N h^{-1} for urea occurred from 12 to 24 h where the maximum ammonia loss rate for K_2SO_4- coated urea was 0.21% of the applied N h^{-1} from 72 to 96 h.

The K_2SO_4- coating delayed ammonia loss for 48 h, reduced the maximum rate of ammonia loss by 0.9% of the applied N h^{-1}, and also delayed the time to reach maximum ammonia loss for 3 days (Fig. 3.4)

The K_2SO_4- coated urea lost significantly greater amounts of ammonia than coatings containing NBPT from 24 to 120 h during trial IV. Maximum ammonia loss rates for the coatings containing NBPT occurred from 144 to 168 h and were 0.17, 0.16, and 0.19% of the applied N h^{-1}, corresponding to 4.05, 3.75, and 4.58% of the applied N for Arborite Ag®, Agrotain®, and K_2SO_4- coated+NBPT (Fig.4). Ammonia losses from coatings containing NBPT were higher than K_2SO_4- coated urea from 168 to 312 h. The higher ammonia loss with NBPT at later sampling intervals is evidence that the inhibitor delayed urea hydrolysis up to 11 to 13 days after N application.

Cumulative ammonia volatilization by 120 h was under 5% of the applied N when NBPT was applied and no differences among treatments receiving NBPT were seen until 168 hours after N application when Agrotain® differed from K_2SO_4-coated+NBPT (Fig. 3.4). The K_2SO_4 –coated urea did delay and significantly decrease the cumulative N lost from ammonia volatilization (Fig. 3.4).

Nitrogen loss was lower with the K_2SO_4- coating, than urea alone; however NPBT further decreased ammonia volatilization compared to K_2SO_4 alone (Fig. 4). It has been postulated that alkali and alkaline earth metals may displace calcium from cation exchange sites, resulting in calcium reacting with bicarbonate produced during urea hydrolysis creating a precipitate (Fenn et al., 1982). The reaction, known as the “Fenn reaction”, minimizes the pH increase around the zone of high urea concentration and decreases the amount of ammonia gas formed (Fenn et al., 1982; Kiss and Simihaian, 2002). The reduction in ammonia volatilization during the K_2SO_4 and $CaSO_4$ trials is most likely due to the reduced

pH in the urea concentrated zone after dissolution. The greater effect observed for potassium sulfate is attributable to its greater solubility in water (11.1 g/100 mL at 20 ^{o}C) versus the solubility of calcium sulfate (0.21 g/100 mL at 20 ^{o}C). Observations during the trial revealed that a shell of $CaSO_4$ was left on the soil surface after the two week trial while there were no physical remains of K_2SO_4. Bayrakli (1990) found that phosphogypsum decreased ammonia volatilization when mixed into the soil with urea. The results from this study indicate that calcium sulfate and potassium sulfate (Figs. 3.3 and 3.4) alone did delay ammonia volatilization intially, and in the case of potassium sulfate (Fig. 3.4) decreased cumulative ammonia volatilization when applied as a coating to urea granules.

3.4.3 Trials V and VI

Trials V and VI were conducted to ascertain the effectiveness of NBPT at different applicatioin rates, using Arborite® Ag. When no inhibitor was applied to the urea granules, the ammonia volatilization rate again peaked at 24 h with an ammonia loss rate 1.4 and 1.2% of the applied N h^{-1} in trials V and VI, respectively. Ammonia volatilization did not differ as NBPT rate increased from 0.02 to 0.1% for the first 48 h in trial V and the first 24 h in trial VI. During the 48 h sampling in trial VI the 0.02% NBPT treatment lost 0.81% of the applied N and was significantly higher than NBPT levels of 0.06, 0.08, and 0.1% with losses of 0.33, 0.31, and 0.30% of the applied N. The 0.02% NBPT rate reached a maximum ammonia loss rate of 0.22% of the applied N h^{-1} (5.35% total N loss) at 144 h during trial V and 0.22% of the applied N h^{-1} (5.38% total N loss) at 120 h during trial VI. The 0.04% reached maximum ammonia loss rates at 144 h in both trials V and VI with rates of 0.21 (5.11% total N loss) and 0.17% of the applied N h^{-1}(4.06% total N loss). The three higher NBPT rates (0.06, 0.08, and 0.1%) all showed maximum ammonia loss rates at 168 h during trial V and VI. The addition of NBPT to the urea granule delayed the maximum ammonia loss rate with each

addition of NBPT up to 0.06%. At NBPT rates of 0.08 and 0.1% no additional effects were seen in ammonia volatilization control compared to the 0.06% NBPT rate.

As the rate of NBPT increased, ammonia volatilization decreased in both trials V and VI. At NBPT rates of 0.02% w/w cumulative ammonia volatilization decreased from 35.6% and 35.1% to 25.4% and 24.1% for trials V and VI, respectively. However, for NBPT rates ranging from 0.04% to 0.1% cumulative ammonia volatilization losses ranged from 23.6% to 19.4% with no differences in either trial V or VI (Figs. 3.5 and 3.6). When NBPT was applied at rates equal to or above 0.06% ammonia volatilization remained below five percent of applied N until 120 hours after N application (Figs. 3.5 and 3.6). By applying more NBPT, ammonia volatilization was delayed an extra 48 hours over the 0.02% NBPT rate, however, cumulative volatilization did not differ between the 0.04% and 0.1% NBPT rates. This delay in N loss could allow for a more flexible time frame for N application in comparison to trying to match rainfall events and N application to a smaller time window.

3.5 Conclusions

Ammonia volatilization (32-35% total N loss) from urea during the six trials was similar in total N loss to other laboratory trials (Antisari et al, 1996; Carmona et al., 1990; and Rawluk et al. 2001). Urea reached a maximum volatilization rate from twelve to 12-24 h after N application when conditions were favorable for volatilization. The inhibitor NBPT delayed ammonia volatilization in all trials; however reduction in ammonia volatilization was not as great as in previous research (Rawluk et al., 2001). The higher ammonia volatilization with NBPT treatment in our studies compared to other studies in the literature may be due to diffusion away from the zone of NBPT in other studies, because of higher soil moisture contents. The conditions of our trials may allow greater interaction of the urea granules with soil urease resulting in higher rates of ammonia volatilization. Urease inhibitors do not stop

urea hydrolysis, but their use allows for the urea in the soil solution to diffuse into a greater volume of soil minimizing the pH increase and the resulting ammonia volatilization.

The physical coatings did not have an additive effect in controlling ammonia volatilization when applied with NBPT. Though when applied alone the physical coatings did delay ammonia volatilization for 24 hours. This supports other research cited by Kiss and Simihaian (2002) which reported that alkali and alkaline earth metals may reduce ammonia volatilization. The physical coatings tested in this trial allow for sulfur, in the form of $CaSO_4$ or K_2SO_4, to be applied with N which may be useful in many regions. However, the low solubility of $CaSO_4$ may limit its effectiveness for ammonia volatilization control. By coating the urea with the aforementioned physical coatings the need for a plant available N and S fertilizer could be met. However, applying a coating to urea adds additional cost as well as lowers the N content of urea. The additional cost associated with $CaSO_4$ and K_2SO_4 coated ureas may be offset by the value of sulfur and potassium in the coating.

Increasing NBPT rates decreased ammonia volatilization in the initial hours after application, but cumulative N loss from the 0.04% to 0.1% rates did not differ significantly. The inhibitor NBPT is a very efficient urease inhibitor and rates as low as 0.02% w/w, NBPT can decrease ammonia volatilization. At NBPT rates of 0.04 to 0.1% w/w ammonia volatilization was delayed an extra 48 hours which could be beneficial. Our data indicate that 0.04-0.06% NBPT produce the same total ammonia volatilization control under laboratory conditions, as the North American industry standard rate of 0.08% NBPT. Further research is needed to confirm these findings and determine optimum NBPT rates for field conditions; and to determine how NBPT in combination with various physical coatings can be most effectively utilized to increase N use efficiency of urea fertilizers in agronomic crop production.

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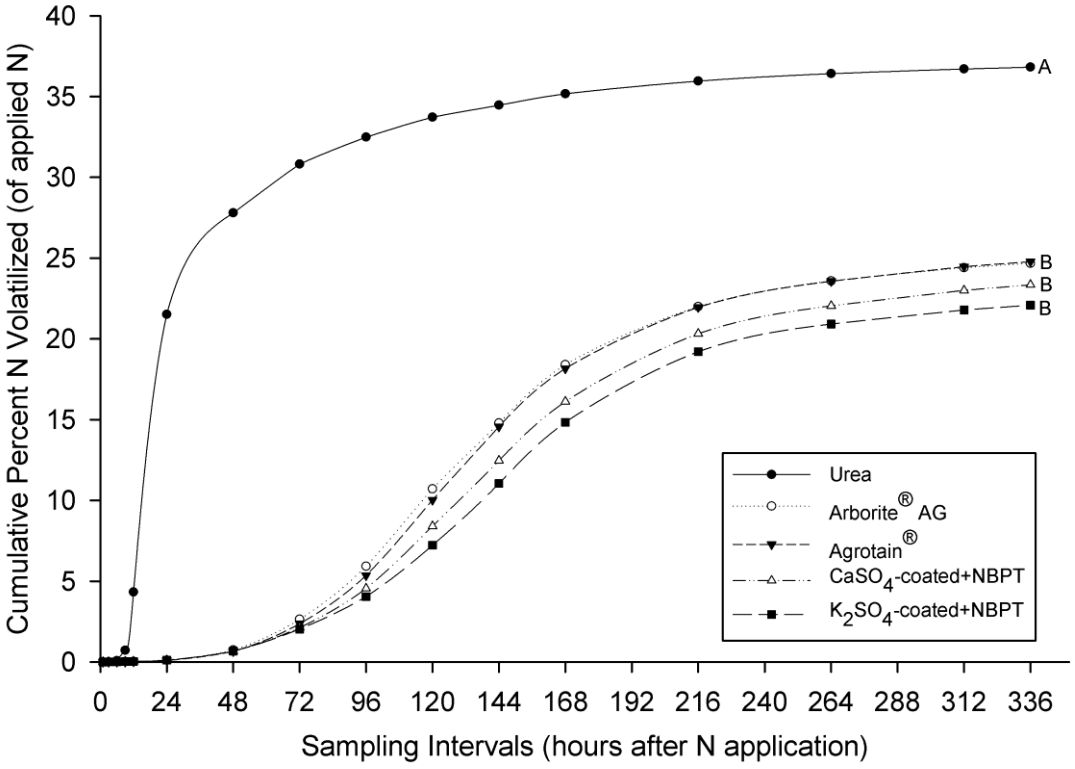

Fig. 3.1: Cumulative N loss (percent of applied) as ammonia from granular urea treated with the urease inhibitor NBPT and $CaSO_4$ and K_2SO_4 physical coatings during trial I.

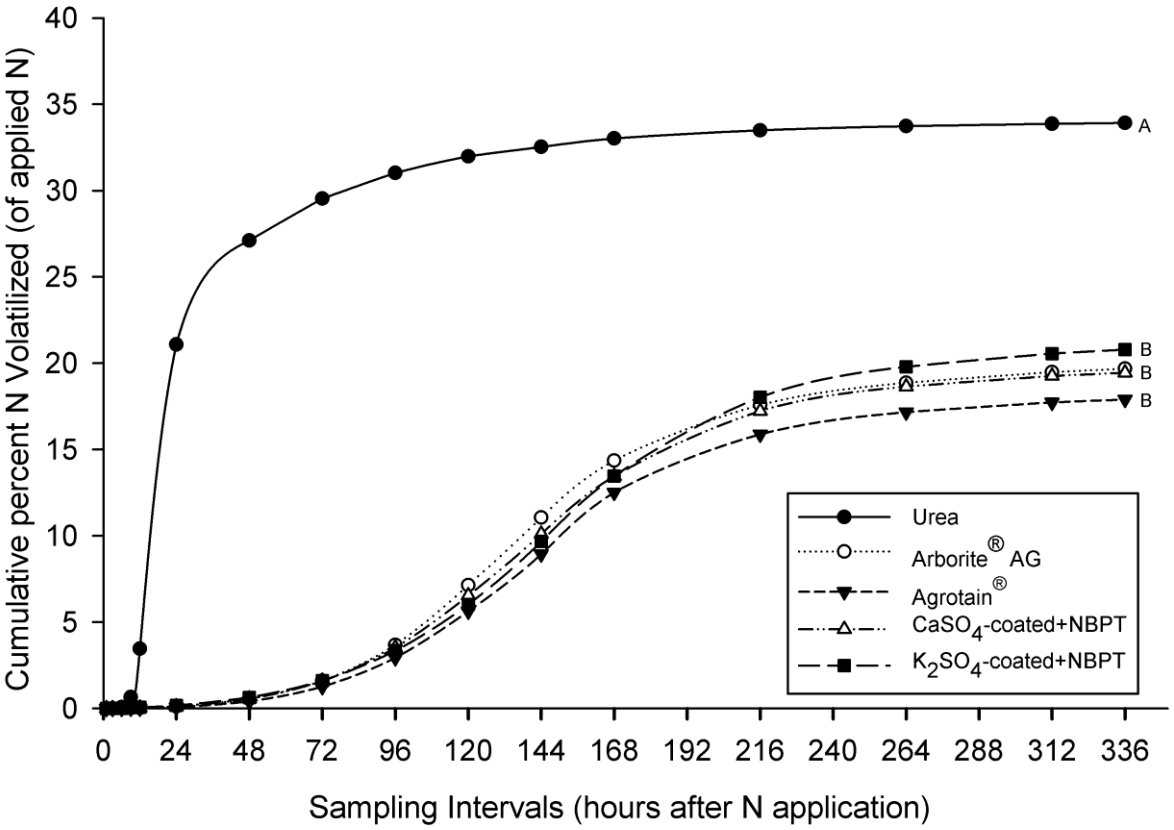

Fig. 3.2: Cumulative N loss (percent of applied) as ammonia from granular urea treated with the urease inhibitor NBPT and $CaSO_4$ and K_2SO_4 physical coatings during trial II.

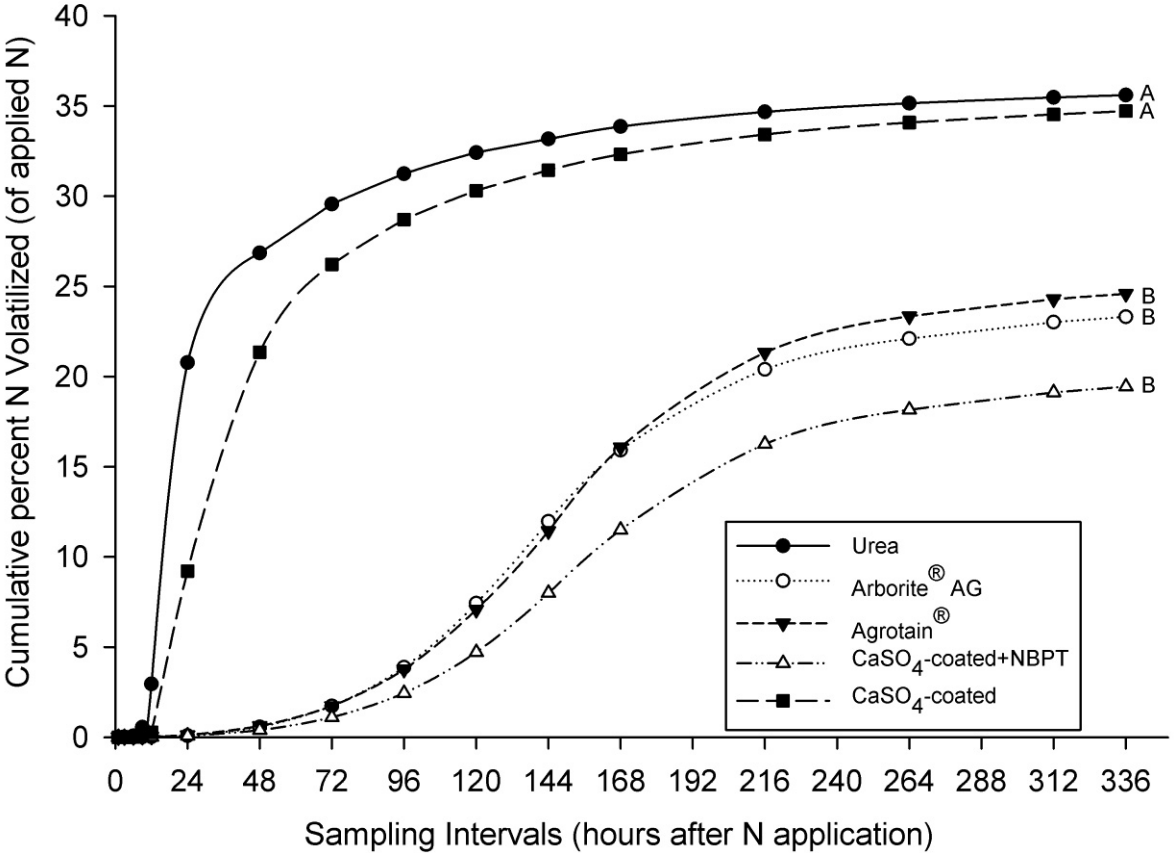

Fig. 3.3: Cumulative N loss (percent of applied) as ammonia from granular urea coated with $CaSO_4$ alone and in conjunction with the urease inhibitor NBPT during trial III.

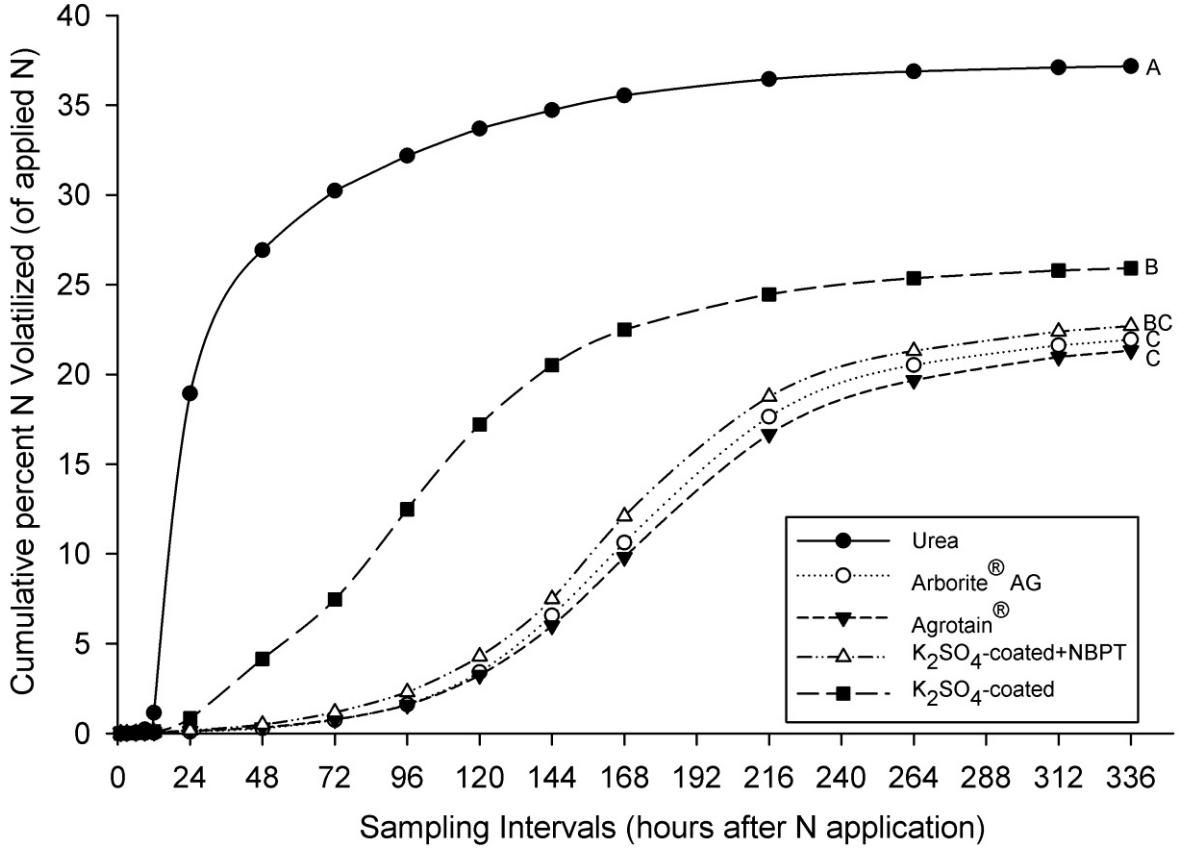

Fig. 3.4: Cumulative N loss (percent of applied) as ammonia from granular urea coated with K_2SO_4 alone and in conjunction with the urease inhibitor NBPT during trial IV.

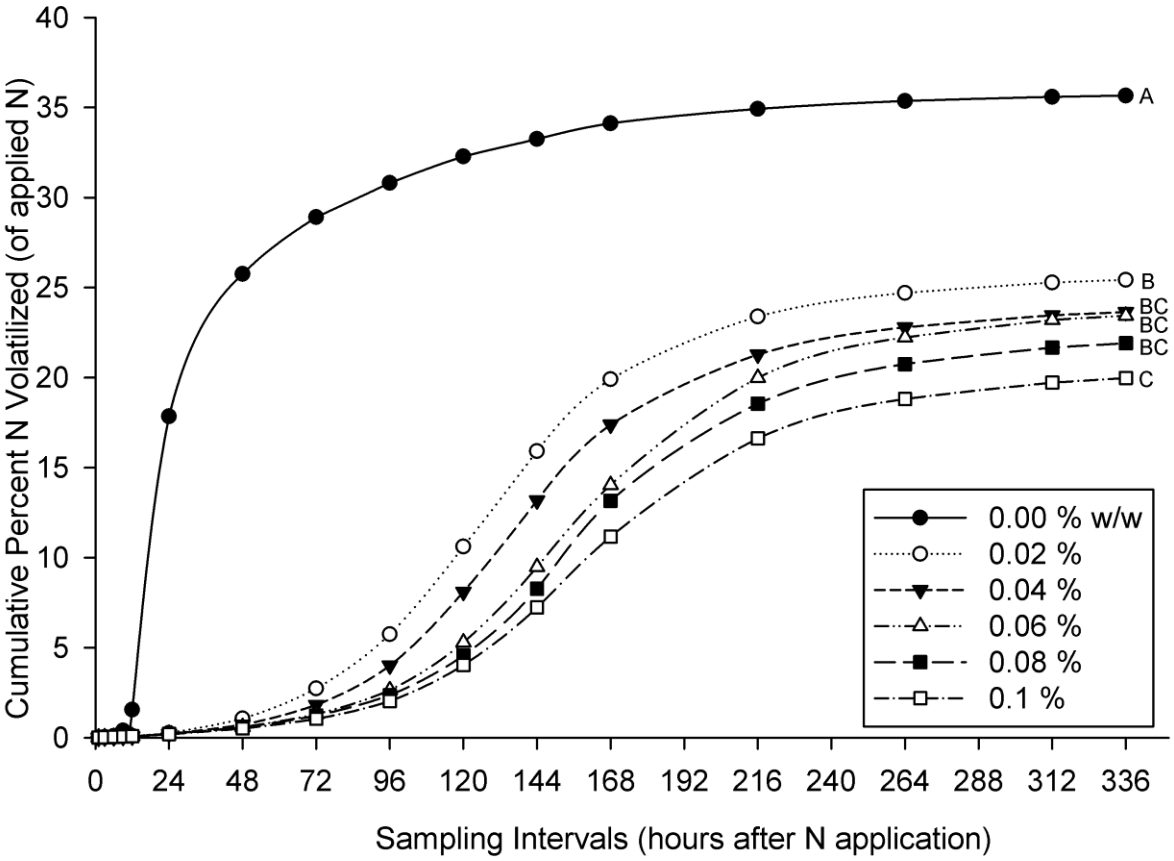

Fig. 3.5: Cumulative N loss (percent of applied) as ammonia from granular urea treated with varying rates of the urease inhibitor NBPT during trial V.

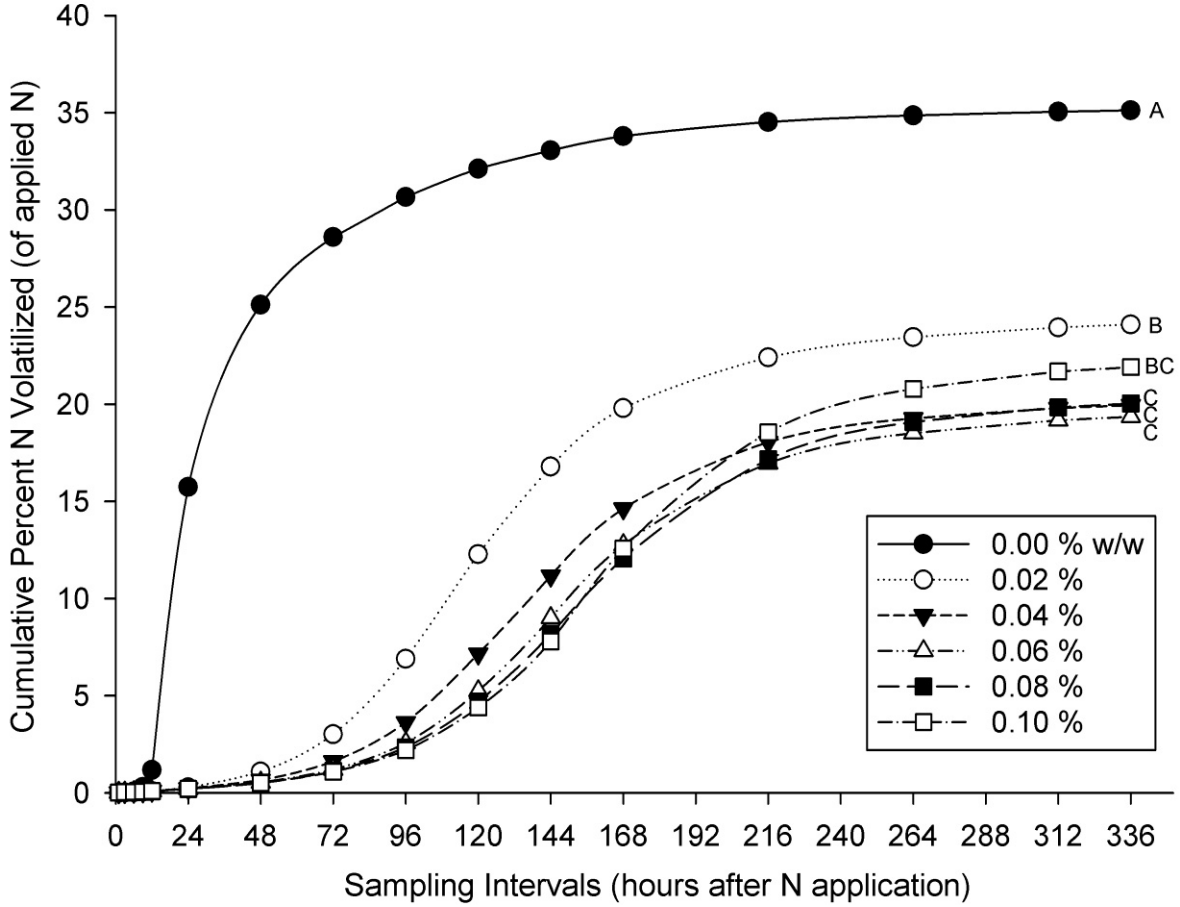

Fig. 3.6: Cumulative N loss (percent of applied) as ammonia from granular urea treated with varying rates of the urease inhibitor NBPT during trial VI.

Table 3.1: Soil pH and physical characteristics for the Wheeling silt loam (Fine-loamy, mixed, active, mesic Ultic Hapludalf) soil used in all ammonia volatilization trials

Soil	pH	Moisture	Sand	Silt	Clay	Carbon
		---------- % ----------				
Wheeling Silt Loam	6.6-6.7	~14 (2/3 F.C. †)	44.2	50.2	5.6	2.33

†F.C. denotes field capacity determined by the pressure plate apparatus at -1/3 bar (Cassell and Nielson, 1998)

Table 3.2: Treatment descriptions for trials I, II, III, and IV.

Coating	Fertlizer Analysis	Trial I	Trial II	Trial III	Trial IV
	(N-P-K-S)				
Check		✓	✓	✓	✓
Urea	46-0-0-0	✓	✓	✓	✓
Arborite® Ag	45.9-0-0-0	✓	✓	✓	✓
Agrotain®	45.8-0-0-0	✓	✓	✓	✓
$CaSO_4$-coated+NBPT†	39.9-0-0-3	✓	✓	✓	
$CaSO_4$-coated	39.9-0-03			✓	
K_2SO_4-coated+NBPT†	36.6-0-7.4-3.2	✓	✓		✓
K_2SO_4-coated	36.6-0-7.4-3.2				✓

† Potassium and calcium physical coatings received the urease inhibitor NBPT in the form of Arborite® Ag

4 Urease Extraction and Activity of Soybean and Corn Residues in Virginia No-till Cropping Systems

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4.1 Abstract

Ammonia volatilization in no-tilled systems, where urea is surface-applied onto crop residue, is higher than in traditional cultivated agronomic systems. Many studies focused on urease activity in fresh plant tissues, but little research has been conducted to describe the urease activity of crop residues in no-tilled agroecosystems. The objectives of the study were to: 1) extract, concentrate, and identify urease from corn and soybean residues using sodium phosphate solutions described in previous research; and 2) analyze the urease activity from soybean and corn residue compared to Jackbean (Canavalia ensiformis) urease using Fourier Transform Infrared Spectroscoy (FTIR). Urease was extracted from dried and ground (1 mm sieve) soybean and corn residues collected after grain harvest, using 50 mM sodium phosphate solutions buffered at pH 7.5. The resulting supernatant was retained as crude extract and subjected to ammonium sulfate fractionations at 0-40%, 40-55%, and 55+% saturation levels to concentrate extracted urease. Crude extracts and ammonium sulfate fractions were compared to Jackbean to identify urease from corn and soybean residues using fast protein liquid chromotagraphy (FPLC) and SDS-PAGE. The activity of urease extracted from corn and soybean residues was compared to Jackbean urease using FTIR, and showed that the activity of soybean urease could be readily measured, while no activity was observed with the corn residue. Analysis of the crop residue extracts with SDS-PAGE confirmed the presence of urease in the soybean 0-40% ammonium sulfate fraction with a molecular weight of approximately 170-180 kDa. Molecular analysis showed no evidence of urease in the corn residue extracts. The data indicated that active urease can be extracted from soybean crop residues with the techniques used in this study. Further research is needed to verify the activity of urease in a range of crop residues with genetic differences and seasonal climatic variations that lead to differential rates of residue decomposition.

4.2 Introduction

When urea is surface applied, as in no-till agronomic systems, urea hydrolysis takes place on the soil or residue surface and ammonia gas is lost through volatilization. The rate of urea hydrolysis and urease activity is dependent on soil moisture, soil texture, and organic matter content. Conservation and no-tilled systems increase soil water holding content and organic matter content of soils which are two factors that affect urease activity and ammonia volatilization from agronomic systems (Stavi et al., 2011). Research has shown that increasing organic matter content in soil increases the urease activity and in turn, potentially, the amount of N loss through ammonia volatilization from urea containing fertilizers (Antisari et al., 1996).

Increasing the amount of organic matter in soils contributes to the number of sites where urease can be adsorbed and remain active, resulting in increased urease activity. Little research has been dedicated to urease activity in crop residues where urea-based N fertilizers are broadcast and do not come into contact with the soil. Broadbent et al. (1985) found that plots covered with chopped wheat (*Triticum aestivum* L.) straw had lower recovery rates of applied N than plots that had no residue cover. Ammonia volatilization was greater in the first four days of a laboratory study conducted by Carmona et al. (1990) when chopped soybean (*Glycine max* L.) straw was added to soil compared to when soil alone was used. Beyrouty et al. (1988) studied the effect of corn (*Zea mays* L.) residue only and soil amended with corn residue, and found that ammonia losses were greater on the residue alone than treatments with soil alone and soil amended with residue. Rochette et al. (2009) found that no-till agronomic systems had higher ammonia volatilization losses compared to moldboard plow systems. Perucci et al. (1982) studied ammonia volatilization from different types of crop residues mixed with soil and found that all residues increased N losses. The percent increase

in N loss was related to the type of residue (corn, wheat, sunflower (*Helianthus annuus)*, and tobacco (*Nicotiana tabacum)*) used with corn increasing N loss by 35% and tobacco increasing N loss by 57% (Perucci et al., 1982).

The differences observed by Perucci et al. (1982) indicated that urease content, and possibly activity, changes depending on species. Leguminous plants generally have higher concentrations of urease than non-leguminous species (Krajewska, 2009). Klose and Tabatabai (2000) concluded that total soil urease activity was significantly affected by cropping rotations. The highest soil urease activities were reported in a four year corn-corn-oats-meadow rotation and the lowest activities were found in monoculture corn and soybean (Klose and Tabatabai, 2000). This suggests that systems containing multiple crops or residue covers may result in higher soil urease activity, and, thus, may require different N management tactics when urea is surface applied to these soils.

The bulk of the research has been conducted looking at the effects of adding residue to soil. The reason for this was to simulate conventionally-tilled agronomic systems. The ammonia volatilization data from conservation and no-tilled agronomic systems, as well as the data from studies looking at soil + residues, suggests that urease activity of plant material contributes significantly to total urease activity of agronomic systems. Substantial research has been conducted to ascertain the role of urease in urea metabolism in actively growing tissues as well as seed development, but little is known about the urease activity of crop tissues after senescence (Ciurli et al., 1999; Krajeswka, 2009).

Jack bean (*Canavalia ensiformi*s) and soybean ureases have been extracted and purified from the meal of seeds for many years. Jackbean seed urease is currently the only plant urease to be structurally described in the literature, and urease from vegetative portions of plants have yet to be completely described (Balasubramanian and Pannuraj, 2010). Extraction of urease from plant material usually involves grinding of fresh plant material

immediately or flash freezing the tissue with liquid nitrogen before processing. Davies and Shih (1984) reported higher urease activities in urease extracts from fresh soybean leaves compared to corn leaves. This difference could be due to higher activity in soybeans or more urease being produced by soybean than corn. Witte and Escobar (2001) extracted and compared urease from fresh leaves of bean (*Vicia faba*), maple pea (*Pisum sativum*), potato (*Solanum tuberosum*), swede(*Brassica napobrassica*), oil seed rape (*Brassica napus)*, winter barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), oat (*Avena sativa)*, and apple (*Malus domestica*). Current research on plant tissue ureases involves extracting ureases from living tissue, rather than the senesced plant material found in agricultural fields after harvest, to which urea containing fertilizers are commonly applied.

The use of buffered solutions containing sodium or potassium phosphate, ethylenediaminetetraacetic acid (EDTA), sodium chloride, and 2-mercaptoethanol are the most common chemical agents utilized in urease extracting solutions (Davies and Shih, 1984; Witte and Escobar, 2001; Follmer et al., 2004). Phosphate buffer solutions have been shown to be competitive inhibitors of urease as the concentration of phosphate increases in the buffering solutions and as pH decreases. Krajewska and Zaborska (1999) demonstrated the competitive inhibitory effects on Jackbean urease and that as the pH of the solution decreases between 7.49 and 5.80, the K_m increases for the reaction. This is due to the inhibitory effect of the orthophosphate ion, $H_2PO_4^-$, which deprotonates as pH approaches 7.2 and upon deprotonation decreases the inhibition of urease (Krajewska and Zaborska, 1999). When using phosphate based buffering systems to extract urease, one must ensure that the pH of the solution is maintained near or above 7 and to use low concentrations of phosphate to maintain conditions where concentrations of the orthophosphate ion $H_2PO_4^-$ is minimal.

Competitive inhibition occurs when the inhibitor blocks the active site of the enzyme preventing the binding of the substrate. To determine the inhibitory effect of a chemical on a

chemical reaction, the V_{max}, maximum reaction rate, and K_m, Michaelis-Menton reaction constant (1/2 V_{max}) must be determined. The K_m of a reaction can also be used to describe the enzyme affinity for the substrate by calculating 1/ K_m. Competitive inhibition of an enzyme increases K_m but does not change the V_{max} of the reaction (Chang, 2000).

Once the urease has been extracted from the plant material, a urease activity assay can be conducted. Urease activity assays consist of adding a known volume to an enzyme extract and determining a reaction rate. The reaction rate is determined by measuring the disappearance of substrate (urea) or the appearance of reaction products (ammonia or bicarbonate). The most common urease activity assay is a colorimetric method to determine the accumulation of ammonia with phenolic compounds (Follmer et al., 2004, Witte and Escobar, 2001). Karmali et al. (2004) used Fourier Transform Infrared Spectroscopy (FTIR) to successfully determine reaction kinetics of the urea hydrolysis reaction. The use of FTIR allows for real-time measurements of the disappearance of substrate (urea) as well as the accumulation of products without disturbance to the reaction vessel or reacting materials.

The development of a method to extract and quantify urease activity from different plant residues would enable cropping systems to be assessed for urea volatilization potential. Understanding different crop urease activities could improve N management practices when applying urea-based fertilizers to no-till production systems. For example, if urease activity differs among residues, different strategies for reducing N volatilization losses might be employed.

The objectives of this experiment were: 1) to extract, concentrate, and identify urease from corn and soybean after plant senescence using sodium phosphate solutions described in previous research; and 2) to determine using FTIR the urease activity from soybean and corn residue compared to Jackbean urease.

4.3 Material and Methods

Crop residues were sampled from two separate fields the same day as machine harvesting of grain. Removing the residue the same day as harvesting reduced the potential soil contamination and degradation of residue by microorganisms, allowing for the determination of urease activity from the crop residue with minimum environmental interferences. Care was taken to omit soybean seed in the residue to prevent contamination by soybean seed urease. To aid in transport and prevent contamination, the corn residue was collected by harvesting mature, whole corn plants and removing the ears prior to processing. The corn residue was coarsely ground using a wood chipper. The ground corn residue and whole soybean residue was air-dried for 72 h. The dried residues were ground to pass a 1-mm sieve using a hammer mill and placed in sealable plastic bags for storage at room temperature. During storage of the crop residues, the residues were visually monitored to ensure that no decomposition that might influence urease activity was occurring inside the storage bags.

4.3.1 Urease Extracting Solutions

Buffered extracting solutions used were sodium phosphate (Na-Phos) solutions of 25mM and 50mM with 50mM sodium chloride, 5mM EDTA, and 0.1% 2-mercaptoethanol at a pH of 7.5. The initial 25 mM sodium phosphate was made using Na-Phos, monobasic. After extracting the corn residue the pH of the crude extract decreased to 6.67 for corn and to 6.95 for soybean. Davies and Shih (1984) report the minimum activity for corn and soybean leaf urease to be at a pH of 6.5; therefore, the corn residue extract pH of 6.67 might differentially affect measured urease activities compared to soybean residue. In light of the results of Davies and Shih (1984) we substituted a 50 mM Na-Phos buffer in the extracting solution to reduce the pH decrease associated with addition of the corn residue.

The 50 mM Na-Phos buffer consisted of using Na-Phos, monobasic in conjunction with Na-Phos, dibasic. Two 50 mM solutions of each Na-Phos compound were made and mixed at a ratio of 1:4, monobasic to dibasic. This ratio produced a buffered solution with a pH near 7.5 that, when combined with 5mM EDTA, disodium salt and 50mM sodium chloride, attained a solution pH of 7.2. The extracting solution was then adjusted to a pH of 7.5 using 2M sodium hydroxide.

The resulting 50 mM Na-Phos buffer increased the buffering capacity of the solution (Fig. 4.1) and maintained the pH of the corn and soybean crude extracts to range from 6.89-6.97 and 6.98-7.06, respectively. Krajewska (2009) reported pH optima for soybean urease activity to be 7.0 and Jackbean urease activity at pH 7.0-7.5, whereas Davies and Shih (1984) reported activity optima for soybean and corn leaf urease to be at 5.5 and 8.8 for soybean and 5.0-5.5, 7.5, and 8.8 for corn. For our studies, the small difference in crude extract pH for corn and soybean residues allows for activity comparisons and is in the range of activity optima reported in previous literature for both species. The 50 mM Na-Phos extracting solution was used exclusively for all extractions and data reported in this study.

4.3.2 Extracting Urease from Crop Residues

The 50 mM Na-Phos, 5 mM EDTA disodium salt, 50 mM sodium chloride extracting solution was stored at 10^{o}C. All steps of the extraction process were carried out at 0-4^{o}C. Prior to the mixing of residues, 2-mercaptoethanol was added to the volume of extracting solution being used at a rate of 0.1% v/v. A ratio of one gram residue material to 10 ml of extracting solution was used to extract urease from corn and soybean residue. An Oster® (Jarden Consumer Solutions, Boca Raton, FL) 6843 12-speed kitchen blender, operated at maximum speed level of 6, was used to homogenize the crop residue and extracting solution mixture. The mixture was blended for three minutes and placed in the refrigerator for 1-5

minutes, and the process was repeated five times for a total blending period of fifteen minutes. Intermittent blending ensured that heating of the residue mixture during blending was minimal. The residue mixture was sieved using a stainless steel French coffee press (Mr. Coffee®, Jarden Consumer Solutions, Boca Raton, FL) to remove large residue material and then passed through a 270 mesh sieve to remove smaller residue material.

The resulting urease solution was then filtered using a #1 Whatman filter paper and vacuum filtration to remove fine particulate material from the urease solution. The urease solution was centrifuged at 15,000*g* at 2°C for 30 minutes using a Sorvall RC 6^+ Superspeed fixed angle centrifuge. The supernatant was retained as the crude extract for both corn and soybean residue and stored at 10°C.

4.3.3 Ammonium Sulfate Fractionation

Urease in plant tissue was not concentrated enough to be identified using routine protein analysis procedures. In order to concentrate the urease extracted from the corn and soybean residues, the protein was precipitated from the extracting solution using ammonium sulfate (Davies and Shih, 1984). Ammonium sulfate fractionations allow for the concentration of urease as well as separation of the urease protein from other molecules in the crude extract. Three ammonium sulfate fractions were examined to find which contained the enzyme urease. The fractions were based on the findings of Davies and Shih (1984) and were at the ammonium sulfate saturation levels of 0-40%, 40-55%, and 55^+%. Seven grams of ammonium sulfate were added to 30 ml of crude extract, dissolved, and centrifuged at 10,000*g* and 2°C for 20 minutes. The supernatant was decanted and the precipitate (0-40% saturation fraction) was re-suspended in 1 ml of extracting solution without 2-mercaptoethanol. To achieve the 55% ammonium sulfate saturation, 2.75 g of ammonium sulfate were added to the 30 ml of supernatant. The supernatant was centrifuged at 10,000*g*

and 2°C for 20 minutes, decanting the supernatant, and re-suspending the precipitate (40-55% saturation fraction) in 1 ml of extracting solution without 2-mercaptoethanol. The supernatant from the second centrifugation was retained as the 55^{+}% saturation fraction. The fractionation steps were replicated three times for each urease extraction from each crop residue.

In order to remove the ammonium sulfate and other smaller molecular weight molecules from the concentrated urease, dialysis was conducted. The use of a size selective membrane and concentration gradient allow for the highly concentrated smaller molecules in the crude extract to diffuse into a larger volume of Na-Phos extracting solution. Once the fractions were re-suspended they were placed in Snakeskin® dialysis tubing (3,500 molecular weight (Da) (3.5 kDa) (Thermo Scientific, Product # 68035) and dialyzed for 24 hours in 2 l of extracting solution without 2-mercaptoethanol. The 2 l of extracting solution were changed once in the 24 h period. After dialysis was complete the fractions were stored at 10°C.

4.3.4 Peak Identification and Standardization with Fourier Transform Infrared Spectroscopy

The urease activity in the extracts was tested using Fourier transform infrared spectroscopy (FTIR). Infrared spectroscopy allows for the detection of specific chemical species based on the absorption spectra of a chemical bond vibrating at certain wavelengths of light energy reported as wavenumbers (cm^{-1}). This study used a Thermo Scientific Nicolet 6700 with a Smart ARK ZnSe 45° sample tray. Each spectrum was conducted at a resolution of 4 cm^{-1} and 64 scans per spectrum. A resolution of 4 cm^{-1} means that there is a ± 4 cm^{-1} variation of where the peak exists, so if a peak is detected at 1,362 cm^{-1} that is accurate ± 4 cm^{-1}. A spectrum was measured every 1-2 minutes during the 20 minute assays to determine if the urease extracts contained active urease.

To identify peaks of reaction products and substrate, 200 mM standards of sodium bicarbonate and urea were analyzed. Prior to analysis of the standards, the Na-Phos extracting solution was analyzed as a background solution. Five peaks were identified from the spectra and standard curves were developed for each peak over a range of sodium bicarbonate and urea concentrations (Figs. 4.2A-C). Each peak represents unique chemical bonds associated with bicarbonate and urea. The correlation of the peak intensities and concentration will allow for future kinetic analyses of urease from crop residues. Karmali et al. (2004) investigated peak heights at 1,625 cm^{-1} and 1,605 cm^{-1} along with the 1,365 cm^{-1} peak for analysis of urea hydrolysis, and our spectra also contained peaks at 1,625 and 1,605 cm^{-1}. However due to the proximity of these peaks and high peak intensities it was difficult to measure peak height accurately. Peak intensity (corrected peak height) was determined using the peak height tool in the OMINIC 8.1 software (Thermo Scientific, 2009). A regression analysis was conducted to determine the correlation between peak intensities and urea/bicarbonate concentrations at 1,466, 1,362, and 1,160 cm^{-1} using Sigma Plot 11 (Systat Software, Inc., 2008). Three of the peaks were selected based on the high linear correlation between reaction products/substrate and peak intensity (Figs. 4.3A-C). The three peaks selected were at 1,466, 1,362, and 1,160 cm^{-1}. The 1,466 and 1,160 cm^{-1} peaks were specific to the substrate, urea, and the 1,362 cm^{-1} was specific to reaction product bicarbonate (Figs. 4.2A and 4.2B). The 1,466 and 1,160 cm^{-1} peaks were associated with the distortion (bending) of the N-H bonds in the urea molecule (Günzler and Gremlich, 2002). Nickolov et al (2003) reported the asymmetrical stretching band for bicarbonate at 1,362 cm^{-1} as well as a shoulder at 1,310 cm^{-1}, which matches the peak selected for bicarbonate in this study (Fig. 4.2B).

4.3.5 Urease Activity Assays with FTIR

Prior to each urease extract assay a background solution was analyzed which consisted of 1 ml of Na-Phos extracting solution and 1 ml of the urease extract of interest. This allowed the analysis to subtract out any background spectra from the assay other than the spectra of bicarbonate and urea. Each assay consisted of 1 ml of the urease extract of interest and 1 ml of the Na-Phos extracting solution containing a concentration of 200 mM urea. A spectrum of the urease assay was scanned every 1-2 minutes for a total assay length of 20 minutes. The scanning period varied slightly because each scan had to be manually started after the one prior had finished, however each time interval was recorded. Three replicate assays were conducted for each urease extract/fraction. The urease concentration of the corn and soybean urease extract/fractions was not known. The Jackbean urease was prepared by dissolving 60 mg of Jackbean urease solid (Urease, Type IX, Sigma Aldrich, U4002) in 250 ml of Na-Phos extracting solution (Quickchem Method 14-206-00-2A). Peak intensities at the three wavenumbers selected were recorded and Fig. 4.2C represents typical spectra for a urease activity assay using FTIR. The peak intensities were plotted at each sampling interval over the duration of the 20 min assays and these plots are referred to as progress curves for the urea hydrolysis reaction.

4.3.6 Urease Identification in Extracts

Urease is a large, complex protein that is composed of six distinct subunits (monomers). Krajewska (2009) and Balasubramanian and Punnuraj (2010) reported the molecular weight of a subunit of Jackbean urease to be 90 kDa and a soybean urease subunit to be 93.5 kDa. To determine if the molecules within each fractionation were similar to the molecular weight of Jackbean urease, two methods were used to identify urease in extracts

from corn and soybean residues. The first method used was fast protein liquid chromatography (FPLC) and the second method was SDS-PAGE.

Urease extracts were separated using a Hi Load 16/60 Superdex 30 column and a GE® ÄKTA Purifier 900. Absorbance of light at 280 nm was measured over the total elution volume (ml of flow through the separation column) to separate out molecules of different molecular weights. Each extract/fraction of corn and soybean urease was compared to Jackbean urease at a concentration of 50 mg solid per 5 ml of extracting solution.

Similar to other gel electrophoresis methods, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separates proteins by the length of the peptide and the charge. The SDS-PAGE analysis was conducted on all extracts/fractions to compare the molecular weight of the proteins in each sample to that of Jackbean urease. The Jackbean urease was prepared in the same manner for SDS-PAGE analysis as in the FPLC analysis. A BIO-RAD Power Pac Basic and BIO-RAD mini-PROTEAN® tetra cell ran at 200 volts for 30 minutes separated the proteins by molecular weight. The stain used was Coomassie G-250 and Tris/Glycerine/SDS solution made by BIO-RAD and 7.5% Mini-PROTEAN® TGX gels (BIO-RAD) were used in the analysis. The procedure was conducted according to the BIO-RAD application guide for SDS-PAGE analysis. The enhanced 0-40% fractionation lane in the SDS-PAGE analyses was achieved by centrifugation of the 0-40% fraction in a centrifuge tube containing a selective membrane that retained molecules above 3.5 kDa. The centrifugation step was done to intensify the band of the 0-40% saturation fraction to ensure correct identification of the band present.

The data collected from the FPLC and SDS-PAGE analysis was analyzed visually for the appearance of bands at certain molecular weights and peaks at certain elution volumes. Three replications of the spectra for concentrations ranging from 0-200 mM of sodium bicarbonate and urea were collected and peak heights measured. Linear regression was used

to determine the relationship between concentration of sodium bicarbonate and urea with peak height. Urease activity assays were conducted in triplicate and the peaks heights at the selected peaks were measured at each sampling interval. Averages and standard deviations were plotted and fit with a spline line to create the urease activity progress curves.

4.4 Results and Discussion

4.4.1 Urease Identification

The urease fractionations from corn and soybean residues were subjected to analysis using a FPLC and SDS-PAGE to determine if urease was present in the extracts. For the purified Jackbean urease, there were only three peaks identified in the FPLC elution curve, with the largest absorbance intensity appearing at 41.6 ml of flow (Fig. 4.4A). The highest absorbance intensities for the soybean residue were at 43, 43.3, 42.5, and 42.3 ml of flow for the crude extract, 0-40%, 40-55% and 55^{+}% fractionations, respectively (Fig. 4.4B). The highest absorbance intensities for the corn residue were found to be 44, 42, 42.5, and 43.5 ml of flow for the crude extract, 0-40%, 40-55% and 55^{+}% fractionations, respectively (Fig. 4.4C). The highest peak intensities for both residues were similar to that of Jackbean urease indicating that similar molecular weight molecules were present in all solutions. The location of the highest peak intensity (41.6-44 ml) for urease in these corn and soybean extracts was similar to the observed elution volume found for urease extracted from *Cajanus cajan* L. (pigeon pea), *Morus alba* (mulberry), and *Momordica charantia* (Das et al., 2002; Hirayama et al., 2000; and Krishna et al., 2011). The fractionations of the crude urease extracts also removed the peaks observed in the crude extracts at greater elution volumes (Fig. 4.4B-C). This could be attributed to the removal of molecules with molecular weights less than 3.5 kDa during dialysis leaving only the peak near the 42 ml of elution volume.

The heights of the peaks for the residues were orders of magnitude higher than the peak height for Jackbean urease (Fig. 4.4). Corn fractions gave the highest peaks, measuring above 3,000 mAU for the 0-40% fraction and above 1,000 mAU for the 40-55% fraction (Fig.4.4). Soybean fractions had peak intensities lower than the corn fractionations but still were above 800 mAU for the 0-40% saturation fraction and 600 mAU for the 40-55% saturation fraction (Fig. 4.4B). Peaks were higher for the 0-40% saturation fraction than the crude extracts for both corn and soybean residues, thus showing that the molecules of this size were being concentrated by 40% ammonium sulfate saturation. The peak heights for both residues were lowest for the 55^{+}% saturation fraction, indicating that most of the urease molecules of this size were concentrated in the lower saturated fractions.

The crop residue extracts were clear and brown in color whereas the Jackbean preparation was colorless. This difference in color potentially affected the amounts of UV light at 280nm being absorbed by the individual fractions. Another explanation for the higher peak heights at 40-45 ml in the corn and soybean residues could be the presence of other large molecular weight molecules. The Hi Load 16/60 Superdex 30 column separates molecules by size, but the peak visible at 40-45 ml could represent multiple molecular weights if molecules with weights greater than 3.5 kDa passed through the column at the same rate. The results from the SDS-PAGE analysis indicated the presence of molecules with molecular weights lower than 25 kDa (dark bands at bottom of gel) in both the soybean and corn residue fractionations (Figs. 5 and 6).

The SDS-PAGE analysis shows three distinct lines for Jackbean urease, indicating molecules with molecular weights near 270-280 kDa, 180-190 kDa, and 90 kDa (Fig. 4.5, lane B). The lowest molecular weight molecule was found to be close to those found by Krajewska (2009), Menegassi et al. (2008), and Balasubramanian and Punnuraj (2010), who reported the molecular weight of a subunit of Jackbean urease to be 90 kDa, a soybean urease

subunit to be 93.5 kDa , and cotton seed urease subunit weight of 98 kDa. The two higher molecular weights present in solution could be explained by the presence of dimers (two subunits bonded together) and trimers (three subunits bonded together) of the Jackbean urease protein. Soybean fractions were analyzed with SDS-PAGE and each fraction appears in the lanes B-G in Fig. 4.5. In the soybean crude extract (Fig. 4.5, lane C) the enzyme urease was apparently not concentrated enough to produce a band. The 0-40% saturation fraction of soybean residue urease was identified at a molecular weight of near 170-180 kDa (Fig. 4.5, lane D). This fraction contained the highest urease activity of the soybean fractions (Fig.4.6) indicating higher concentrations of urease and was supported by the SDS-PAGE analysis (Fig. 4.5, lane D and G). The 40-55% ammonium sulfate fraction has a very faint band near 90-100 kDa signifying a low concentration of single subunits of soybean urease in this fractionation (Fig. 4.5, lane E). Polymers of urease subunits (dimers and trimers) may precipitate at lower saturation levels than single subunits of urease. Thus, explaining the presence of a band at 90-100 kDa in the 0-40% fractionation (Fig. 4.5, lane C) and the faint band at 90-100 kDa visible in the 40-55% fractionation (Fig. 4.5, lane E). No bands were present in the 55+% fractionation (Fig. 4.5, lane F). This was supported by the lack of activity in this fraction of soybean residue urease (Fig. 4.6). Polymers of urease were successfully separated from crude extracts with ammonium sulfate saturation levels up to 55%. Krishna et al. (2011) found urease extracted from *Momordica charantia* to have a molecular mass of 174.5 kDa with native-PAGE; Hirayama et al. (2000) confirmed that urease extracted from leaves of mulberry (*Morus alba*) existed as a homo-dimer enzyme. The band produced in the 0-40% saturation fraction of soybean residue was also of similar size (170-180 kDa) as urease extracted from *M. charantia* whose molecular weight inferred the presence of two bonded urease subunits (dimers).

The SDS-PAGE analysis for the corn residue did not produce visible bands in any of the fractionations (Fig. 4.7, lanes C-G). The absence of visible bands could be due to the low concentration of the enzyme urease in corn or the enzyme was destroyed during plant senescence or extraction. The corn residue used in this study was from one variety and one field, and thus is a small sampling of the possible genetic material available for examination. It is known that actively growing corn tissue contains urease but further research is needed to isolate and quantify urease from corn residue (Davies and Shih, 1984).

4.4.2 Assays to detect urease activity

During urea hydrolysis assays, peak heights detected by FTIR were measured to quantify disappearance of the urea and appearance of bicarbonate. Correlating the absorbance of infrared light with different concentrations of bicarbonate and urea allows for kinetic data to be interpolated with this assay method. There was no difference in the percent absorbance from 0 mM to 25mM urea at 1,466 cm^{-1} and 1,160 cm^{-1}. When plotting urea standard curves, only concentrations from 25mM to 200mM were used and 25mM was found to be the detection limit for the detector used in this study (Figs 4.3A and 4.3B). The peaks at 1,160 cm^{-1}, 1,362 cm^{-1}, and 1,466 had R^2 values above 0.99, showing that the absorbance was highly correlated to concentration of bicarbonate or urea at these wavenumbers (Fig. 4.3). Of the three absorbance peaks identified and investigated to quantify the activity of the enzyme urease, the peak at 1,362 cm^{-1} was the most responsive (greatest ΔA over concentrations) and was the best indicator of urease activity in the control urease assay of Jackbean urease (Figs. 4.2C and 4.3A-C). Karmali et al. (2004) also found that the 1,365 cm^{-1} bicarbonate peak was also the most suitable for monitoring urea hydrolysis. The bicarbonate peak at 1,362 cm^{-1} was the only peak selected to describe the urease activity in crop residues for this study.

The peak height at 1,362 cm^{-1} increased over time during the Jackbean and soybean residue urea hydrolysis assays (Fig. 4.2C and 4.4). The Jackbean urease was the most active urease when comparing peak heights at 1,362 cm^{-1}; however, bicarbonate concentrations increased in the soybean crude extract, 0-40% and 40-55% saturation fractions also (Fig. 4.6). The difference in activity among urease fractions was attributed to different enzyme concentrations because the substrate concentration was kept constant during each assay. The urease activity of the soybean 40-55% saturation fraction was lower than that of the soybean crude extract indicating that a majority of the urease was removed in the first fraction step (Fig. 4.6). The soybean 55^{+}% saturation fraction did not increase in peak height at 1,362 cm^{-1} indicating that there was no urease remaining in that fraction. Davies and Shih (1984) also found the 40% fractionation to be the most useful concentrating step for soybean leaf urease and found that saturations > 55% produced no urease activity.

Davies and Shih (1984) reported higher ureolytic activities in soybean than in corn in all extracts or fractionations. In this study the FTIR data at 1,362 cm^{-1} showed no ureolytic activity in the corn urease over the duration of a 20 min assay as evidenced by no increase in peak intensity at 1,362 cm^{-1} compared to Jackbean urease (Fig. 4.8). This supports the SDS-PAGE analysis for the corn residue where no molecular evidence of urease was found (Fig. 4.7). Davies and Shih (1984) showed urease activity in corn when measured from fresh leaf tissue, and Perucci et al. (1982) showed that by adding corn residue to soil the urease activity was increased above that of soil alone. Perucci et al. (1982) allowed the soil and residue mixture to incubate for 15 days before toluene was added to halt microbial activity. The increase in urease activity observed by Perucci et al. (1982) may have been due to microbes releasing urease upon their death rather than an actual contribution of urease from the corn residue. The lack of ureolytic activity in the corn residue could also be due to shorter incubation times in the study than to the 40 minute incubations used by Davies and Shih

(1984). Finally, the pH optimum for corn residue urease activity may be higher than used in the current study. However, our results show that no urease activity could be detected with our methods.

4.5 Conclusions

Fourier transform infrared spectroscopy enabled the monitoring of reaction substrate disappearance and product appearance during urea hydrolysis within minutes of the initiation of the reaction. This method of monitoring urea hydrolysis allowed for peak intensities to be measured every one to two minutes without stopping the reaction. No chemical reagents were needed to quantify the concentrations of reaction products or substrates. The infrared wavenumbers selected were highly correlated to urea and bicarbonate concentrations though monitoring the production of bicarbonate at 1,362 cm^{-1} was more responsive than monitoring the consumption of substrate urea. In future studies of crop residue urease the bicarbonate peak at 1,362 cm^{-1} would be the optimum peak to analyze in order to develop kinetic models for crop residue ureases.

The use of FPLC and SDS-PAGE allowed for the identification and comparison of crop residue urease with Jackbean urease. The FPLC elution curves indicated that molecules in the urease extracts had elution volumes similar to that of Jackbean urease. Determining concentrations of urease in extracts with the FPLC was not feasible due to the lack of separation of molecules with a mass 3.5 kDa and greater, and the dark color of the crop residue extracts may have affected absorbance at 280 nm.

The ammonium sulfate fractions were extremely effective in concentrating urease in the soybean extracts. The ammonium sulfate fraction from 0-40% saturation concentrated the soybean urease increasing the reaction rate as well as allowing for the identification of the protein with SDS-PAGE. The presence of a band at 170-180 kDa in the soybean SDS-PAGE

indicates the presence of urease as a homodimer. Urease subunits have been reported for Jackbean urease at 90 kDa, soybean urease subunits at 93 kDa and cotton urease subunits at 98 kDa (Balasubramanian and Punnuraj, 2010; Krajewska, 2009; Menegassi et al., 2008). Krishna et al. (2011) and Hirayama et al. (2000) reported urease as homodimers supporting the findings from soybean residue urease. However, corn residue showed no urease activity during the assays and molecular analysis yielded no evidence of the protein. Corn has lower protein content than soybean and the lack of urease being extracted and detected in corn residue in our study may be due to low concentrations of the enzyme in corn residues.

Extracting urease with sodium phosphate solutions was successful in extracting active urease from soybean crop residue. The results from this study indicate that differences in urease activity and concentration may be present between corn and soybean residues. Future research is needed to ascertain the correlation between ammonia volatilization from crop residues and their respective urease activities. If large differences in potential volatilization losses were found for different residues, N management practices in no-till productions may need to be adjusted. For example, different rates of the urease inhibitor, N-(n-butyl) thiophosphoric triamide (NBPT), might be needed to optimize the reduction in ammonia volatilization from surface-applied urea to different residues. Research with different residues over different sites is needed to determine if such differences exist.

4.6 References

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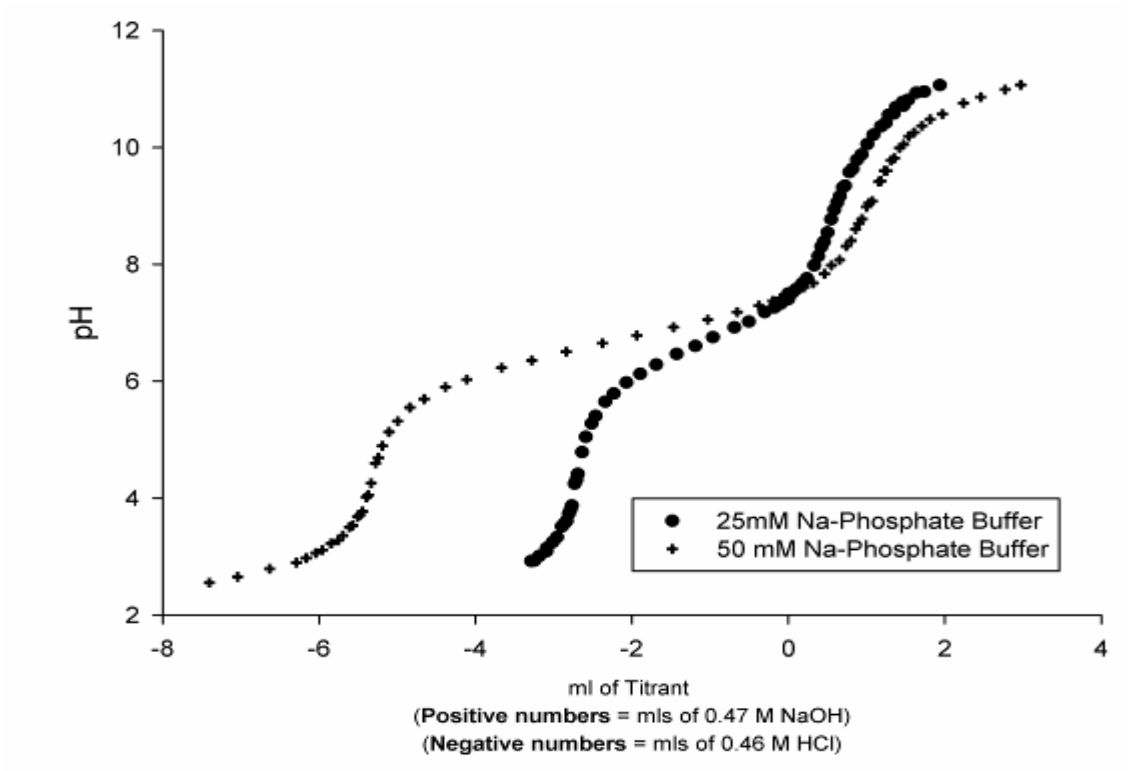

Fig.4.1: Buffering capacity of the 25mM and 50mM Na-Phos solutions prepared to extract urease from crop residues.

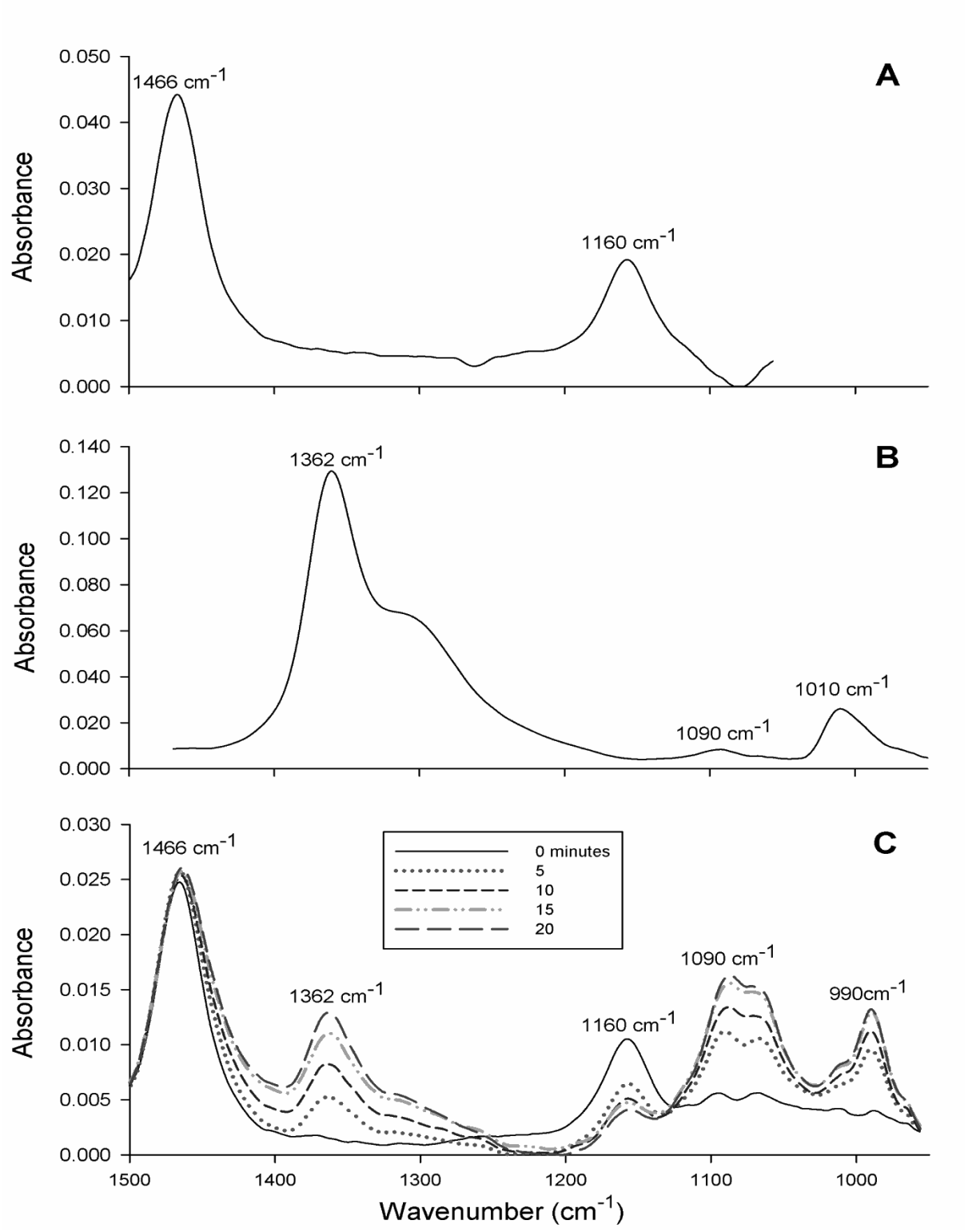

Fig.4.2: FTIR peaks identified and measured to characterize urea hydrolysis for 200mM Urea (A), 200mM sodium bicarbonate (B) and an assay of Jackbean urease showing the change in peaks

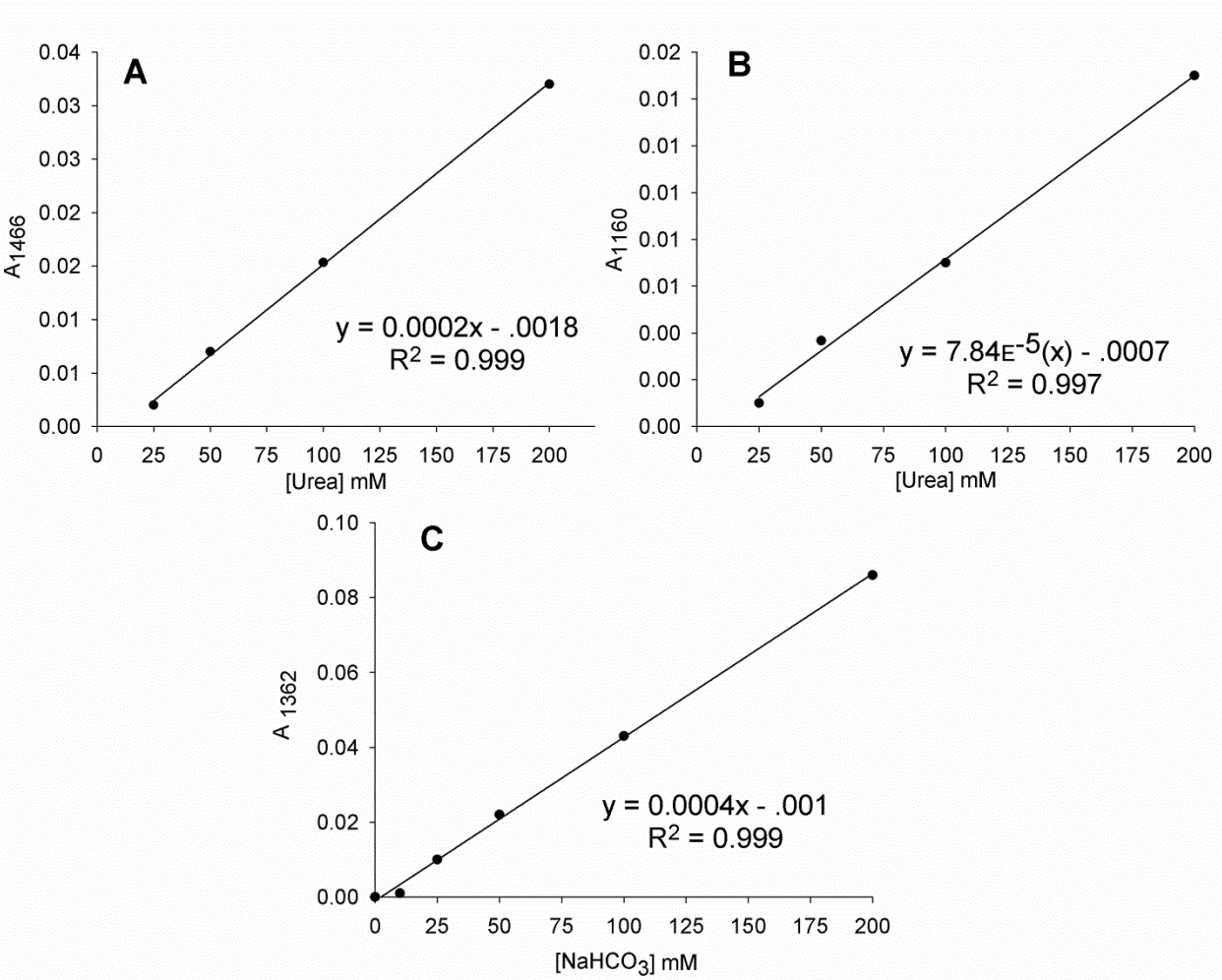

Fig.4.3: Regression analysis for three FTIR peaks; correlating absorbance peak intensities to a range of urea concentrations at 1,466 cm^{-1} (A) and 1,160 cm^{-1} (B) and sodium bicarbonate at 1,362 cm^{-1} (C).

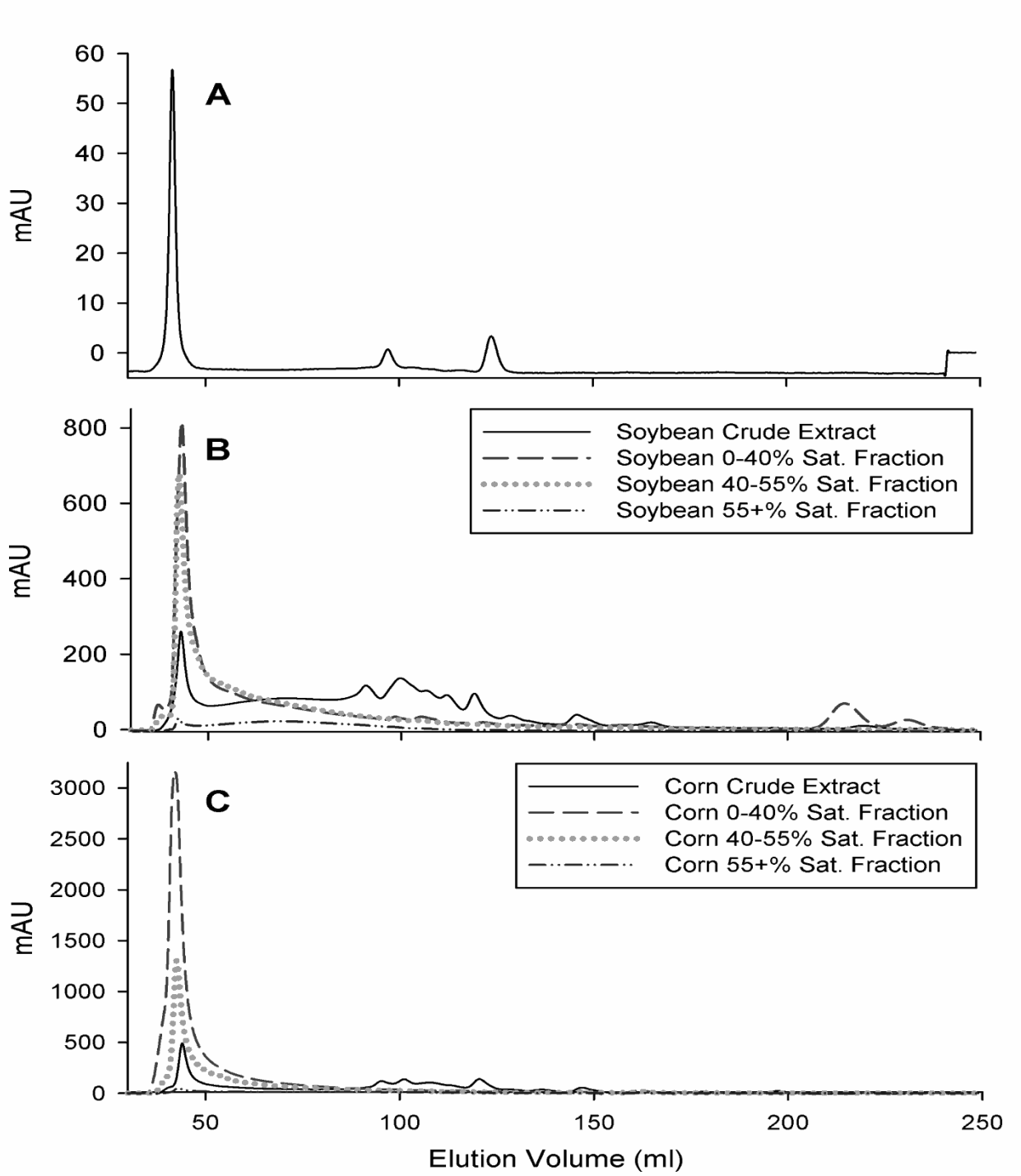

Fig. 4.4: Fast Protein Liquid Chromatography (FPLC) elution curves measuring absorbance at 280 nm for the Jackbean urease (A) and fractionations of soybean (B) and corn (C) residues in 50 mM sodium phosphate extracting solution.

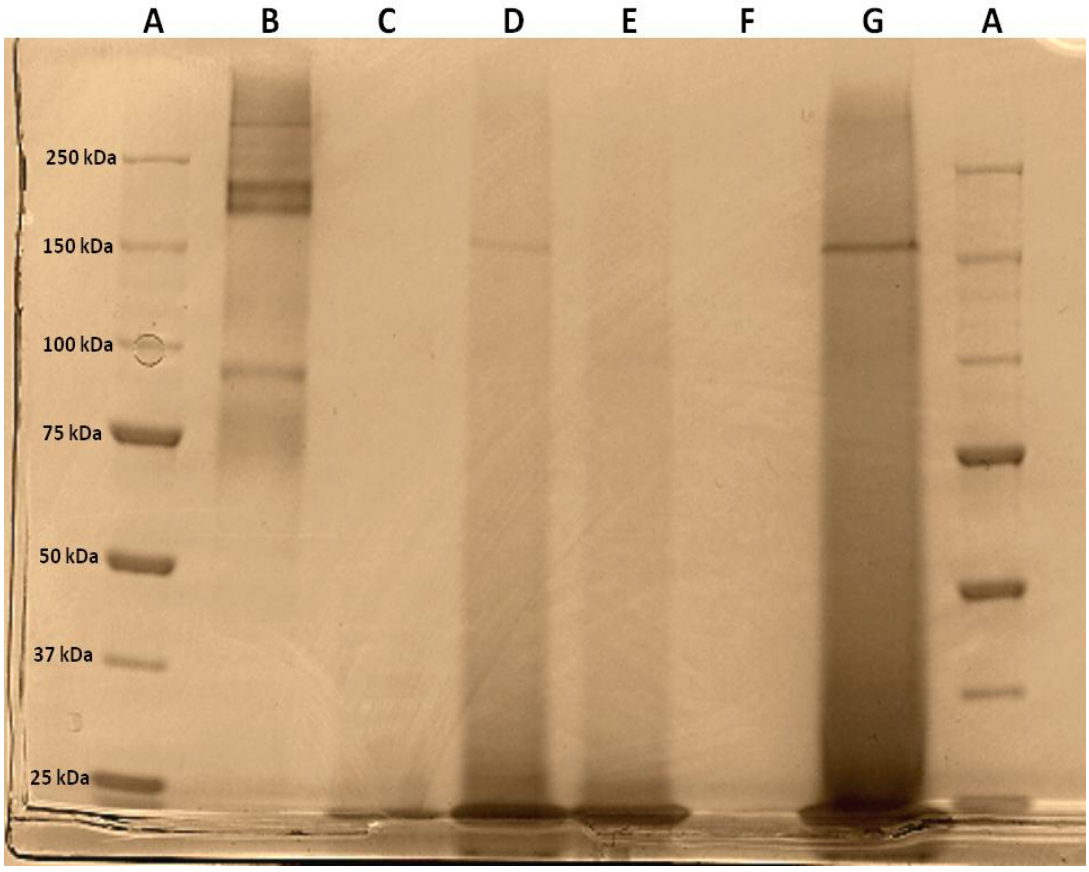

Fig.4.5: SDS-PAGE analysis for a standard with known molecular weights (A), Jackbean urease (10 mg ml^{-1}) (B), crude soybean extract (C), 0-40% $(NH_4)_2SO_4$ saturation soybean fraction (D), 40-55% $(NH_4)_2SO_4$ saturation soybean fraction (E), 55^{+}% $(NH_4)_2SO_4$ saturation soybean fraction (F), and 0-40% $(NH_4)_2SO_4$ saturation soybean concentrated fraction (G).

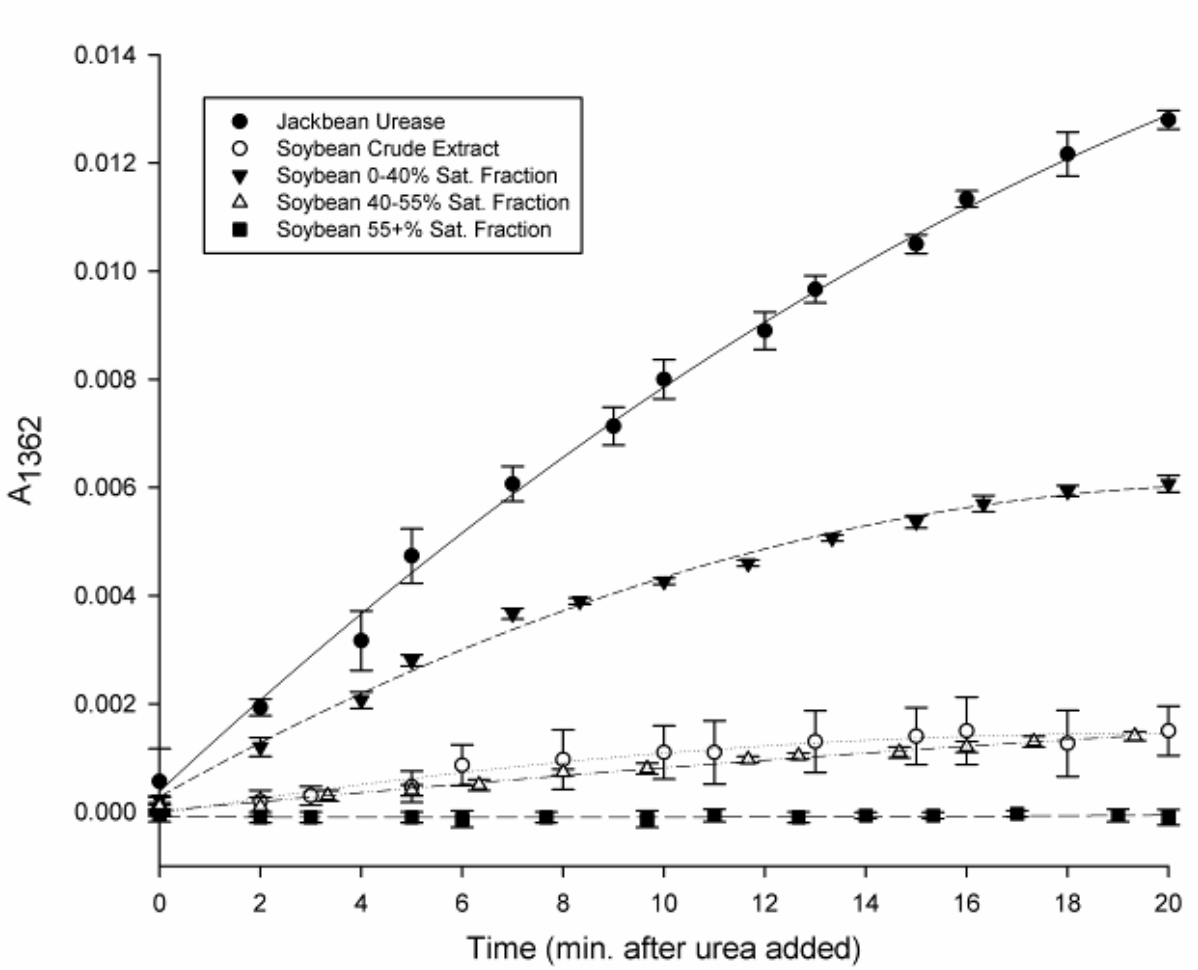

Fig. 4.6: Progress curves for urea hydrolysis monitoring the formation of bicarbonate at 1,362 cm^{-1}. The reaction mixture contained 1 ml of 200mM urea with 1 ml of Jackbean urease or soybean urease fractionation at 25 ^{o}C for 20 minutes

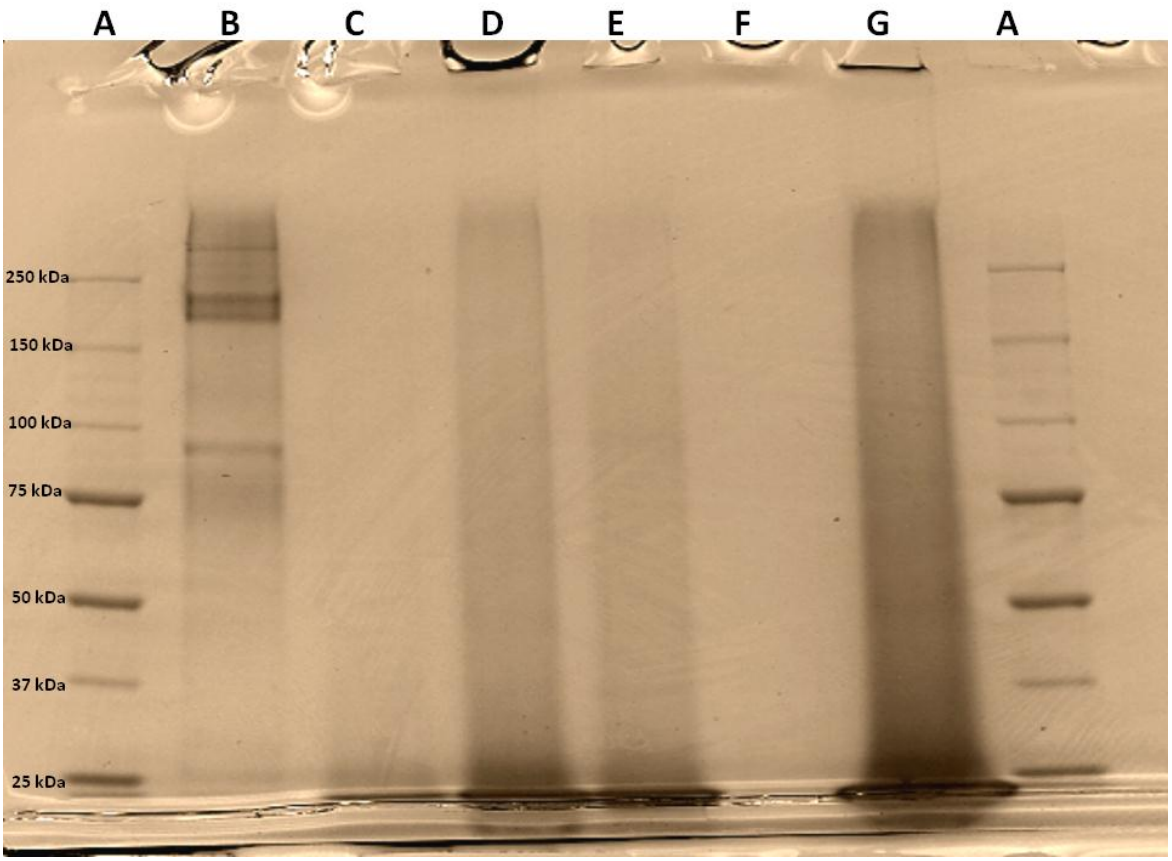

Fig. 4.7: SDS-PAGE analysis for a standard with known molecular weights (A), Jackbean urease (10 mg ml^{-1}) (B), crude corn extract (C), 0-40% $(NH_4)_2SO_4$ saturation corn fraction (D), 40-55% $(NH_4)_2SO_4$ saturation corn fraction (E), 55^{+}% $(NH_4)_2SO_4$ saturation corn fraction (F), and 0-40% $(NH_4)_2SO_4$ saturation corn concentrated fraction (G).

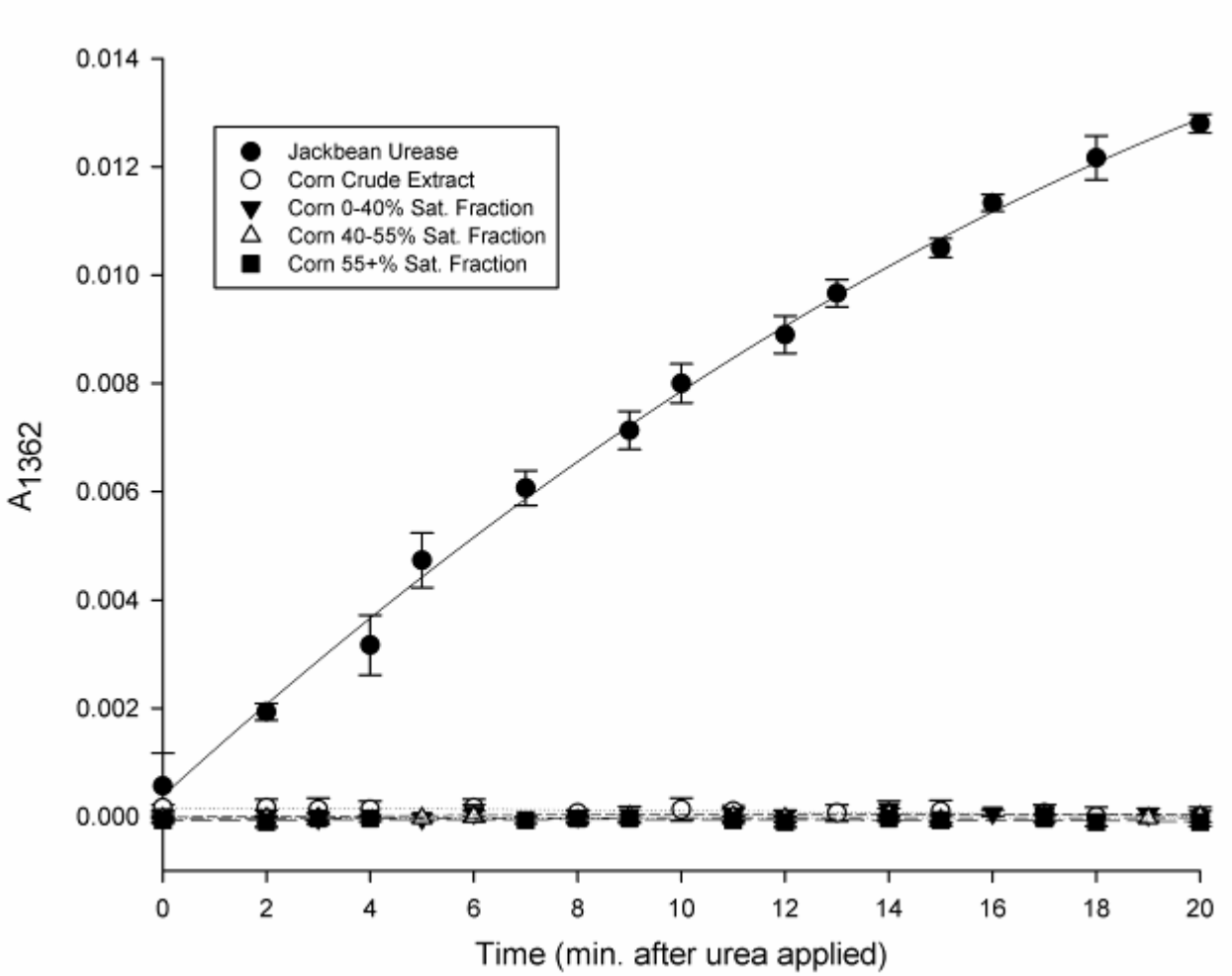

Fig. 4.8: Progress curves for urea hydrolysis monitoring the formation of bicarbonate at 1,362 cm^{-1}. The reaction mixture contained 1 ml of 200mM urea with 1 ml of Jackbean urease or corn urease fractionation at 25^{o}C for 20 minutes.

5 Agronomic Evaluation of Coated Urea to Reduce Ammonia Volatilization from Side-dress Applications to *Zea mays L.*

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5.1 Abstract

Urea is the dominant nitrogen (N) fertilizer used globally because of its high N concentration, ease of handling, and cost. The objectives of this research were: 1) to compare the effect of urea with and without the urease inhibitor, N-(n-butyl) thiophosphoric triamide (NBPT) on corn ear leaf N content and grain yield; and 2) to compare the effect of sulfate salts as coatings with and without NBPT on N concentration in corn ear leaves and corn grain yield in field studies. The current study was conducted at 10 locations over 3 years in Virginia and North Carolina to evaluate NBPT as a coating on granular urea in corn production systems. Urea and Arborite® Ag were applied at four N rates: 56, 112, 168, and 224 kg N $ha^{-1}$and all other coatings were applied at 112 kg N ha^{-1}. All treatments were surface broadcast at V5-V7, and all other nutrients were applied based on soil test recommendations. Percent N in corn ear leaves and grain yield were measured to evaluate the effect of the coatings on N availability. Low rainfall early in the growing season at locations 3-10 did not seem to be detrimental to grain yield as all locations had higher than the expected grain yields for their respective soil types. The concentration of N in corn ear leaves was significantly increased using Arborite® Ag at 5 out the 10 locations during the study at $\alpha = 0.1$. Grain yield and N concentration in corn ear leaves increased at a decreasing rate with N rates from 0-224 kg N ha^{-1}. Physical and chemical coatings had no effect on N concentration in corn ear leaves and grain yield at 112 kg N ha^{-1}.

5.2 Introduction

Commercial N fertilizers are an essential part of agricultural cropping systems worldwide. Nearly 40% of the increase in grain yield over the past sixty years can be attributed to N fertilization. (Brown, 1999; Mosier et al., 2004; Peoples et al., 2004; Smil, 2002). Nitrogen is lost through the following pathways: (1) denitrification; (2) leaching; (3) ammonia volatilization; (4) runoff; and (5) erosion. Of these loss pathways, denitrification

and ammonia volatilization are the greatest gaseous loss mechanisms worldwide (IFA/FAO, 2001). Losses of N from ammonia volatilization are higher than losses from denitrification due to the increased use of urea during the past two decades (IFA/FAO, 2001).

Urea has become the dominant N source worldwide because of its high N concentration, positive handling characteristics, and cost compared to alternative N sources. Urea undergoes a hydrolysis reaction when applied to the soil surface, converting urea to bicarbonate and ammonium. The reaction is catalyzed by the enzyme urease, found ubiquitously in soil, plants and animals. Soil urease is more stable and persistent than other ureases found in bacteria and plants (Kiss and Simihaian, 2002). Adsorption of the enzyme to organic matter and clay colloids protect the enzyme from microorganisms and subsequent degradation. The protected soil urease enables the hydrolysis reaction to proceed at rates 10^{14} times faster than the non-catalyzed reaction, producing large quantities of ammonium in a small zone surrounding the applied urea fertilizer granule and bicarbonate that produces an abrupt increase in pH in the zone of urea hydrolysis (Ciurli et al., 1999; Mobley and Hausinger, 1989). The pH increase around the concentrated zone of urea converts ammonium to ammonia, and if this occurs on the soil surface, the ammonia is lost to the atmosphere. Applying urea on the soil surface can result in losses greater than 50% of the applied fertilizer N via ammonia volatilization, although average losses are generally much lower, i.e. 20 to 25% (Antisari et al., 1996; Bayrakli, 1990; Beyrouty et al., 1988; Carmona et al., 1990; Christianson et al., 1990; Christianson et al., 1994).

A management tactic to prevent rapid hydrolysis in soil systems is to apply a urease inhibitor with the urea fertilizer. A urease inhibitor application is usually accomplished as a coating on urea granules or adding the inhibitor into urea-ammonium nitrate (UAN) solutions. Urease inhibitors block the active site of the enzyme preventing hydrolysis of urea. Urease inhibitors have been studied for agricultural systems in the field and simulated

systems in laboratories. Turner et al. (2010) found that NBPT slowed urea hydrolysis and decreased ammonia loss compared to untreated urea during a trial using winter wheat. Rawluk et al. (2001) also found that the rate of hydrolysis and ammonia volatilization losses were reduced 75-85% for urea treated with NBPT rates ranging from 0.05 to 0.15% w/w, when compared to untreated urea applied at 100 kg N ha^{-1}. Buresh et al. (1988) concluded that NBPT increased agronomic efficiency and potentially could increase yields of lowland rice. However, little information has been compiled on the effect of NBPT on corn (*Zea Mays L.)* production in Virginia and North Carolina.

Until recently the only source of NBPT available to producers was Agrotain® products (Koch Agronomic Services, LLC, Wichita, KS). Recently, Weyerhaeuser Co. (Federal Way, W.A.) and Brooks Whitehurst and Associates, Inc. (New Bern, N.C.) have developed a binder technology to coat urea granules with NBPT. This new product will be marketed under the trade name Arborite® Ag, with a NBPT application rate of 0.08% w/w. The coating technology has been successfully used in commercial fertilization of loblolly pine (*Pinus taeda*) production, and also has the benefit of being able to coat urea with secondary and micronutrients such as sulfur, or other powder formulated nutrients (Personal Communication, Robert Campbell, 2010). Calcium sulfate coated urea has shown promise as an ammonia volatilization control as well as providing plant available sulfur. Laboratory studies have shown that ammonia volatilization was reduced with the use of the calcium sulfate and ammonium sulfate coatings when NBPT was also applied in the coating (Frame et al., 2012, Agron. J. In review). Field evaluations of these fertilizers have not been conducted.

The objectives of this research were: 1) to compare the effect of urea with and without the urease inhibitor, NBPT on corn ear leaf N content and grain yield; and 2) to compare the effect of sulfate salts as coatings on urea with and without NBPT on N concentration in corn ear leaves and corn grain yield in field studies.

5.3 Material and Methods

A total of 10 field trials from 2009-2011 were conducted to evaluate the use of surface-applied urea with multiple physical coatings with and without the urease inhibitor NBPT. Out of the 10 sites, 4 were conducted in 2009, 5 in 2010, and 1 in 2011. Five sites over 2 years were conducted near New Bern, N.C., in the Neuse River Basin, with the remaining four in Virginia (Table 5.1). One site was in the eastern piedmont of Virginia with the remaining four being conducted in Blacksburg, Va., at Virginia Tech's Kentland Farm, in the Ridge and Valley physiographic province. Dominant soil series, taxonomic description, and coordinates for each site are shown in table 5.1. Trials were conducted using a randomized complete block design with four replicates of each treatment at each location. Nitrogen application rates were nested within treatments for both years of the study with urea and Arborite® Ag urea applied at 56, 112, 168, and 224 kg N ha^{-1}. The urease inhibitor, NBPT, was applied at an application rate of 0.08% w/w as this is the commercial standard rate used in the U.S. All other coated treatments were applied at 112 kg N ha^{-1}. In 2009, there were 12 total treatments (Table 5.2) and 15 total treatments in 2010 and 2011 (Table 5.2).

Treatments were applied to 6 row plots with row widths of 0.76 and 0.91 m. An individual plot consisted of 6 rows of corn and varying row widths were a result of dual purpose planters for cotton and corn in North Carolina. Plot lengths varied from 9.14 m and 7.62 m depending on the available area at each location. Treatments were hand-applied at growth stage V5-V7 and every effort made to avoid precipitation in the weather forecast around the time of application. Phosphorus and potassium were applied at the recommended rates based on soil tests to ensure no other nutrient was limiting plant growth.

Corn ear leaf samples were collected from the second and fifth rows of plots, dried at 60°C until a constant weight was reach, ground to pass a 0.5mm sieve and analyzed for g N 100 g^{-1} tissue using a Vario Max CNS-analyzer (Elementar, Hanau, Germany). All corn ear

leaves were collected at green silking stage of development. Yield data were collected on the third and fourth rows, the two middle rows, of plots using two different methods. A plot combine was used to harvest where possible. The combine shelled the grain and weighed the grain from the total length of the plot using a load cell. A subsample was then taken from each plot for analysis of moisture and test weight using a Dickey John GAC® 2000. For the remaining sites two 3.05 meter sections were hand-harvested from the third and fourth rows of plots. The total weight of ears was recorded and six representative ears were sub-sampled and weighed. The grain and cob were separated by hand shelling the six ear sample and the cobs were weighed to calculate a percent shelled weight from the subsample. The grain subsample was processed using the Dickey John GAC 2000. Yields were calculated as Mg grain ha^{-1} based on an adjusted moisture content of 15.5%.

Treatments were analyzed in two groups based on treatment design. Analysis of variance was conducted using Proc Mixed, with a factorial treatment design, for all combinations of N rates, urea and Arborite® Ag in SAS 9.2. Polynomial regression analyses were conducted to determine the response to N fertilization as urea and Arborite® Ag using Sigma Plot 11 (Systat Systems, 2008). Regression analyses for both urea and Arborite® Ag were conducted only at sites where the main effect of coating was significant at $\alpha = 0.1$. At locations where the coating main effect was not significant a combined regression analysis was conducted to described the N rate response on N content in corn ear leaves and grain yield. Analysis of variance was conducted for all coatings at 112 kg N ha^{-1} using Proc Mixed in SAS 9.2 and mean separation was conducted using the Tukey-Kramer mean separation procedure at alpha = 0.05.

5.4 Results and Discussion

5.4.1 Sidedress N rates and Coatings

Nitrogen content of corn ear leaves increased at a decreasing rate (quadratic response) from 0-224 kg N ha^{-1} at all locations except location 9 (Figs 5.1 and 5.2). Nitrogen concentration in corn ear leaves was significantly higher using Arborite® Ag than urea alone at 5 out of the 10 locations during the study (Table 5.3 and Fig. 5.1). The five locations had the strongest relationship between N rate and N content of corn ear leaves out of the ten locations (Fig. 5.1). The N response at locations where Arborite® Ag and urea were not significantly different in N content of corn ear leaves can be found in Fig. 5.2 and Table 5.3. The relationship between N content in corn ear leaves and N sidedress application rates was weaker (lower R^2 values) at these sites than the locations where Arborite® Ag and urea were significantly different (Figs. 5.1 and 5.2).

The N sufficiency range for N content of corn ear leaves in Virginia is defined as 3.00-3.50 g N 100 g^{-1} tissue (Donohue, 2000). The average y-intercept of the regression analyses at the locations where Arborite® Ag and urea did not differ was 2.59±0.51 while at the locations in Fig. 5.1 the average y-intercept was 2.04±0.32. This difference in N content at 0 kg N ha^{-1} suggests that at the sites where Arborite® Ag and urea did not different, there was more N available from mineralization than at the locations where Arborite® Ag and urea differed.

Grain yield with Arborite® Ag and urea significantly differed at 3 out of the 10 locations during the study (Fig. 5.3). At locations 1 and 3 Arborite® Ag significantly increased grain yields while at location 6, urea produced significantly higher grain yields (Table 5.3). The regression analysis of Arborite® Ag over sidedress N application rates was not significant at location 6 and the difference between urea and Arborite® Ag cannot be biologically explained as urea did not produce significantly higher in N content of corn ear

leaves or higher grain yields at any other location. At the other 7 locations Arborite® Ag and urea did not different in grain yield and regression analyses show that yield response to N sidedress application was quadratic at all sites except locations 2 and 9. There was not a significant relationship between grain yield and sidedress N rate at location 9 (Fig. 5.4), this was the only location in the study that did not have a significant grain yield response to N fertilization. The coefficient of variation for grain yield at each location ranged from 3-14% with an average of 9.9% during the study. Rozas et al. (1999) found that NBPT did not significantly increase N uptake and grain yield of corn. Watson et al. (1998) reported mixed responses to NBPT in terms of dry matter yield and N offtake in a perennial ryegrass (*Lolium perrenne*) system. Dawar et al. (2010) found that dry matter yield of herbage in a mixed pasture system was increased with the addition of NBPT coated on granular urea. The variable grain yield response to NBPT during the study was most likely due to environmental conditions during the 2009-2011 growing seasons such that volatilization losses of N from the side-dress applications was not a major limiting factor to corn grain yields.

5.4.2 Physical coatings at 112 kg N ha^{-1}

Responses to physical coatings were sparse during the study with only 3 out the 10 locations having significant differences in N concentration in corn ear leaves (Table 5.4). The N concentration in corn ear leaves using urea was significantly lower than the K_2SO_4-coated at location 4 (Table 5.4). These two treatments were the only treatments to be significantly different at this location. The other two locations where differences occurred were locations 5 and 6 in 2010 where ESN® had significantly lower N concentrations in corn ear leaves than all other coated treatments (Table 5.4). The lower N concentrations in corn ear leaves with ESN® was most likely due to lower than average precipitation (Table 5.5) in June and July which was not adequate to diffuse the urea through the polymer coating so that the N would be plant available. The data set on ESN® was limited since only two locations contained the

treatment and more data are needed to make general conclusions about the effect of ESN® on N content in corn ear leaves from surface broadcast, sidedress applications.

Grain yields were not significantly different at 112 kg N ha^{-1} between the selected physically coated materials at each location from 2009-2011 (Table 5.6). The lack of response to the coated urea materials was indicative that ammonia volatilization losses were not great enough to influence yields at each location in this study. Also, at a majority of the sites in this study N concentrations and grain yields were optimized at 112 kg N ha^{-1} (Figs. 5.1-5.4). While we expected that the 112 kg N ha^{-1} rate would be on the linear part of the response curve where differences between coatings were likely to be observed, the rate was not low enough for these sites. This means that the coated treatments were applied at an N rate where the response to N loss would not be as great as at lower N rates (the linear portion of the N response curves). The coefficient of variation at each location ranged from 3-16% with an average of 10.9% which was lower than expected given the moisture stress during June and July (Table 5.5). Also, the sulfur coated materials showed no advantage compared to bulk blended fertilizer in respect to N concentration in tissues or grain yield.

All locations, except locations 1 and 2, received lower than average precipitation during June (Table 5.5). Precipitation was lower than the 30 year averages for July at all locations in 2009-2011. This time period was directly after N sidedress application and visible signs of drought stress were present at many of the locations. Comparing the grain yields at each location with the expected yields determined by soil type in table 5.1, the grain yields in the current study are higher than expected yields. The average grain yields for Virginia and North Carolina in 2009, 2010, and 2011 were 8.2, 4.2, and 7.4 Mg ha^{-1} and 7.3, 5.7, and 5.3 Mg ha^{-1}, respectively (National Agricultural Statistic Service, 2012). The higher than average and published expected grain yields in this study from 2009-2011 indicate that there was enough moisture available to support higher than average yields in Virginia and

North Carolina at individual locations. The precipitation at the locations in this study was lower than normal levels at all locations, and at locations 3, 4, 7, 8, and 9 the poorly drained soils may have helped prevent drought stress from limiting grain yield at these locations. Location 2 was irrigated during the growing season to ensure yields were not limited by drought. The expected yields of soil series may need to be updated to account for the higher genetic yield potential and drought tolerance of current varieties given the lower than average rainfall and higher grain yields measured during the study.

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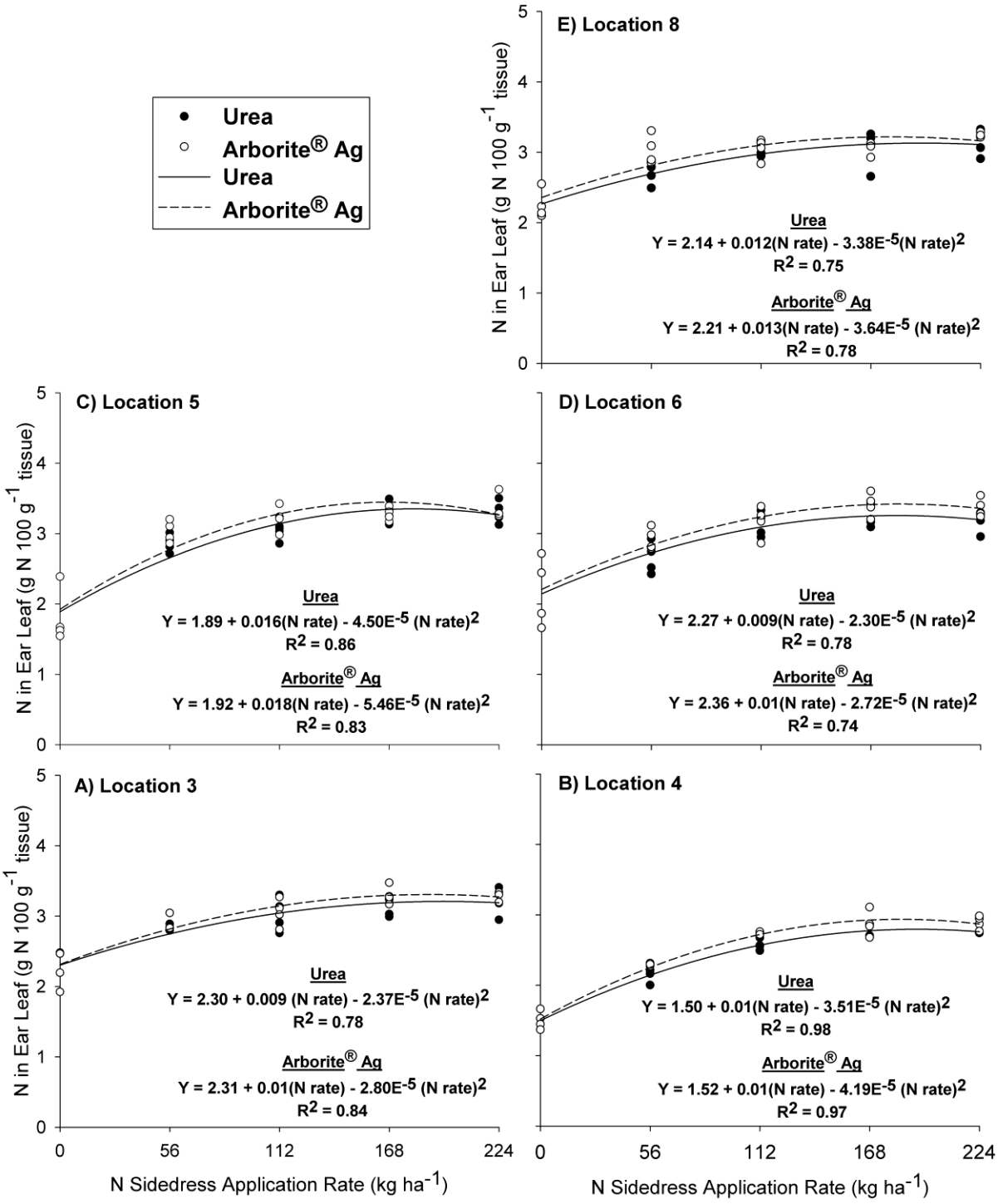

Fig. 5.1: Corn leaf N concentration at V5-V7 following granular urea application at rates from 0 to 224kg N ha^{-1} for sites where urea and Arborite® Ag were significantly different at $\alpha = 0.1$.

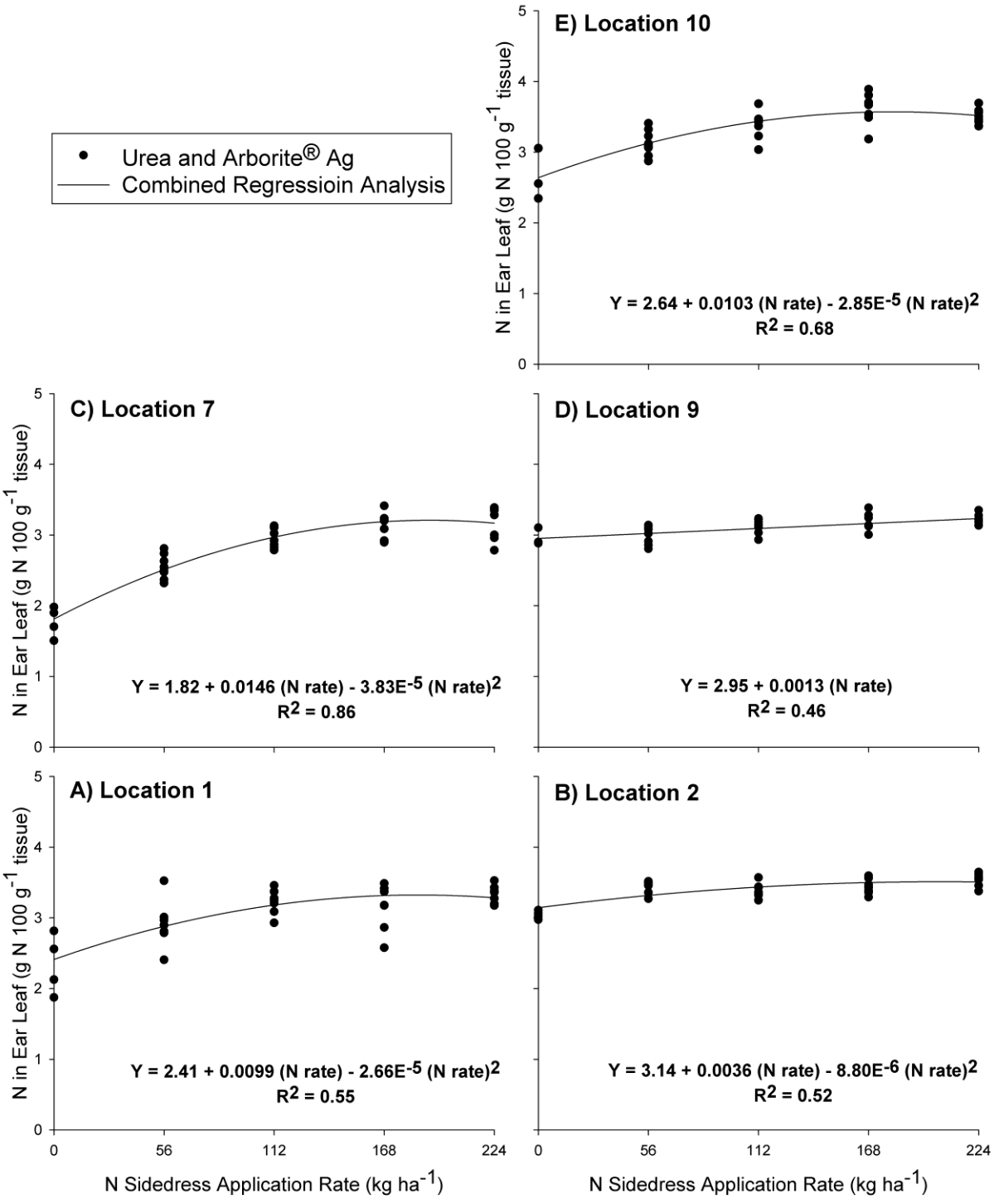

Fig. 5.2: Corn ear leaf N concentration at V5-V7 following granular urea application at rates from 0 to 224kg N ha^{-1} for sites where urea and Arborite® Ag were not significantly different at $\alpha = 0.1$.

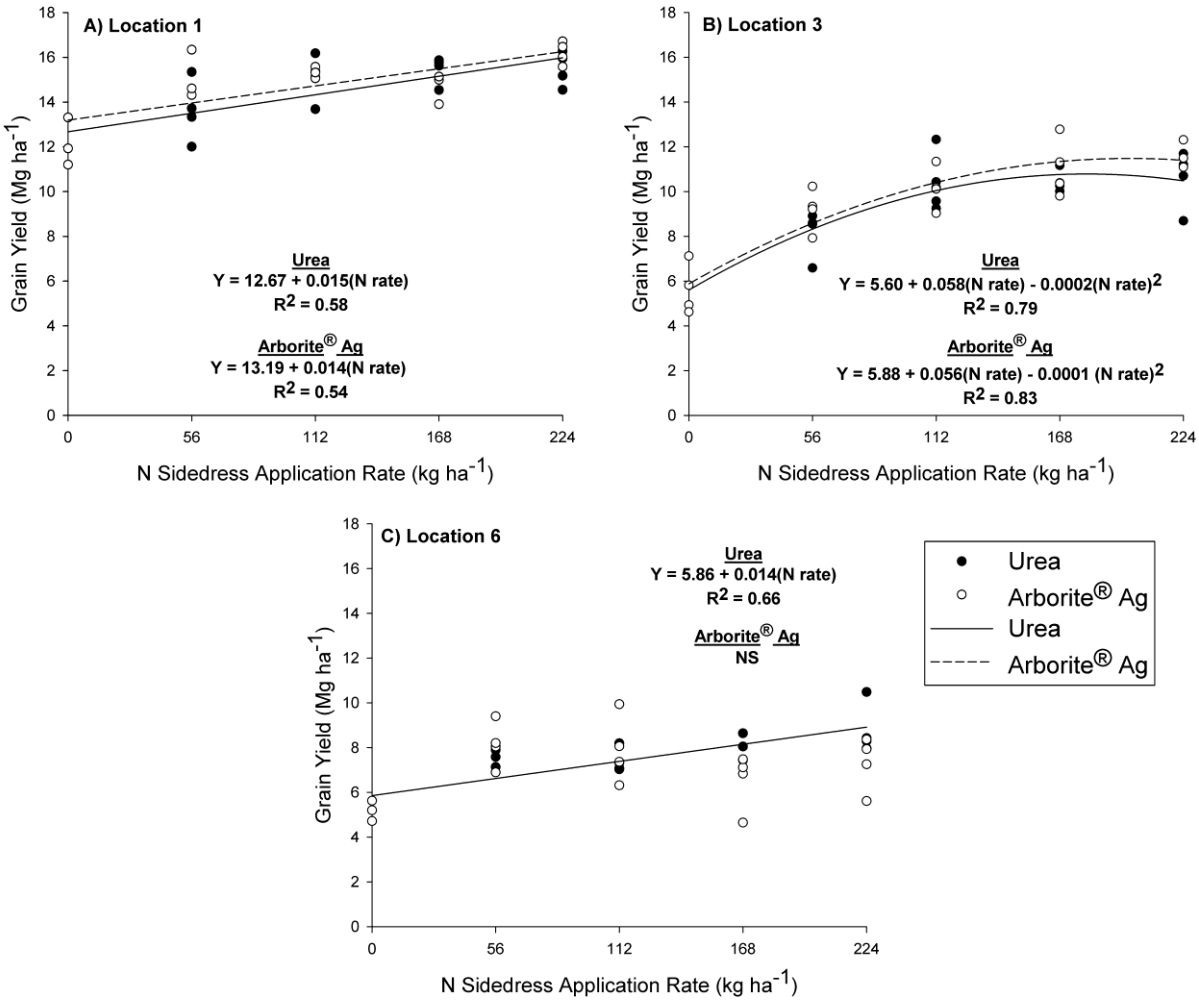

Fig. 5.3: Corn grain yield (Mg ha^{-1}) and granular urea application at rates from 0 to 224kg N ha^{-1} for sites where urea and Arborite® Ag were significantly different at $\alpha = 0.1$.

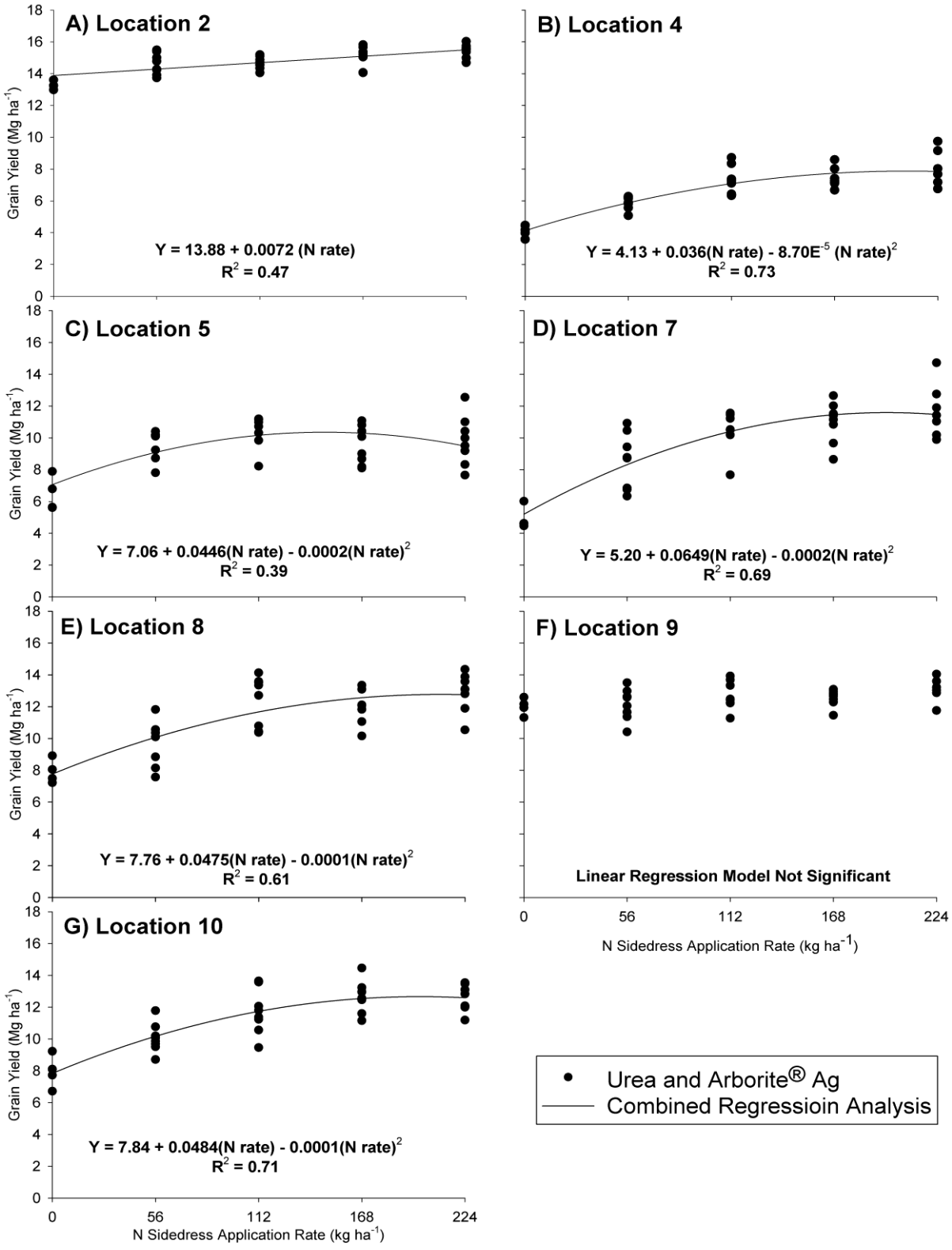

Fig. 5.4: Corn grain yield (Mg ha^{-1}) and granular urea application at rates from 0 to 224kg N ha^{-1} for sites where urea and Arborite® Ag were not significantly different at $\alpha = 0.1$.

Table. 5.1: Location names, coordinates, and soil descriptions for all locations during 2009-2011

Location #	Year and Location Coordinates	Dominant Soil Series	Taxonomic Description	Expected Yields (Productivity Class†)
	2009			Mg ha^{-1}
1	**Kentland Farm, VA** 37° 12'07.53" N, 80° 33'58.13"W	Hayter loam	Fine-loamy, mixed, active, mesic Ultic Hapludalfs	9.41 (Ib)
2	**Hanover, VA** 37° 46'40.11"N, 77° 18'47.77"W	State loamy fine sand	Fine-loamy, mixed, semiactive, thermic Typic Hapludults	10.04 (Ia)
3	**B. Campbell, NC** 35° 13'46.39"N, 77° 03'00.51"W	Lynchburg fine sandy loam	Fine-loamy, siliceous, semiactive, thermic Aeric Paleaquults	7.8‡
4	**Cove City, NC** 35° 10'12.31"N, 77° 17'31.65"W	Rains fine sandy loam	Fine-loamy, siliceous, semiactive, thermic Typic Paleaquults	7.8‡
	2010			
5	**Kentland Farm, VA** 37° 12'15.31"N, 80° 33'39.36"W	Hayter loam	Fine-loamy, mixed, active, mesic Ultic Hapludalfs	9.41 (Ib)
6	**Childress Farms, VA** 37° 05'06.11"N, 80° 29'45.06"W	Frederick/ Vertrees Silt Loam	Fine, mixed, semiactive, mesic Typic Paleudults/Fine, mixed, semiactive, mesic Typic Paleudalfs	8.16 (IIb)
7	**River rd, NC** 35° 14'41.97"N, 77° 09'22.67"W	Leon sand	Sandy, siliceous, thermic Aeric Alaquods	4.4‡
8	**Cow Pen Landing rd, NC** 35° 15'04.79"N, 77° 09'14.63"W	Arapahoe fine sandy loam	Coarse-loamy, mixed, semiactive, nonacid, thermic Typic Humaquepts	8.8‡
9	**State Camp rd, NC** 35° 16'23.11"N, 77° 10'11.34"W	Arapahoe fine sandy loam	Coarse-loamy, mixed, semiactive, nonacid, thermic Typic Humaquepts	8.8‡
	2011			
10	**Kentland Farm, VA** 37° 11'32.21"N, 80° 34'39.17"W	Guernsey silt loam/Hayter loam	Fine, mixed, superactive, mesic Aquic Hapludalfs/ Fine-loamy, mixed, active, mesic Ultic Hapludalfs	8.16 (IIb)

† Soil productivity class and expected yields were determined by soil series in the Virginia Department of Conservation and Recreation (2002)

‡ Expected yields were taken from the North Carolina Nutrient Management Workgroup (2003)

Table 5.2: Treatment descriptions for 2009, 2010, and 2011corn trials.

Trt	N Rate	Coating	NBPT	Analysis
	kg ha^{-1}			
1	0	None	-	N/A
2	56	Urea	no	46-0-0
3	112	Urea	no	46-0-0
4	168	Urea	no	46-0-0
5	224	Urea	no	46-0-0
6	56	Arborite® Ag	yes	45.85-0-0
7	112	Arborite® Ag	yes	45.85-0-0
8	168	Arborite® Ag	yes	45.85-0-0
9	224	Arborite® Ag	yes	45.85-0-0
10	112	Agrotain®	yes	45.82-0-0
11	112	K_2SO_4-coated+NBPT	yes	36.62-0-9-3.2S
12	112	K_2SO_4-coated†	no	36.62-0-9-3.2S
13	112	$CaSO_4$-coated+NBPT†	yes	39.87-0-0-3S
14	112	$CaSO_4$-coated†	no	39.87-0-0-3S
15	112	Urea + S ($CaSO_4$)	no	46-0-0
16	112	ESN®†‡	no	44-0-0

† Treatments not applied in 2009

‡ ESN® only applied at locations 5 and 6

Table 5.3: Main effects for urea and Arborite® Ag coating applied at four sidedress N application rates on N concentration in corn ear leaves and grain yield at all locations.

Location	Urea	Arborite® Ag	Urea	Arborite® Ag
	N Content in Corn Ear Leaves		Grain Yield	
2009	-------- g N 100 g^{-1} tissue --------		-------- Mg ha^{-1} --------	
1	3.21 ns	3.14	14.9 b†	15.3 a
2	3.46 ns	3.47	14.9 ns	15.0
3	3.05 b†	3.14 a	9.9 b	10.8 a
4	2.58 b	2.72 a	6.88 ns	6.99
2010				
5	3.12 b	3.22 a	9.6 ns	10.0
6	2.98 b	3.10 a	8.0 a	7.5 b
7	2.92 ns	3.01	10.3 ns	10.5
8	3.06 b	3.22 a	11.7 ns	11.8
9	3.12 ns	3.13	12.6 ns	12.6
2011				
10	3.38 ns	3.44	11.6 ns	11.9

† Means within the same row and dependent variable with different letters are significantly different at alpha = 0.1

ns Means within the same row and dependent variable are not significantly different at alpha = 0.1.

Table 5.4: : Effect of different physical and chemical coatings applied at 112 kg N ha^{-1} on N concentration of corn ear leaves at all locations.

Coating Treatments	Locations									
	1	2	3	4	5	6	7	8	9	10
	---------- N Content of Corn Ear Leaves (g N 100 g^{-1} tissue) ----------									
Urea	3.23 ns	3.45 ns	3.03 ns	2.57 b†	3.02 a†	3.01 ab†	2.93 ns	3.13 ns	3.10 ns	3.45 ns
Arborite® Ag	3.26	3.43	3.05	2.73 ab	3.21 a	3.05 a	3.01	3.17	3.15	3.34
Agrotain®	3.39	3.52	3.13	2.80 ab	3.26 a	3.03 ab	2.91	3.15	3.15	3.44
K_2SO_4-coated+NBPT	3.38	3.51	3.13	2.85 a	3.13 a	3.06 a	2.89	3.14	3.17	3.14
K_2SO_4-coated	N/A	N/A	N/A	N/A	3.26 a	3.09 a	3.14	3.29	3.17	3.41
$CaSO_4$-coated+NBPT	N/A	N/A	N/A	N/A	3.21 a	3.06 a	3.1	3.2	3.09	3.43
$CaSO_4$-coated	N/A	N/A	N/A	N/A	3.10 a	3.08 a	2.95	3.16	3.22	3.44
Urea + S ($CaSO_4$)	3.15	3.35	3.05	2.68 ab	3.02 a	3.04 a	3.05	2.99	3.13	3.36
ESN®	N/A	N/A	N/A	N/A	2.50 b	2.76 b	N/A	N/A	N/A	N/A

†Means within the same column with the same letter are not significantly different at alpha = 0.1.

N/A Treatment was not applied at this location.

Table 5.5: : Monthly rainfall totals at individual location during June, July and August from 2009-2011.

Location	June	July	August
	Precipitation (mm)		
2009			
1	92 (95)†	93.7 (101)	74 (89)
2‡	182 (89)	65 (106)	114 (102)
3	70 (121)	119 (226)	115 (190)
4§¶	83 (121)	93 (226)	205 (190)
2010			
5	37 (95)	61 (101)	86 (89)
6#	46 (91)	52 (81)	90 (85)
7¶	37 (121)	160 (226)	45 (190)
8¶	37 (121)	160 (226)	45 (190)
9¶	40 (121)	169 (226)	60 (190)
2011			
10	27 (95)	112 (101)	44 (89)

† Numbers in parentheses are 30 year monthly precipitation totals for that location

‡ Monthly and 30 year average precipitation data taken from Southeast Region Climate Center for the Ashland 1 SW, VA weather station in 2009.

§ Monthly precipitation data taken from Southeast Region Climate Center at the New Bern 3 SW weather station in 2009

¶ 30 year average precipitation data taken from Southeast Region Climate Center at the New Bern 3 SW weather station

Monthly and 30 year average precipitation data taken from Southeast Region Climate Center for Radford, VA weather station in 2010.

Table 5.6: Effect of different physical and chemical coatings applied at 112 kg N ha^{-1} on corn grain yield (Mg ha^{-1}) at all locations during 2009

Coating Treatments	Locations									
	1	2	3	4	5	6	7	8	9	10
	---------- N Content of Corn Ear Leaves (g 100 g^{-1} tissue)----------									
Urea	14.9 ns	14.9 ns	10.4 ns	6.87 ns	10.2 ns	7.4 ns	10.3 ns	12.7 ns	12.3 ns	11.8 ns
Arborite® Ag	15.3	14.4	10.2	7.62	10.5	7.9	10.9	12	13.1	11.6
Agrotain®	15.8	15.5	11.4	6.14	9.9	8.5	10.7	11.6	12.3	11.5
K_2SO_4-coated+NBPT	15.2	14.9	10.3	6.41	10.7	8	10.6	12	12.7	11.1
K_2SO_4-coated	N/A	N/A	N/A	N/A	9.9	8.1	10.5	11.7	12.9	11.6
$CaSO_4$-coated+NBPT	N/A	N/A	N/A	N/A	9.8	8.7	9.2	13.5	12.1	11.7
$CaSO_4$-coated	N/A	N/A	N/A	N/A	9.3	8.1	9.8	12.5	13.2	11.3
Urea + S ($CaSO_4$)	14.6	15.1	10.1	7.66	10.1	8.1	11.2	10.5	13.1	11.6
ESN®	N/A	N/A	N/A	N/A	10.5	8	N/A	N/A	N/A	N/A

†Means within the same column with the same letter are not significantly different at alpha = 0.1.

N/A Treatment was not applied at this location.

6 Conclusions

The human population eclipsed 7 billion in 2011 and with that increasing population is the need for increased food production globally. Since most of the arable land in the world is already cultivated there needs to be increased crop yields on currently available arable land. The use of synthetic inorganic N fertilizers, such as urea, has enable non-legume crop yields to sustain human population growth. The dominant N source used globally is urea; however when urea is surface-applied up to 50% of the applied N can be lost via ammonia volatilization. This dissertation focused on management strategies to decrease ammonia volatilization from agroecosystems, specifically corn production systems, in Virginia and North Carolina utilizing NBPT coated granular urea, and selected physical coatings containing sulfate salts. To accomplish this, a series of experiments were designed to evaluate coated granular urea under controlled environmental conditions, determine the agronomic impact of such coatings, and quantify the urease activity from two crop residues commonly found in conservation tillage systems in Virginia and North Carolina.

The evaluation of coated granular urea was conducted using a novel laboratory ammonia volatilization system. A series of two week trials were conducted to determine the efficiency and variation inherent in the laboratory system at two air flow rates (1.00 and 2.00 L min^{-1}), two acid trap volumes (50 and 100 ml), and at two temperatures (26 and 30 ^{o}C). When the system was operated at 1.00 L min^{-1}, with 100 ml of 0.02M phosphoric acid, and at 26^{o}C the system could account for 97% of the applied N with a standard deviation of 1.6% over all chambers. The standard deviation of mg N captured at each sampling interval was also minimized at these system parameters. Given the efficiency and variation it was determined that 1.00 L min^{-1}, with 100 ml of 0.02M phosphoric acid, and at 26^{o}C were the optimum system parameters for future trials conducted with the laboratory ammonia

volatilization system, and that the system provided for reproducible comparison of ammonia volatilization from urea fertilizers.

Using the system described and validated in chapter II, a series of trials were designed to look at two chemically coated urea formulations consisting of NBPT, Arborite® Ag and Agrotain®, as well as urea coated with calcium and potassium sulfate with and without the inhibitor, NBPT. The final experiments quantified the effects of varying rates of NBPT as Arborite® Ag on ammonia volatilization losses. Ammonia loss from granular urea occurred within the first 9 h after N application and the maximum loss rate occurred from 12 to 24 h. During this 12 h period 17-18% of the applied N was lost and this was equal to 1.5% of the applied N h^{-1}. Cumulative losses of N from urea ranged from 33-37% of the applied N over the duration of a two week trial. When NBPT was applied as a coating to urea, ammonia volatilization losses were delayed for 96h, the maximum loss rate averaged 0.16% of the applied N h^{-1} at 120 to 168 h after N application, and cumulative losses were reduced to 19-25% of the applied N. Calcium sulfate coated urea delayed ammonia volatilization for 24 h after N application but did not reduce the cumulative loss of N during the two week trial. Potassium sulfate coating delayed ammonia volatilization for 48 h after N application and significantly reduced cumulative loss of N compared to urea. There was no difference in cumulative losses of ammonia from urea coated with NBPT rates from 0.04 to 0.1% w/w. Further research needs to be conducted to see if NBPT rates lower than the commercial standard of 0.08% w/w could be used to achieve similar control of volatilization under field conditions.

Under field conditions ammonia volatilization losses are generally lower than those reported in laboratory studies. However, in no-till cropping systems ammonia volatilization losses are higher than conventionally tilled cropping systems. In order to understand why losses of ammonia are greater in no-till systems, an experiment was designed to extract and

assay the activity of urease from crop residues commonly found in Virginia. Urease was extracted using sodium phosphate based extracting solutions and urease activity assays were conducted using Fourier transform infrared spectroscopy (FTIR). It was found that a 50 mM sodium phosphate solution provided enough buffering capacity to minimize pH change in crude extracts. Using FTIR one could monitor the disappearance of reactants and appearance of reaction products in real time without altering the reaction mixture. Monitoring the production of bicarbonate at 1362 cm^{-1} was optimum for determining the rate of urea hydrolysis compared to monitoring the disappearance of urea. Urease was successfully extracted and assayed from soybean residues, however urease from corn residues could not be identified in the extract and urease activity assays did not produce bicarbonate as seen in the Jackbean and soybean urease activity assays. Further research needs to be conducted over a more diverse pool of genetic material for corn and soybeans as well as other potential residue covers. However, the initial results of the extractions indicated that there might be differences in urease content and thus urease activity in different residues. Further studies are needed to determine if urease activity in different residue covers needs to be considered when selecting N management tactics to minimize losses via ammonia volatilization.

The final study conducted for this dissertation was a field study to measure the impacts of Arborite® Ag, Agrotain®, and the selected physical coatings on the agronomic performance of corn receiving urea N fertilization. Ten locations during 2009-2011 were selected in Virginia and North Carolina for the agronomic trials where urea and coated urea was applied at corn growth stage V5-V7 as surface broadcast applications. Sampling of corn ear leaves at silking for N content revealed a trend that applying Arborite® Ag at four N rates increased the percent N in leaf tissue. Yield responses to Arborite® Ag were inconclusive as there was no clear advantage when Arborite® Ag was applied compared to urea. A literature review revealed that other field trials utilizing NBPT were also mixed with some reporting

substantial increases in grain or biomass yields while others reported little to no effect of NBPT. When Arborite® Ag, Agrotain®, and sulfate coatings were compared at 112 kg N ha^{-1} there were no differences in N concentration of corn ear leaves and grain yields. The polymer coated urea, ESN®, was significantly lower in N concentration in corn ear leaves, however the treatment was only applied at two locations and a larger data set is needed to confirm this difference. The lack of response to different coatings on granular urea was most likely due to applying the fertilizers at an N rate above the linear portion of the N response curve.

Coating granular urea with NBPT reduced ammonia loss allowing more N to be available for plant uptake. While the reduction in ammonia loss from surface applied NBPT-coated urea could be clearly measured in laboratory studies, and corn ear leaf N concentrations were generally higher in field studies, the influence on grain yields was limited. This lack of clarity in grain yield responses probably reflects the complex influence of climate, soil and management on grain yield. The use of NBPT coatings when surface applying urea fertilizers is one component to increasing N use efficiency in crop production, and continuing research will add to the knowledge base to refine the conditions and rates under which the use of this technology provides economic and/or environmental benefits.