

WHEAT, RICE AND CORN RESPONSE TO THE UREASE INHIBITOR N-(N-
BUTYL THIOPHOSPHORIC TRIAMIDE) IN A DIMETHYL
SULFOXIDE/PROPYLENE GLYCOL SOLUTION

By

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ABSTRACT

Surface applied granular urea is a nitrogen fertilizer commonly used throughout the world. Unfortunately, a significant portion of the urea can be lost to the atmosphere through ammonia volatilization. Much research has been conducted in an attempt to reduce or stop this process and increase the efficiency of urea fertilizer. Urease inhibitors have been shown to be one possible method to reduce nitrogen volatilization. The chemical compound N-(n-butyl) thiophosphoric triamide (NBPT) has been shown to be an effective urease inhibitor. The purpose of this research was to test the effect that a new commercial NBPT containing product, N-Yield™, had on the growth of the common field crops including wheat, rice, and corn. Additionally, N-Yield was compared to the existing product, Agrotain Ultra®. Three studies were performed; a greenhouse study in Verde variety spring wheat, a greenhouse study of CL 111 variety rice, and a field study of dent corn. In the wheat study, three treatments, consisting of urea, urea with Agrotain (4.17 mL/kg), and urea with N-Yield (4.17 mL/kg) applied at 56, 84 and 112 kg nitrogen/ha, were compared to a no fertilizer treatment. The study was a CRD design, with three replications of each treatment. Above and below ground biomass were measured, and protein was analyzed using a Near Infrared Spectrometer (NIR). Significant differences were found in the mean above ground biomass for the Olton Clay Loam soil type ($p=0.05$). A significant difference between treatment means in protein content was found for both the Olton Clay Loam ($p=0.08$) and Amarillo Fine Sandy Loam soil types ($p=0.001$). In the rice study, three treatments consisting of urea, urea

with Agrotain Ultra (4.17 mL/kg), and Urea with N-Yield™ (4.17 mL/kg) applied in either one or two applications of 22.41 kg nitrogen/ha, were compared to a no fertilizer treatment. The study was a CRD design, with four replications of each treatment. Above ground biomass and grain weight per plant was measured. Significant differences were observed between the treatment means of the grain weight ($p=0.06$) and for above ground biomass ($p=0.09$). The field corn study was set up in a RCBD design, using the prevailing south wind as a blocking factor. Three treatments and a no fertilizer control were used. Treatments consisted of urea, urea with Agrotain Ultra© (4.17 mL/kg), and urea with N-Yield™ (4.17 mg/kg) applied at a rate of 112 kg of nitrogen per hectare. There were three replications of each treatment for a total of 12 plots. Twenty plants were harvested from the middle of each plot and grain weight per ear was collected. Significant differences were found for the means of the grain weight per plant ($p=0.008$).

Keywords: NBPT, ammonia volatilization, urea, Agrotain, urease inhibitor, N-yield, wheat, rice, corn.

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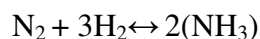
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CHAPTER I

REVIEW OF LITERATURE

Urea as a Fertilizer

In 1872, G.K. Rutherford discovered that nitrogen is essential for plant growth (Fageria and Baligar, 1997). Nitrogen is generally recognized as the most limiting macronutrient for crop production. Its use is often required in order to achieve profitable crop yields (Fageria and Baligar, 2005). Therefore, it is common practice to supplement nitrogen fertilizer to field crops. According to the United States Department of Agriculture (USDA), 99% of all corn acreage in Texas received some form of nitrogen fertilizer (USDA ERS, 2014). There are many ways to supplement nitrogen to a field. The most commonly used fertilizer worldwide is urea ($\text{CO}(\text{NH}_2)_2$). Over 60 million metric tons of urea were produced in 2011 for use on crops like wheat, rice and corn (USDA ERS, 2014). Urea can be applied in either a granular form or as urea ammonium nitrate liquid (UAN). Worldwide, urea granules are more common, accounting for over 50% of all the nitrogen fertilizer used over the last decade (Gilbert et al., 2006). This is likely because they are safe to handle and easy to store, transport and apply (Soares et al., 2012). Urea granules are produced in two steps. First, the Haber-Bosch process catalyzes the formation of ammonia (NH_3) from atmospheric nitrogen. The atmospheric nitrogen is exposed to high temperature and pressure using an iron catalyst:



Urea is formed from a two-step reaction with carbon dioxide (CO_2):

Step one: $2\text{NH}_3 + \text{CO}_2 \leftrightarrow \text{NH}_2\text{COONH}_4$

Step two: $\text{NH}_2\text{COONH}_4 \leftrightarrow \text{H}_2\text{O} + \text{CO}(\text{NH}_2)_2$

The urea is concentrated and granulated by spraying molten urea onto seed granules that are supported on a bed of air (Coppelstone and Kirk, 2014). The end product is a fertilizer that is 46% nitrogen by weight (Overdahl et al., 2014).

Nitrogen Loss from Urea Fertilizer

Although granular urea has advantages over other types of nitrogen fertilizers, it also has a major drawback. Urea granules applied to a field can lose nitrogen in many ways. These include ammonia volatilization, denitrification, soil leaching, surface runoff, immobilization and gaseous plant emission (Raun and Johnson, 1999). Denitrification from applied nitrogen fertilizer has been shown to be as high as 10% to 22% of applied nitrogen in corn (Hilton et al., 1994). Loss from surface runoff can account for between 1% and 13% of the total applied nitrogen (Chichester and Richardson, 1992; Blevins et al., 1996). The studies vary widely on the rate of nitrogen loss from ammonia volatilization in urea. Watson et al. (1994) found that the rate and amount of ammonia loss from urea granules depended on soil pH and soil type, and the greatest losses were 38% of the applied nitrogen, from a soil with a high pH and low titratable acidity. This work agreed with studies by Fox and Hoffman (1981) and Fox et al. (1986) who found ammonia volatilization loss from surface applied urea as high as 30% in no till corn. Fox and Piekielek (1993), reported ammonia volatilization losses from surface applied urea of 28.7%, 11.3%, and 27.3% in 1989, 1990, and 1991, respectively. Fowler and Brydon

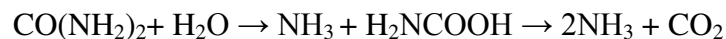
(1989), reported when urea is applied to the soil surface, loss of nitrogen as ammonia can exceed 40%. Nitrogen not taken up by plants represents a \$15.9 billion annual loss worldwide, and even a 1% increase in efficiency of nitrogen applied on crops would save \$2.3 million annually (Raun and Johnson, 1999).

In order to meet the demand for increasing grain yields while using fewer resources, continuing research regarding the most advantageous use of nitrogen fertilizer to enhance production is warranted.

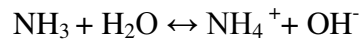
Ammonia Volatilization

When urea is applied to the soil, it undergoes several chemical reactions and produces the plant available ammonium (NH_4^+). Ammonium is an ion that readily reduces to ammonia. At room temperature, ammonia is a gas and is quickly lost to the atmosphere (Fageria and Balinger, 2005). This process is called ammonia volatilization.

Upon application to the soil surface, urea undergoes a hydrolysis reaction to form NH_3 and CO_2 (Bremmer, 1995). The following reactions occur:



In a field study performed on a sandy loam soil type, this occurred within one or two days after urea application (Zaman et al., 2008). When urea is applied to the soil surface, a biological enzyme, urease, combines with H_2O and catalyzes the breakdown of urea to carbamic acid (H_2NCOOH). The rapid decomposition of carbamic acid forms two ammonia molecules and one carbon dioxide molecule (Tisdale et al., 1985). In the presence of water, ammonia reacts to form ammonium and hydroxide (OH^-):



An equilibrium occurs between the ammonia dissolved in water and the ammonium ion (USEPA, 1979). This reaction leads to high levels of ammonium in the soil, as well as a spike in the pH levels due to the presence of hydroxide, creating conditions that are conducive to volatilization. It is important to note that ammonium is a plant available form of nitrogen while ammonia is not (Brady and Weil, 2008).

The rate of this reaction is dependent on many things. The concentration of urease in the soil plays a role. Dick (1983) showed that in no-till corn, the concentration of urease was highest at the soil surface. Watson et al. (2008) showed that the total ammonium volatilization increased with increasing temperature and that soil type played a role. Ammonium volatilization was higher in sandy loam than in clay soils. Additionally, Watson (2008) found that highest nitrogen losses occurred in soils with a pH of 7.0 or greater. Christensen et al. (1993) found highly alkaline soils to increase urea hydrolysis (and therefore increasing ammonia volatilization), while acidic soils significantly slowed the rate of hydrolysis. Hayashi et al. (2008) found that wind velocity effected the rate of volatilization, higher rates of volatilization were observed in higher wind velocities. Soil organic matter has been shown to have an effect. (Dick, 1983; Watson et al., 1994; Singh et al., 2013). Fenn and Hossner (1985) found that ammonia volatilization was higher in soils with a high organic carbon content. The type of tillage effects volatilization. Conventional tillage has the highest rates of nitrogen loss (Rochette et al., 2009). Soil moisture plays a large role in the amounts of nitrogen lost. If urea granules are broadcast onto a moist soil, volatilization rates increase as the urea

hydrolyzes before it is able to move below the soil surface (Keller and Mengel, 1986).

Conversely, rainfall or irrigation immediately following application greatly reduces the rate of volatilization. Losses increased as the number of days between application and the time it took to get 10 mm of rain increased (Fox and Hoffman, 1981; Fox et al., 1986). In an experiment performed by Sanz-Cobena et al. (2011), the highest ammonia emissions came from urea with no irrigation. Black et al. (1987) found that initial soil moisture increased volatilization of ammonia, but 16 mm of irrigation immediately following application of fertilizer greatly reduced ammonia loss. When too much rainfall is received, nitrogen can still be lost from leaching (Macnack et al., 2013). When rainfall following urea application allows the urea to move below the soil surface, it may still return to the surface in response to evaporation (Ferguson and Kissel, 1986; McInnes et al., 1986). Macnack et al. (2013) reviewed over 40 published papers and evaluated several factors that influence the rate of ammonia volatilization. They developed a model to estimate the rate of potential volatilization based on pH, soil temperature and wind speed. This model is available at www.nue.okstate.edu/ammonia_loss.htm.

Mitigation Strategies

Rainfall following surface applied urea, while the urea hydrolysis reaction is occurring, will help reduce the amount of nitrogen lost. Urea is mobile in the soil. Rainfall will move it deeper into the soil, where it can hydrolyze with less opportunity for volatilization (Hendrickson, 1992). Fox and Piekielek (1993) found that nitrogen losses from ammonia volatilization were greater when there were more than two or three days

without rain following application of fertilizer. While it is impossible to control the weather, farmers can reduce their nitrogen loss from NH_3 volatilization by irrigating soon after urea fertilizer application in order to move the fertilizer below the soil surface.

The incorporation of urea fertilizer below the soil surface reduces the rate of ammonia volatilization (Moll et al., 1982; Datta, 1986; Raczowski and Kissel, 1989; Sommer and Hutchings, 1995). Loss of nitrogen as ammonia can exceed 40% when urea is applied to the soil surface without incorporation or rainfall (Fowler and Brydon, 1989). In field crops, agronomic practices often utilize side dressing nitrogen after the emergence of the crop (Sawyer et al., 2006). Additionally, timely incorporation is not possible in reduced-till or no-till crops (Black et al., 1987; Bremner, 1995). In situations where surface applied urea granules do not receive adequate water or incorporation to reduce N losses from volatilization, fertilizer additives must be considered in order to increase the efficiency of the applied urea.

Many possible additives have been studied. These include nitrification inhibitors, coated urea, acidification and urease inhibitors. Most have shown some degree of success (Rao and Ghai, 1985; Salman et al., 1989; Hendrickson 1992; Xiaoyan et al., 1993; Xiaolin et al., 2010). Reddy and Prasad (1975) evaluated several different types of coated urea and nitrification inhibitors on their ability to reduce the degradation of urea in a sandy clay loam soil. Urea coated with sulphur or shellac degraded two to three times slower than untreated urea. Additionally, they found the nitrification inhibitors N-Serve, sulphathiazole, coal tar and neem cake all retarded the nitrification of urea to some

degree. Few urea additives are consistently effective and safe to use on field crops and effective in small quantities.

Urease Inhibitors

Urease inhibitors temporarily reduce the activity of the urease enzyme, allowing a percentage of the applied nitrogen to remain as urea for several days. Urease inhibitors appear to be the most promising method for reducing nitrogen losses from volatilization in situations where incorporation is not possible. Therefore they have been the focus of much research over the last 20 years. Many compounds have been evaluated for their effectiveness as a urease inhibitor. Phosphoryl di- and triamides meet the requirements for effective soil urease inhibition while being economically and environmentally friendly (Byrnes and Freney, 1995; Christianson et al., 1990; Qui-Xiang et al., 1994).

One common phosphoryl triamide, N-(n-butyl thiophosphoric triamide) (NBPT), has been shown to be effective at delaying urea hydrolysis and therefore loss of nitrogen from ammonia volatilization, even in small concentrations (Christianson et al., 1990; Zhengping et al., 1991; Watson et al., 1994). NBPT must be converted to N-(n-butyl) phosphoric triamide (BNPO) in the soil to be effective as a urease inhibitor (Byrnes and Christianson, 1988). Douglass and Hendrickson (1991) found that applying NBPT controls urea hydrolysis more effectively than directly applying BNPO. The reason is unknown.

NBPT is currently sold as a urea fertilizer additive under several brand names. One of those brands is Agrotain Ultra (Koch Agronomic Services, LLC, Wichita, KS).

Agrotain Ultra contains 26.7% NBPT and is suspended in propylene glycol (PG) and n-methyl 2-pyrrolidone (NMP) (U.S. patent # 5698003A). Agrotain Ultra is a liquid formulation that is designed to be coated onto urea granules prior to field application. One downfall to Agrotain Ultra is that NMP has been shown to cause some level of toxicity in rats, exposed both dermally (Maternal toxicity was indicated at 750 mg of N-methylpyrrolidone/kg) and through inhalation (reproductive toxicity) (Becci et al., 1982; Hass et al.). Leira et al. (1992) found NMP to be an irritant to the eyes and respiratory system in humans.

A new NBPT urea fertilizer additive, N-Yield (Eco Agro Resources, High Point, NC) was recently introduced to the market. N-Yield contains 20% NBPT suspended in a dimethyl sulfoxide (DMSO) and propylene glycol solution. Studies have shown DMSO could be an alternative to NMP as it has long been used in agriculture (Smale et al., 1975). N-Yield shows promise as having similar properties and applications as Agrotain (Roy, 2013).

CHAPTER II

WHEAT RESPONSE TO THE UREASE INHIBITOR N-(N-BUTYL THIOPHOSPHORIC TRIAMIDE) IN A DIMETHYL SULFOXIDE/PROPYLENE GLYCOL SOLUTION

INTRODUCTION AND RESEARCH OBJECTIVES

Effects of Urease Inhibitors on Wheat

Limited research has been performed to evaluate the use of NBPT for improving wheat yield and protein. Schlegel et al. (1987) analyzed several phosphoramidate urease inhibitors, including NBPT, on their ability to increase grain protein and grain yield in winter wheat. Schlegel et al. (1987) found that grain yield was a more responsive indicator of nitrogen addition than grain protein, but no significant improvement in yield or protein was found for the addition of a urease inhibitor to winter wheat. They state this was because ammonia volatilization was not a problem at the application times of urea fertilizer.

In a two year field study in Italy, researchers evaluated the ability of NBPT applied with urea to reduce ammonia volatilization. A 42-55% reduction in ammonia volatilization rates were observed. No grain yield benefit was found. The authors suggested this was due to the wheat not responding to the conserved nitrogen, but that yield response would be expected in conditions where the wheat would be able to respond (Nastri et al., 2000). Gezgin and Bayraklı (1995) tested several compounds on their ability to reduce nitrogen losses from ammonia volatilization and affect grain

protein and yield. They found the most reduction in ammonia volatilization with NBPT, reducing losses by up to 63% over untreated urea. Additionally, they found a highly significant, negative correlation ($r=-0.69$) between total nitrogen loss and grain protein content. Compounds evaluated had no effect on grain yield.

Objectives

To date, no published studies have been conducted on the effect of N-Yield on spring wheat. The objectives of this research were as follows:

1. Evaluate the effectiveness of urea fertilizer coated with NBPT in a DMSO/PG solution (N-Yield) by measuring the above ground biomass and protein content of spring wheat.
2. Compare the performance of N-Yield to that of Agrotain Ultra.

MATERIALS AND METHODS

Experimental Design

The experimental design consisted of a completely randomized design (CRD) with ten treatments, conducted in two different soil types. The explanatory variable was fertilizer type and amount. Response variables were above ground biomass and whole plant protein content. The experimental unit was the pot. There were a total of 60 pots (10 treatments, three replications per treatment, two soil types).

The treatments were as follows:

- Treatment 1 – No fertilizer (Nf)
- Treatment 2 – Untreated urea at the nitrogen application rate of 56.0 kg N/ha (50 lb/ac) (U50)
- Treatment 3 – Untreated urea at the nitrogen application rate of 84.1 kg N/ha (75 lb/ac) (U75)
- Treatment 4 – Untreated urea at the nitrogen application rate of 112.1 kg N/ha (100 lb/ac) (U100)
- Treatment 5 – Urea treated with N-Yield (4.17 mL/kg of urea), at a nitrogen application rate of 56.0 kg N/ha (Ny50)
- Treatment 6 – Urea treated with N-Yield (4.17 mL/kg of urea), at a nitrogen application rate of 84.1 kg N/ha (Ny75)
- Treatment 7 – Urea treated with N-Yield (4.17 mL/kg of urea), at a nitrogen application rate of 112.1 kg N/ha (Ny100)
- Treatment 8 – Urea treated with Agrotain Ultra (4.17 mL/kg), at a nitrogen application rate of 56.04 kg N/ha (At50)
- Treatment 9 – Urea treated with Agrotain Ultra (4.17 mL/kg), at a nitrogen application rate of 84.06 kg N/ha (At75)
- Treatment 10 – Urea treated with Agrotain Ultra (4.17 mL/kg), at a nitrogen application rate of 112.08 kg N/ha (At100)

Soil Preparation

Two soil types were selected for their relatively different properties. The United States Department of Agriculture (USDA) defines Olton Clay Loam as clayey eolian soil, with moderate medium granular and subangular blocky structure.

Amarillo Fine Sandy Loam is defined by the USDA as a loamy eolian soil formed from the blackwater draw formation of the Pleistocene era, with a weak fine granular structure and is very friable.

Amarillo Fine Sandy Loam and Olton Clay Loam bulk samples were collected from two separate locations at the West Texas A&M University (WTAMU) Nance Ranch, based on the NRCS Web Soil Survey map. The soils were taken to the WTAMU greenhouse, where they were sifted and composited in order to provide a uniform substrate. Three soil samples were taken from the prepared soil for later analysis. Sixty pots with a diameter of 15.2 cm (6 in.) were acquired and a length of heavy paper was placed in the bottom of each pot. The paper covered the drain holes to prevent soil loss, while still allowing for excessive water to drain. Half of the pots were filled with one of each of the two soil types, for a total of 30 pots for each soil type. Each pot was filled to a uniform soil depth of 15.2 cm (6 in.).

Planting and Greenhouse Set-Up

The *Verde* variety of hard red spring wheat (*Triticum aestivum* L.) was acquired from the Texas A&M Agrilife Research wheat breeding program in Bushland, Texas. Due to time of year constraints, spring wheat was chosen over winter wheat, despite not being commercially grown on the southern high plains. Spring wheat has a much shorter growing season, requiring only 90 days after emergence to reach maturity, as well as not requiring a vernalization period.

On March 6, 2013, seeds were planted 1.3 cm (½ in) deep in three locations in each pot, with three seeds in each location. After planting, each pot was watered with 300 mL of water. Pots were arranged randomly on greenhouse benches. One week after emergence, pots were thinned until only one plant in each location remaining (three plants in each pot).

Plants were staked using a 0.6 cm (0.25 in.) dowel rod and gardening wire as required to prevent lodging. Thirty-six days after planting, greenbugs were observed on three pots in the Olton Clay Loam soil. The greenbugs were physically removed and the whole greenhouse was treated with an Abamectin insecticide bomb. Over the duration of the experiment, it should be noted that a few plants among various treatments in the Olton Clay Loam soil type died before completing their life cycle. Analysis was performed on the biomass those plants produced before dying.

Watering Schedule

Pots were regularly watered once every 48 hours. After the initial watering of 300 mL, water amounts were reduced to 150 mL per application. Reverse osmosis water was used throughout the study. After each watering, the pots were re-randomized with respect to location on the greenhouse bench in order to homogenize variation due to position on the bench. Pots were watered one day prior to treatment application, followed by a 10-day drought period to simulate field conditions where rainfall was not received directly following fertilizer application. Watering was stopped five days prior to harvest to allow the pots to dry.

Treatment Application

On March 27, at the onset of tillering, fertilizer treatments were applied. The fertilizer treatments were prepared in WTAMU's Commercial Core Lab by filling three containers with 454 g of a commercial urea fertilizer. One container had 1.87 mL N-Yield added (Treatments 5, 6 and 7). Another container (Treatments 8, 9 and 10) had

1.87 mL Agrotain added giving the equivalent of 4 qts/ton of urea. The third container (Treatments 2, 3 and 4) was left untreated. Treated or untreated urea was weighed into vials of the correct amount for each pot. The 112.08 kg/ha treatments received 818 mg of fertilizer per pot. The 86.04 kg/ha treatments received 613.5 mg of fertilizer, and the 56.04 kg/ha treatments received 409 mg of fertilizer. The control received no fertilizer.

Treatments were applied to pots in each soil type randomly, as selected using a random number generator in Microsoft Excel. Urea was surface applied. At the time of treatment, pots were labeled for the treatment they received (Figure 2.1).



Figure 2.1 Olton Clay Loam wheat pots immediately after treatment application.

Harvest

Harvest occurred 90 days after planting the wheat. Pots were arranged by order of soil type and then by treatment. Every plant in each pot was clipped at the soil surface and placed in an individually labeled paper bag. Samples were then placed in a Shel Lab FX28-2 forced air drying oven at 50°C until they maintained a stable weight. At the completion of the experiment, soil samples were collected from each pot and composited by treatment and soil type for later analysis. Soil samples were analyzed for nitrate-N (Cd reduction method), phosphorous (Mehlich 3), and potassium (ammonium acetate). A Total N and total kjeldhal nitrogen (TKN) analysis was also performed.

Above Ground Biomass and Protein Analysis

Wheat samples were analyzed in the Commercial Core Lab at WTAMU. Bags were removed from the drying oven ten at a time to avoid the possibility of absorbing moisture from the air. Sample dry weights were measured using a Mettler Toledo Classic Plus balance (Mettler-Toledo LLC., Columbus, OH). Samples were ground using a Cyclone Sample Mill (Udy Corp., Ft. Collins, CO) and analyzed for protein content using a Foss XDS Near-Infrared Rapid Content Analyzer (Foss, Eden Prairie, MN). Results for the protein analysis were based on a Dairy One wheat protein calibration. A moisture adjusted crude protein percentage was collected. Soil samples were analyzed for nitrogen content at Servi-Tech Laboratories in Amarillo, Texas.

Statistical Analysis

Data analysis was performed in SAS (v9.3 SAS Institute, Inc., Cary, NC), on total above ground biomass as well as grain yield. Means separation was calculated using the LSD (least significant difference) procedure. A statistically significant difference was determined at $p \leq 0.10$.

RESULTS AND DISCUSSION

Soil Properties

A nutrient analysis was conducted on soil samples collected from each soil type prior to planting wheat (Table 2.1, Table 2.2). The analysis showed the Olton Clay Loam to have more organic matter than the Amarillo Fine Sandy Loam. Olton Clay Loam averaged 2.16% organic matter while Amarillo Fine Sandy Loam averaged 0.46%. The nitrate-N was also considerably higher in the Olton Clay Loam soil type, averaging 15.7 ppm. The Amarillo Fine Sandy Loam averaged 7.3 ppm.

Table 2.1 Results of nutrient analysis on the Olton Clay Loam soil collected prior to planting of the wheat.								
Sample Name	Organic Matter %	Nitrate-N ppm	CEC	pH	Ca ppm	P ppm	Mg ppm	K ppm
1	2.13	18.0	15.7	6.58	2153	53	330	609
2	2.10	16.0	15.9	6.65	2155	50	337	620
3	2.25	13.0	15.7	6.63	2138	51	335	623
Mean	2.16	15.7	15.7	6.62	1248	51	334	617
Standard Deviation	0.08	3	0.1	0.04	9	2	4	7

Table 2.2 Results of nutrient analysis on the Amarillo Fine Sandy Loam soil prior to planting of the wheat.								
Sample Name	Organic Matter %	Nitrate-N ppm	CEC	pH	Ca ppm	P ppm	Mg ppm	K ppm
1	0.26	10.0	10.5	7.75	1345	18	282	579
2	0.61	7.0	10.8	7.89	1396	16	276	616
3	0.52	5.0	11.7	7.82	1519	18	302	625
Mean	0.46	7.3	11.0	7.82	1420	17	286	606
Standard Deviation	0.15	2	0.5	0.06	73	1	11	20

The Olton Clay Loam soil type had a higher nutrient content at the start of the study when compared to the Amarillo Fine Sandy Loam. It had a higher organic matter,

nitrate nitrogen content, calcium, phosphorous and magnesium contents. Additionally Oldham Clay Loam had a more ideal pH and cation exchange capacity.

The results of the soil analysis performed on the samples taken after harvest for the Olton Clay Loam soil type are presented in table 2.3, and for the Amarillo Fine Sandy Loam in table 2.4.

Table 2.3 Results of nutrient analysis performed on Olton Clay Loam soil after harvest of the wheat.					
Sample Name	Total N ppm	TKN ppm	Nitrate-Nitrogen ppm	Phosphorous ppm	Potassium ppm
Nf	978	973	5	54	645
U50	1068	1012	56	52	580
U75	1060	995	65	51	631
U100	1036	968	68	53	646
Ny50	1126	1075	51	51	631
Ny75	1282	1216	66	50	593
Ny100	1175	1069	106	53	615
At50	1112	1052	60	46	622
At75	1284	1218	66	45	606
At100	1273	1199	74	49	632
Mean	1139	1078	62	50	620
Standard Deviation	110	99	25	3	22

Table 2.4 Results of nutrient analysis performed on Amarillo Fine Sandy Loam soil after harvest of the wheat.					
Sample Name	Total N ppm	TKN ppm	Nitrate-Nitrogen ppm	Phosphorous ppm	Potassium ppm
Nf	523	520	3	19	486
U50	449	526	23	14	435
U75	963	790	173	16	554
U100	564	536	28	14	460
Ny50	453	432	21	15	441
Ny75	484	432	52	15	443
Ny100	499	446	53	16	433
At50	396	376	20	16	424
At75	2019	1956	63	20	464
At100	588	524	64	16	440
Mean	694	654	50	16	458
Standard Deviation	492	471	48	2	38

The Olton Clay Loam soil type showed a higher nitrate-N content at the conclusion of the study in all treatments except for no fertilizer. The pots that did not receive any fertilizer had a very low (3 ppm) ending nitrate-N test. A total nitrogen analysis was not performed at the start of the study, so variation from pre-planting to after harvest is unknown. The potassium and phosphorous content varied little from before to after the study in the Olton Clay Loam soil. The Amarillo Fine Sandy Loam soil saw a reduction in both potassium and phosphorous over the duration of the study.

The Amarillo Fine Sandy Loam soil type exhibited very similar changes as the Olton Clay Loam. The nitrate-N levels increased during the study in all but the no fertilizer treatment. An outlier was observed in the At75 treatment total nitrogen. The reason for this unusually high number is unknown. The Amarillo Fine Sandy Loam soil

type also saw a decline in the level of potassium. There was little change in the concentrations of other nutrients.

Above Ground biomass

The two soil types were analyzed separately. A p value of $p=0.056$ was observed for the Olton Clay Loam soil above ground biomass results (Table 2.5). The above ground biomass results for the Amarillo Fine Sandy Loam soil type yielded a p value of $p=0.161$ (table 2.6).

Table 2.5 Above ground biomass ANOVA table for the Olton Clay Loam soil type.					
Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	9	6.769	0.752	2.32	0.0561
Error	20	6.482	0.324		
Total	29	13.251			

Table 2.6 Above ground biomass ANOVA table for the Amarillo Fine Sandy Loam soil type.					
Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	9	3.611	0.401	1.67	0.161
Error	20	4.795	0.239		
Total	29	8.406			

At a significance of 0.10, a difference was found in above ground biomass among treatments in the Olton Clay Loam soil type. The Amarillo Fine Sandy Loam soil type showed no statistical difference among treatments in the above ground biomass analysis.

The reason a difference was observed in one soil type and not the other is likely due to the different soil properties. The Olton Clay Loam soil type had higher rates of all nutrients tested. It is possible that nitrogen wasn't the limiting nutrient that affected the growth of wheat plants in the Amarillo Fine Sandy Loam soil.

The results for the LSD means separation test for above ground biomass for the Olton Clay Loam soil type are presented below (table 2.7). LSD was not run for the Amarillo Fine Sandy Loam soil as there was no overall difference among the means for the above ground biomass. Means for the treatments are presented in table 2.8.

Table 2.7 Results of the means separation test for above ground biomass of the Olton Clay Loam soil type wheat study.	
Treatment	Mean (g)
At75	2.68 A
At50	2.49 AB
At100	2.28 ABC
U75	1.82 ABC
U50	1.66 BC
Ny50	1.55 BC
Nf	1.53 BC
Ny100	1.39 C
Ny75	1.34 C
U100	1.31

Means with a different T grouping letter differ at $p \leq 0.10$.

Means were not as expected based on fertilizer rate. The Nf treatment was expected to have a significantly lower mean than any other treatment. The treatments that

were applied at the highest application rates (U100, Ny100, and At100) should have shown the highest above ground biomass. This suggests that something other than nitrogen fertilizer type and rate was the main factor affecting the growth of the plants in this greenhouse study. Some of this variation could be explained by the early death of three plants in the Ny75 treatment.

Table 2.8 Treatment means for the above ground biomass for the Amarillo Fine Sandy Loam soil.	
Treatment	Mean (g)
Ny50	2.78
At75	2.62
Ny75	2.60
U50	2.55
U75	2.23
U100	2.10
At100	2.07
At50	2.06
Ny100	1.91
Nf	1.65

Similarly, for the Amarillo Fine Sandy Loam soil type, there appears to be no correlation between amount or type of fertilizer that the pots received and the weight of the above ground biomass.

Protein Content Analysis

The two soil types were analyzed separately. The null hypothesis was rejected when the p value was $p \leq 0.10$. The ANOVA table for the Olton Clay Loam soil type can

be seen in table 2.9. The ANOVA for the Amarillo Fine Sandy Loam soil is presented in table 2.10.

Table 2.9 Results of the ANOVA for protein content in the Olton Clay Loam soil type.					
Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	9	31.330	3.481	2.05	0.087
Error	20	33.978	1.699		
Total	29	65.309			

Table 2.10 Results of the ANOVA for protein content in the Amarillo Fine Sandy Loam soil type.					
Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	9	90.682	10.076	50.63	<0.001
Error	20	3.980	0.199		
Total	29	94.663			

A significant difference ($p=0.087$) was found in the Olton Clay Loam soil type, based on the ANOVA analysis for protein. The effects of fertilizer type were significant for grain protein content in the Amarillo Fine Sandy Loam soil type ($p<0.001$), based on the ANOVA.

LSD means separation was performed. It was important to see where the differences lay. Several significant groupings were found based on the LSD procedure for the Amarillo Fine Sandy Loam soil type (table 2.11), and for the Olton Clay Loam soil type (table 2.12).

Table 2.11 Results of the means separation test for protein content in the Amarillo Fine Sandy Loam soil type.	
Treatment	Mean (%)
Ny100	12.64 A
At100	12.45 A
At75	12.27 AB
Ny75	11.68 BC
Ny50	11.16 CD
At50	11.14 CD
U75	10.64 DE
U50	10.45 E
U100	10.32 E
Nf	6.26 F

Means with a different T grouping letter differ at $p \leq 0.10$.

The means separations for the Olton Clay Loam soil type are more similar to the expected. The fertilizer treatments that contained a urease inhibitor (Ny, At) were significantly higher than the urea only treatments (U100, U75, U50). Furthermore, the no fertilizer treatment had the lowest mean protein percentage and was statistically lower than any fertilized treatment.

Table 2.12 Results of the means separation test for protein content of the Olton Clay Loam soil type.	
Treatment	Mean (%)
Ny100	14.10 A
At75	13.78 AB
U50	13.45 ABC
At100	12.39 ABC
Ny50	12.34 ABC
At50	12.31 ABC
U100	12.03 BCD
U75	11.90 CD
Ny75	11.87 CD
Nf	10.42 D

Means with a different T grouping letter differ at $p \leq 0.10$.

The highest protein content was found in the Ny100 treatment and the Nf control had the lowest average protein content. Much like the Olton Clay Loam means separation for above ground biomass, the means are not grouped based on fertilizer rate. It was expected the U100, Ny100, and At100 would perform better than their respective 84.1 kg/ha treatment (U75, Ny75, At75), which should have yielded better than their respective 56.0 kg/ha treatment (U40, Ny50, At50). This suggests there were other factors affecting the protein results beside fertilizer treatment. The soil properties made the greenhouse study difficult. Additionally, temperatures in the greenhouse warmed above the ideal conditions for spring wheat.

SUMMARY AND CONCLUSIONS

The objectives of this study (Chapter II) were to evaluate the effect urea fertilizer coated with the urease inhibitor NBPT in a DMSO/PG solution (N-Yield) had on the above ground biomass and grain protein content of spring wheat. Additionally we wanted to evaluate how N-Yield performed compared to an existing urease inhibitor product.

Based on the ANOVA procedure, a significant difference among the treatment means was found in the above ground biomass for the Olton Clay Loam soil type. No significant difference was found for the Amarillo Fine Sandy Loam above ground biomass. A significant difference between treatment means in protein content was found for both the Olton Clay Loam and Amarillo Fine Sandy Loam soil types. Although the highest mean came from the Ny100 treatment and the lowest was the Nf treatment, no pattern was found in the means separation analysis between the means of treated vs. non treated urea at the same nitrogen application rate.

CHAPTER III

RICE RESPONSE TO THE UREASE INHIBITOR N(N-BUTYL THIOPHOSPHORIC TRIAMIDE) IN A DIMETHYL SULFOXIDE/PROPYLENE GLYCOL SOLUTION

INTRODUCTION AND RESEARCH OBJECTIVES

Effects of Urease Inhibitors on Rice

There is much potential for urease inhibitors in rice. Rice demands large amounts of nitrogen (Choudhury and Kennedy, 2005). The National Agriculture Statistic Service (NASS) reported that in 2013, 97% of United States rice acreage received nitrogen fertilizer, at an average of 195 kg N/ha. Due to the aquatic nature of the crop, and that it's often grown in hard to access areas, urea granules are the most convenient form of nitrogen fertilizer (Gilbert et al., 2006). Fillery and Vlek (1986) found that urea broadcasted onto flooded rice could see up to 50% nitrogen loss from ammonia volatilization. These losses occur when the fertilizer is top-dressed onto a young crop and poorly incorporated. Several studies have been performed to evaluate the effect of NBPT urease inhibitors in rice. One study found that flooded rice fields treated with NBPT lost only one third the amount of ammonia compared to untreated fields, 5.4% instead of 15% (Phongpan et al. 1995). A greenhouse study conducted by Byrnes et al. (1988) found an increase in grain yield and straw when NBPT was applied with urea to flooded rice. Freney et al. (1995) found a reduction in ammonia losses when applying fertilizer treated with NBPT. Buresh et al. (1988) reported a significant ($p<0.05$) increase in grain yield

when urea amended with NBPT was applied 10 days after rice transplant, and five to 10 days after panical initiation, in a silty soil. Conversely, they observed no benefit in a clay soil. There was no increase in grain protein for either soil type.

Objectives

To date, no published studies have been conducted on the effect of N-Yield on lowland rice. The objectives of this research were as follows:

1. Evaluate the effect of urea fertilizer coated with NBPT in a DMSO/PG solution (N-Yield) on the above ground biomass and grain weight of lowland rice.
2. Compare the effects of N-Yield to Agrotain Ultra.

MATERIALS AND METHODS

Experimental Design

This greenhouse study consisted of a completely randomized design (CRD) with seven treatments. The independent variable was fertilizer type. Dependent variables consisted of above ground biomass, grain yield and grain protein content. Bucket was the experimental unit. This gave a total of 28 buckets (seven treatments, four replications per treatment).

The seven treatments were as follows:

- Treatment 1 – No fertilizer (Nf)
- Treatment 2 – Untreated urea, applied once at a nitrogen rate of 22.41 kg/ha (20 lb/acre) (U20)
- Treatment 3 – Untreated urea, applied twice at a nitrogen rate of 22.41 kg N/ha per application (total 40 lb N/acre) (U40)

- Treatment 4 – Urea treated with N-Yield, applied once at a nitrogen rate of 22.41 kg N/ha (Ny20)
- Treatment 5 – Urea treated with N-Yield (4.17 mL/kg), applied twice at a nitrogen rate of 22.41 kg N/ha per application (total 40 lb N/acre) (Ny40)
- Treatment 6 – Urea treated with Agrotain Ultra (4.17 mL/kg), applied once at a nitrogen rate of 22.41 kg N/ha (At20)
- Treatment 7 – Urea treated with Agrotain Ultra (4.17 mL/kg), applied twice at a nitrogen rate of 22.41 kg N/ha per application (total 40 lb N/acre) (At40)

Soil Preparation and Watering

Olton Clay Loam soil, a thermic aridic paleustoll, was used for this experiment. Soil was collected from the West Texas A&M University (WTAMU) Nance Ranch from an area delineated by the NRCS Web Soil Survey. The soil was taken to the WTAMU greenhouse where it was sifted and thoroughly mixed for uniformity. Three soil samples were taken for lab analysis. Twenty eight, 18.9 L (5 gal) buckets were filled with 15.2 cm (6 in.) of the prepared soil mixture.

The buckets were flooded to simulate typical conditions used for rice cultivation. Reverse osmosis water was added to the buckets in increments, until 5.1 cm (2 in.) of water remained above the soil surface (Figure 3.1). The 5.1 cm flooding was maintained for the duration of the experiment, water levels were adjusted twice a week. After each water level adjustment, the pots were re-randomized with respect to location on the greenhouse bench in order to minimize variation due to position on the bench. One week before harvest, watering was stopped to allow the buckets to dry.



Figure 3.1 Photograph of rice buckets after the addition of soil and water, and prior to planting the seedlings.

Planting and Greenhouse Set Up

Clearfield Cl 111 variety rice (*Oryza sativa*), was acquired from Dr. W. Ray McClain of the Rice Research Station (Louisiana State University Agricultural Center, Rayne, LA). On May 7, seeds were broadcast planted into a seed tray filled with a commercial seed starter potting mix. The seed tray was watered to field capacity and placed under a grow light for germination.

On May 25, five healthy seedlings were selected from the seed starter tray and planted in each bucket, arranged in a star pattern, with approximately 20 cm between seedlings. Buckets were placed on the greenhouse bench and equally spaced to receive similar amounts of sunlight (Figure 3.2).



Figure 3.2 Photograph showing how rice buckets were arranged on the greenhouse bench.

The greenhouse temperature was set to 30°C during the daytime (12 hrs) and 24°C at night (12hrs), to simulate the environment in which rice is grown.

On July 22, evidence of spider mite damage was observed on many plants, necessitating pesticide application. On July 24, all rice plants were sprayed with a 0.31 mL/L (4 fl. oz/100 gal) mixture of an Abamectin product.

Treatment Applications

On June 17, at the onset of tillering, initial nitrogen treatments were applied to the buckets. The treated urea was prepared by filling three containers with 227g of urea granules. One container was treated with 0.935 mL of N-Yield, the equivalent of four qts/ton of urea (Treatments 4 and 5). Another container was treated with 0.935 mL of Agrotain (Treatments 6 and 7), and the last container was left untreated (Treatments 2 and 3). Urea (272.48 mg) was measured from each container and placed in an individually labeled vial. This was repeated four times, once for each replication. Urea from each vial was applied to the rice buckets. Treatments were applied to the rice buckets in a random order, as determined using a random number generator in Microsoft Excel. Buckets were labeled with treatment at time of application. Buckets remained flooded during treatment application.

Treatments requiring a second application of fertilizer received those on August 5th, when the rice plants were in the panicle elongation stage. Treatments were applied at the same rate of 272.48 mg per bucket, giving those buckets the equivalent nitrogen of 44.83 kg/hectare (40 lb nitrogen/acre).

Harvest

Plants were harvested on October 1. Individual plants were cut at the soil surface and placed in a labeled paper bag. Bags were placed in a Shel-Lab model FX28-2 drying

oven at 50°C for 96 hours. Three soil samples were taken from each bucket and composited by treatment for analysis at Servi-Tech Laboratories in Amarillo, Texas.

Forage and Grain Analysis

Samples were removed 10 at a time from the drying oven and total dry weight of the above ground biomass was determined using a Mettler Toledo Classic Plus balance (Mettler-Toledo LLC, Columbus, OH). Samples were then placed in plastic bags to avoid exposure to moisture. After above ground biomass was recorded, the grain was separated from the leaves and stems. An Almaco seed cleaner (ALMACO, Nevada, IA) was used to separate the grain from the hulls. The number of seeds was recorded using a Key-Mat seed counter. Grain weights were recorded using an Ohaus Navigator XT balance (OHAUS Corporation, Parsippany, OR). After dry weights were collected, all samples from each treatment were composited and sent to Servi-Tech Laboratories in Amarillo, Texas for grain protein analysis. The soil was analyzed to determine levels of nitrate-N, plant available phosphorous (mehlich 3 method), and plant available potassium (ammonium acetate method). A total N and total kjeldhal nitrogen (TKN) analysis was also performed.

Statistical Analysis

Data analysis was performed in SAS (v9.3 SAS Institute, Inc., Cary, NC) using the GLM procedure. Analysis was performed on total above ground biomass, as well as grain yield. Means separation analysis was performed using the LSD procedure. A statistically significant difference was determined to be $p \leq 0.10$.

RESULTS AND DISCUSSION

Soil Properties

The results of the soil analysis performed on samples from before planting can be seen in table 3.1.

Table 3.1 Results of nutrient analysis on soil collected prior to planting of the rice.					
Sample Name	Total N ppm	TKN ppm	Nitrate-Nitrogen ppm	Phosphorous ppm	Potassium ppm
1	1317	1247	70	82	827
2	1545	1476	69	83	823
3	1624	1555	69	83	854
Mean	1495	1426	69	83	835
Standard Deviation	159	160	0.5	0.5	17

Soil samples collected at the completion of the experiment were also analyzed for total N, TKN, nitrate-nitrogen, phosphorous and potassium (Table 3.2).

Table 3.2 Results of the nutrient analysis performed on soil collected at the completion of the experiment, after the rice had been harvested.					
Sample Name	Total N ppm	TKN ppm	Nitrate-Nitrogen ppm	Phosphorous ppm	Potassium ppm
Nf	1245	1180	65	57	546
U20	1162	1100	62	55	518
U40	1204	1187	17	59	480
Ny20	1009	1006	3	61	439
Ny40	1092	1021	71	47	475
At20	1261	1192	75	54	507
At40	1300	1240	60	53	541
Mean	1181	1132	50	55	501
Standard Deviation	102	91	28	5	39

A reduction in nitrogen, phosphorous and potassium was observed from the soil samples taken prior to planting to the samples taken after harvest. The no fertilizer control saw little reduction in nitrogen levels. Without having any added nitrogen, it was expected to have the highest demand on the existing nitrogen in the soil. The At20 and Ny40 treatments both ended with more nitrogen in the soil than at the beginning of the experiment. The ending soil analysis showed that one treatment, Ny20 ended with only 3 ppm nitrate-nitrogen while the other, At20, ended with 75 ppm nitrate nitrogen. These treatments were expected to be more similar.

Grain Weight Analysis

The GLM procedure in SAS (v9.3 SAS Institute, Inc., Cary, NC) was used to analyze the data. A significant difference was found ($p=0.065$) among the treatments in the grain weight of the rice (Table 3.3).

Table 3.3 Results of the ANOVA for total grain weight per plot of the rice study.					
Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	6	792.842	132.140	2.380	0.065
Error	21	1166.500	55.547		
Total	27	1959.343			

Because the p value showed a relationship between the treatment and grain weight, the LSD procedure for means separation was performed to determine where the differences existed (Table 3.4).

Table 3.4 Results of the means separation test for grain weight of the rice study.	
Treatment	Mean (g)
At40	32.63 A
Ny40	29.96 AB
At20	24.66 AB
U40	22.88 BC
Nf	22.51 BC
Ny20	21.67 BC
U20	15.10 C

Means with a different letter differ at $p \leq 0.10$.

Several significant groupings were found using the LSD means separation test. The At40, Ny40, and At20 treatments yielded the highest grain weight. The U40, Nf, Ny20, and U20 treatments returned the lowest grain weight. With the exception of the Ny20 treatment, all of the treatments that included a urease inhibitor were in the grouping

with the highest mean grain weight. This suggests that the addition of a urease inhibitor increases the grain weight of lowland rice. Additionally, there was no statistical difference between the performance of Agrotain Ultra and N-yield at the highest application rate (two treatments of 22.41 kg N/ha).

Above Ground Biomass

When tested using the GLM procedure in SAS (v9.3 SAS Institute, Inc., Cary, NC), the model of above ground biomass = treatment, yielded a p value of $p=0.099$ (Table 3.5).

Table 3.5 Results of the ANOVA for above ground biomass of the rice study.					
Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	6	1935.928	322.655	2.080	0.099
Error	21	3256.750	155.083		
Total	27	5192.679			

At a significant level of 0.10, differences were found between the treatments. A means separation test was performed to determine where those differences lie (Table 3.6).

Table 3.6 Results of the means separation test for above ground biomass in the rice study.	
Treatment	Mean (g)
Ny40	131.25 A
U40	130.75 A
Ny20	130.50 A
U20	123.75 A
At20	122.00 A
At40	121.25 A
Nf	105.75 B

Means with a different T grouping letter differ at $p \leq 0.10$.

The Ny40 produced the highest mean biomass and the Nf treatment yielded the lowest. None of the fertilized treatments' means were significantly different from each other. The Nf treatment was the only treatment with a significantly lower above ground biomass. This suggests that the application of the urea was the cause for increased biomass production in the rice plants, and that the addition of a urease inhibitor did not add any plant growth. This could possibly be attributed to the fact it was a greenhouse experiment. Urease inhibitors perform better when conditions are more suitable for ammonia volatilization than what was simulated in the greenhouse for the duration of the experiment.

Grain Protein Analysis

The grain was composited by treatment and analyzed by Servi-Tech Laboratories for crude protein content. Results for the dry matter crude protein are presented in figure

3.3. Statistical analysis was not performed on this data as grain was consolidated by treatment before lab analysis.

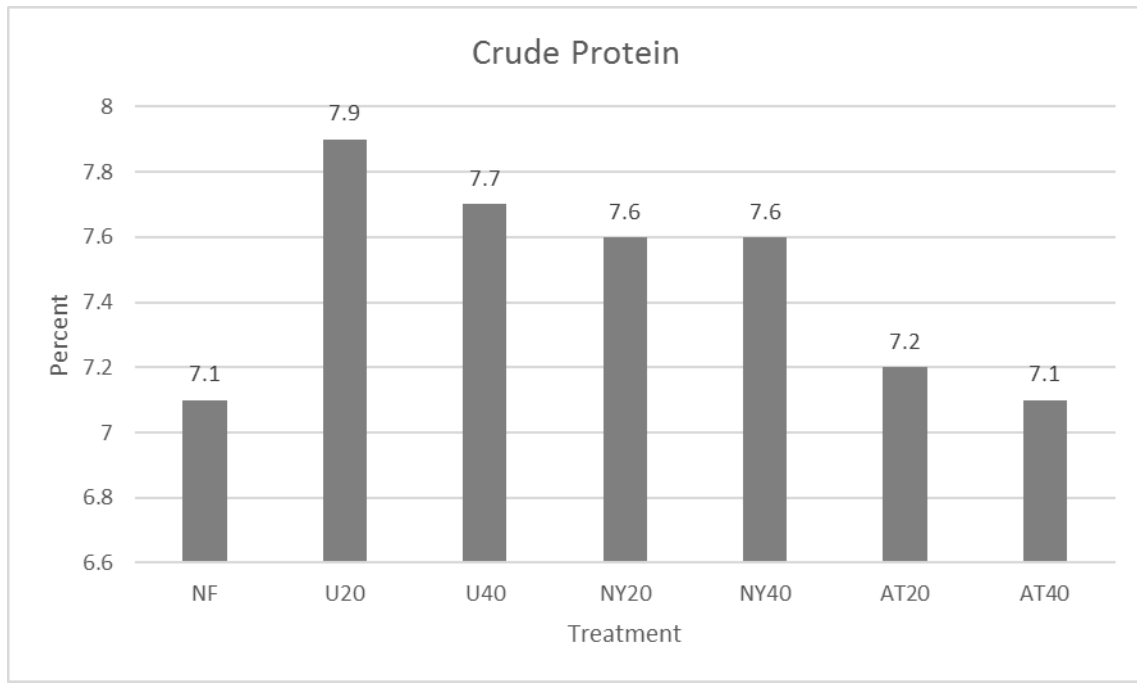


Figure 3.3 Rice grain crude protein analysis.

The Nf treatment had a lower crude protein content than the fertilized treatments. The urea only treatment tested as having the highest protein, with the U20 treatment yielding 7.9%, and the U40 treatment yielding 7.7% crude protein. The Agrotain coated urea only performed slightly better than no fertilizer. The At20 treatment of Agrotain treatment yielded 7.2% crude protein while the At40 treatment failed to perform any better than the Nf control, both testing at 7.1% crude protein. According to the USDA, the average crude protein content of rice is 7.1% (USDA ARS, 2014). In a study where nitrogen was the limiting nutrient, it was expected that Agrotain and N-Yield treatments

would both test higher for crude protein than the urea only treatments at the same application rate. More research needs to be performed on effect that NBPT has on the crude protein content in the grain of rice, in a way that can be statistically analyzed.

SUMMARY AND CONCLUSIONS

The objectives of this study were to evaluate the effect urea fertilizer coated with the urease inhibitor NBPT in a DMSO/PG solution (N-Yield) had on the above ground biomass, grain yield, and grain protein content of lowland rice. An additional objective was to evaluate how N-Yield compared to a similar urease inhibitor product.

Based on the ANOVA procedure, there was a significant difference between the treatment means of the grain weight and above ground biomass of lowland rice. The results of the means separation test were not entirely as expected. Means for the grain weight did show the Ny40 and At40 treatments were not statistically different from each other, and were in the grouping with the highest mean grain weight. The means for above ground biomass suggest that the addition of the urea influenced the biomass, and that the treatments with a urease inhibitor had no added benefit.

Based on these results, it can be concluded that the applying urea, with or without a urease inhibitor, had some effect on the grain weight and above ground biomass of lowland rice. Additionally, N-yield and Agrotain performed similarly to each other.

CHAPTER IV

CORN RESPONSE TO THE UREASE INHIBITOR N-(N-BUTYL THIOPHOSPHORIC TRIAMIDE) IN A DIMETHYL SULFOXIDE/PROPYLENE GLYCOL SOLUTION

INTRODUCTION AND RESEARCH OBJECTIVES

Effects of Urease Inhibitors on Corn

Dent corn is a large consumer of nitrogen fertilizer. As stated earlier, 99% of all corn acreage in Texas received some form of nitrogen fertilizer (USDA ERS, 2014). With such a large consumer of nitrogen, increasing the efficiency of applied nitrogen fertilizers should be beneficial. Hendrickson (1992) analyzed corn response to NBPT in 78 trials, from 1984 to 1989. When he averaged all urea application rates and NBPT application rates, he found an average of 269.89 kg/ha increase in grain yields by using NBPT. Additionally, Hendrickson found the yield response to NBPT was greater where crops were able to utilize the nitrogen conserved by NBPT. He found that maximum grain yields could be achieved using an average of 82.94 kg/ha less nitrogen when NBPT was included with the urea.

Schlegal et al. (1986) compared six urease inhibitors to surface applied urea and UAN in both conventional and no-till systems on the grain yield of corn. They found that urea outperformed UAN with or without a urease inhibitor, and urease inhibitors applied with granular urea increased corn yields more often than when applied with UAN. The addition of a urease inhibitor increased the grain yields when ammonia volatilization was

a problem, and in conditions conducive of high ammonia volatilization, NBPT performed as well or better than other urease inhibitors. Schlegal et al. also stated that urease inhibitors were more effective in no-till practices.

Murphy and Ferguson (1997) looked at the addition of NBPT with urea and UAN, over three years and in three soil types, on the yield of ridge-tilled corn. They found highly variable results and concluded that the effect of NBPT depended highly on climatic conditions. In a three year study, Bronson et al (1990) evaluated ear leaf nitrogen, grain nitrogen and grain yield when urea was applied with or without NBPT. In 1987, they found no difference among the treatments, as rainfall three days after fertilizer application reduced the risk of ammonia volatilization. In 1988, they found higher ear leaf nitrogen when urea was applied with NBPT. Higher grain protein and grain yield was observed in 1989, but not in any other year.

Objectives

To date, no published studies have been conducted on the effect of N-Yield on field crops. The objectives of this research were as follows:

1. Evaluate the effect of urea fertilizer coated with NBPT in a DMSO/PG solution (N-Yield) on the average ear weight of dent corn.
2. Compare the effect of N-Yield to Agrotain Ultra.

MATERIALS AND METHODS

Experimental Design

A randomized complete block design (RCBD) was used for this experiment. Blocking was performed with the prevailing southerly wind as the nuisance variable. Fertilizer type was the independent variable. Grain yield and grain protein content were the dependent variables. Four treatments were used, replicated once per each of the three blocks.

Treatments were as follows:

Treatment 1 – No Fertilizer (Nf)

Treatment 2 – Untreated urea applied at a rate of 112.1 kg N /ha (100 lb N/acre) (U)

Treatment 3 – Urea treated with N-Yield applied at a rate of 112.1 kg N/ha (Ny)

Treatment 4 – Urea treated with Agrotain Ultra applied at a rate of 112.1 kg N/ha (At)

Site Preparation

This experiment took place at the West Texas A&M University (WTAMU) Nance Ranch the summer of 2013. A 9.1 m by 24.1 m (30 ft X 80 ft) area of previously fallow ground was selected for the experiment plots. The size of the study area was limited by the amount of irrigation water available at the site. On June 10, the site was worked several times with a disc to provide a proper seedbed (Figure 4.1). Plots were staked and three soil samples were collected from the top 15.2 cm (6 in.) of the soil profile. Samples were composited by plot for later lab analysis.



Figure 4.1 Pre-planting ground preparation for the corn study.

Planting

Channel 216-49VT3P variety dent corn (*Zea mays L.*) was planted on June 10. Seeds were planted 5 cm (2 in.) below the soil surface at a rate of 26,000 plants per acre using a four row planter (Cole Planter Company, Albany Georgia). Rows were spaced 76.2 cm (30 in.) apart, allowing four 6.1 m (20 ft) rows in each plot. The seed corn had a reported 95% germination rate, per the label.

Irrigation

Two lines of surface poly tubing were placed in each plot between the outside and inside rows of plants. Nozzles were placed on the drip tape every 30.5 cm (24 in). Water meters were installed with two water lines sharing a single meter (Figure 4.2). Plots were irrigated twice per week, 2.5 cm (1 in) for each irrigation, or 5.08 cm (2 in) of water per week. A rain gauge was installed near the plots, and irrigation amounts were adjusted for rainfall. On occasions where rainfall exceeded the amount to be irrigated, no water was added. Two weeks before harvest irrigation was stopped to allow the plots to dry.



Figure 4.2 Water meter for the corn plots. Each block had two drop lines attached to a single meter.

The plots were irrigated the day before applying the nitrogen treatments and then allowed a ten day drought period to simulate conditions where irrigation or rainfall failed to dissolve the urea granules. This maximized the potential for ammonia volatilization and increased the likelihood of observing a difference among treatments.

Treatment Application

The nitrogen fertilizer treatments were prepared at the WTAMU Commercial Core Lab. Urea was coated with N-Yield or Agrotain at a rate of 4.17 mL/kg of urea (4 qts/SI ton). Commercial urea granules are 46% nitrogen. In order to apply 112.1 kg N/ha, 243.56 kg urea was applied per hectare. On a 0.002 ha (200 sq ft) plot, this translated to 1.12 kg (0.996 lb) of urea per plot.

Fertilizer treatments were surface applied using a broadcast spreader to each plot on July 10, when the corn plants were in the V6 stage, having six fully grown leaves with a visible collar. Treatments were applied randomly to each plot within a block. A random number generator in Microsoft Excel was used to designate which plot received which treatment (Figure 4.3). Plywood barriers were temporarily erected around each plot as it was being treated to prevent fertilizer drift into neighboring plots (Figure 4.4).

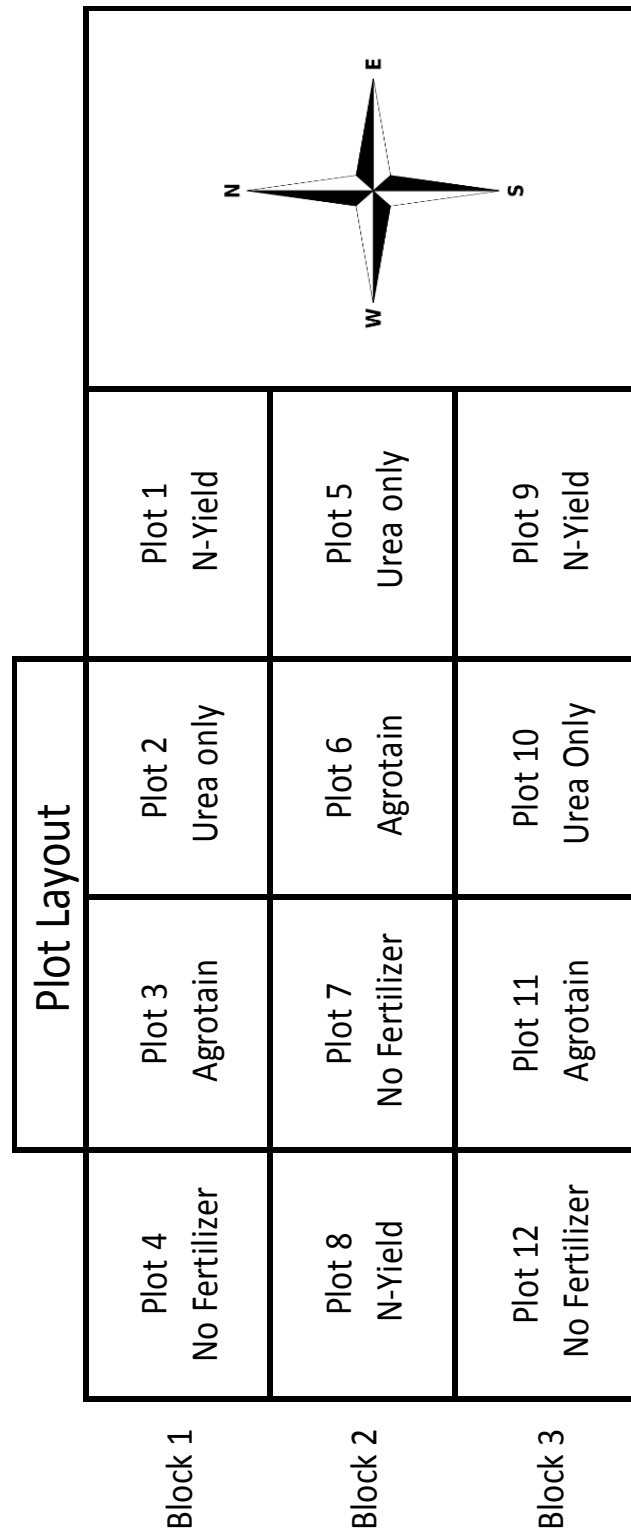


Figure 4.3 Field layout for the corn experiment.



Figure 4.4 Applying nitrogen fertilizer treatment to a corn plot using a handheld fertilizer spreader. A handheld plywood sheet was used on both sides of each plot to prevent drift.

Observations

Field bind weed and native grasses in the plot area became a problem. On July 1, the plots were sprayed with both 2-4-D and Roundup using a 3.8 liter (1 gallon) handheld sprayer. The herbicide was mixed using the recommendations on the label. The plots were walked and weeds were spot sprayed close to the ground to best avoid drift onto the corn plants. Weeds were subsequently managed by hoeing.

On July 14, four days after urea application, it was observed that corn plants that received nitrogen fertilizer were noticeably darker and greener. Many plots had plants with some degree of browning on the leaf edges (Figure 4.5), probably from nitrogen burn. The plots experienced mild hail and wind damage from a rain storm on July 24.



Figure 4.5 Nitrogen burn on a corn plant four days following treatment application.

Corn Ear Harvest

Plants were harvested on October 10. Ten plants were selected from the middle ten feet of each of the inside two rows in each plot, for a total of 20 plants per plot. To avoid bias, each plot was measured in 1.5 m (5 ft) from the outside edge and the next 10

plants were selected for harvest (Figure 4.6). Three plants in plot four (Nf) were skipped due to early lodging from the July 24 storm. One plant in plot five (U) was skipped due to incomplete growth, having died before ears were mature. Each plant was cut at ground level then placed into a burlap sack for transport to the lab. Ears were removed from the stalk and husked. Three soil samples were collected from the top 15.2 cm (6 in.) of soil in each plot and composited for later lab analysis.



Figure 4.6 Preparing a burlap sack for a corn plant during above ground biomass harvest.

Corn Grain Analysis

Ears were placed in a Shel-Lab model FX28-2 (Sheldon Manufacturing, Cornelius OR) drying oven at 50°C for 144 hours, until constant weight was observed. Ears were removed from the drying oven 10 at a time to reduce the chance of absorbing

moisture from the air. The number of rows and columns of kernels on each ear was recorded. Kernels were then removed from the ears and dry weights of the cob and kernel were collected using an Ohaus Navigator XT balance (OHAUS Corporation, Parsippany, NJ). After dry kernel weights were recorded, samples were composited by treatment and one sample from each treatment was sent to Servi-Tech Laboratories in Amarillo, Texas for protein analysis. Funding constraints prevented us from analyzing each sample individually. Soil samples were also sent to Servi-Tech for nitrogen analysis. The soil was analyzed for nitrate-nitrogen, phosphorous, and potassium. A total nitrogen (N) and total kedjal nitrogen (TKN) analysis was also performed.

Statistical Analysis

Data analysis was performed in SAS using the MIXED procedure. Analysis was performed on the average ear weight per plot. In order to see where differences between means occurred, means separation was calculated using the LSmeans procedure. A statistically significant difference was determined to be $p \leq 0.05$

RESULTS AND DISCUSSION

Soil Properties

Results of the analysis on the soil samples taken prior to planting of the corn can be seen in table 4.1. Only slight variations occurred between the mean and each plot.

Table 4.1 Results of nutrient analysis on soil collected prior to planting of the corn.						
Plot Number	Block	Total N ppm	TKN ppm	Nitrate-Nitrogen ppm	Phosphorous ppm	Potassium ppm
Ny	1	863	828	35	76	481
U	1	711	671	40	78	448
At	1	709	672	37	69	385
Nf	1	832	781	51	80	432
U	2	730	665	65	77	462
At	2	668	621	47	69	390
Nf	2	638	601	37	65	346
Ny	2	747	686	61	66	408
Ny	3	743	666	77	64	380
U	3	761	709	52	71	398
At	3	738	670	68	79	412
Nf	3	781	727	54	69	386
Mean	na	743	691	52	72	411
Standard Deviation	na	59	60	13	6	37

Soil samples collected at the completion of the experiment were also analyzed for total N, TKN, nitrate-nitrogen, phosphorous, and potassium (Table 4.2).

Table 4.2 Results of nutrient analysis on soil collected at the completion of the experiment, after the corn had been harvested.						
Plot Number	Block	Total N ppm	TKN ppm	Nitrate-Nitrogen ppm	Phosphorous ppm	Potassium ppm
Ny	1	893	822	71	81	434
U	1	875	814	61	76	394
At	1	798	768	30	72	395
Nf	1	771	747	24	92	474
U	2	811	767	44	78	468
At	2	886	830	56	84	498
Nf	2	819	814	5	83	456
Ny	2	839	805	34	81	442
Ny	3	963	923	40	75	451
U	3	1062	993	69	75	347
At	3	963	876	87	77	424
Nf	3	761	731	30	80	472
Mean	na	876	824	46	80	438
Standard Deviation	na	86	72	22	5	41

The means nitrate-nitrogen was depleted over the course of the study. Total nitrogen, TKN nitrogen, phosphorous, and potassium all increased over the duration of the study. The Nf treatments, especially in block 2 showed the largest decrease in nitrate-nitrogen. This indicates that they had the largest demand on the existing nitrogen in the soil.

Grain Weight Analysis

The average grain weight per ear was analyzed using the MIXED procedure in SAS (v9.3 SAS Institute, Inc., Cary, NC). A significant difference between treatment was observed for ear weight ($p=0.008$) using the type 3 test of fixed effects.

The N-Yield treatment had the highest average grain weight per ear, with a mean of 117.33 g (std. dev. 17.65). The lowest grain weight was from the no fertilizer control at 66.74 g (std. dev. 21.23). Intermediate grain weight values were found for both the urea only and Agrotain treatments measuring 84.45 g (std. dev. 30.66) and 86.83 g (std. dev. 26.05) of grain, respectively (Figure 4.7). Figure 4.8 shows the treatment means by block.

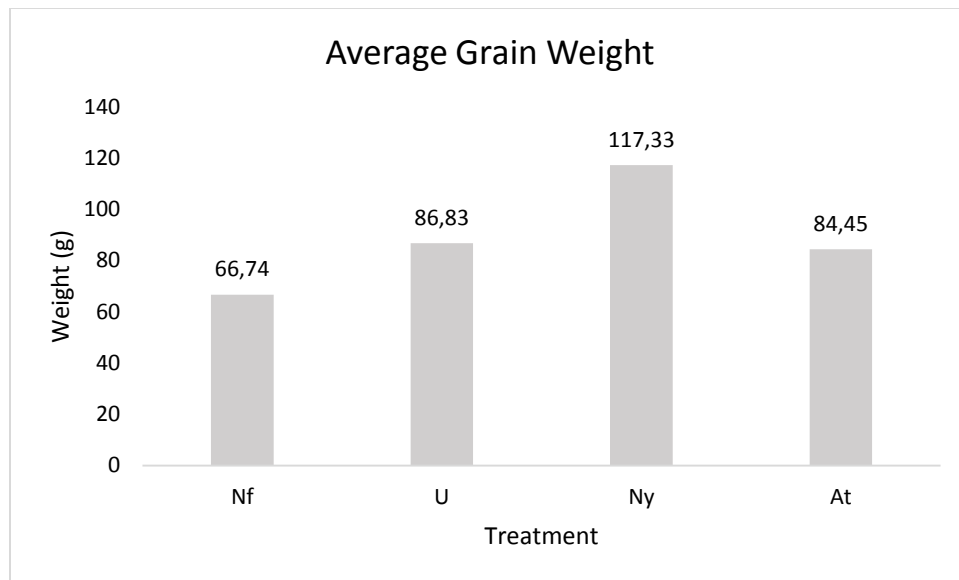


Figure 4.7 Treatment means of the corn study, expressed as average grain weight per ear.

The means were calculated from 20 ears harvested in each plot.

The N-yield treatment resulted in the highest grain weight, while the No Fertilizer treatment had the lowest grain weight. The urea only treatment slightly outperformed the Agrotain treatment.

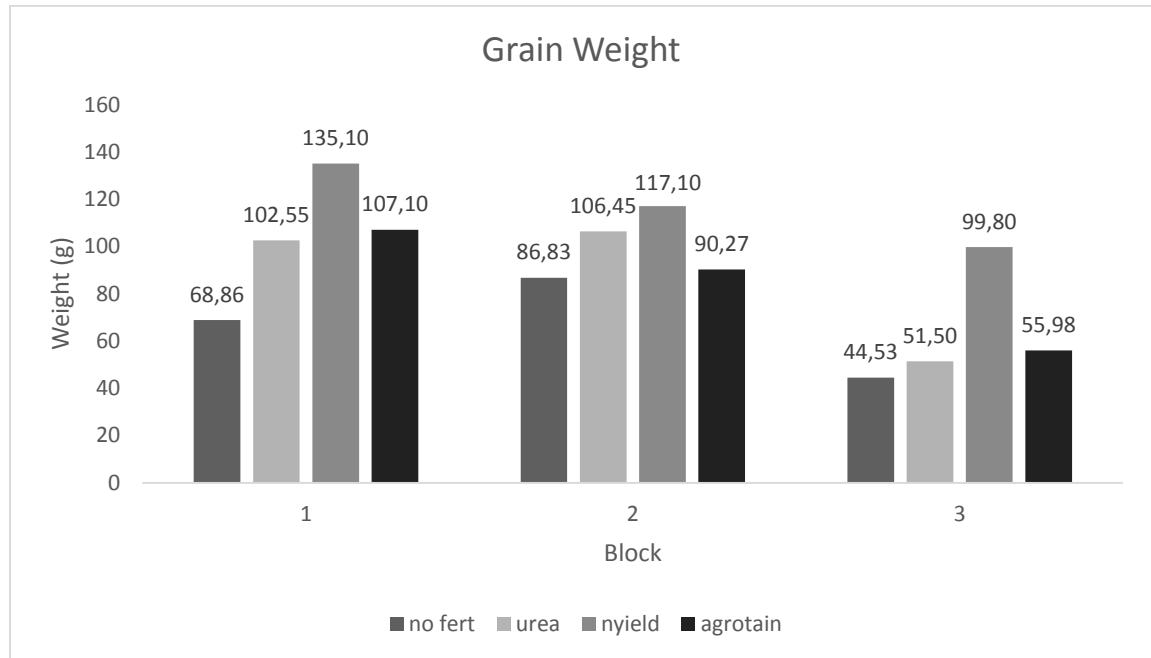


Figure 4.8 Means of the corn study, expressed as grain weight per ear, by block and treatment. The means were calculated from 20 ears harvested in each plot.

The variation in treatments in each block can be seen in Figure 4.8. Block one was on the north side of the plots while block three was on the south side. Block three was exposed to the primarily southerly wind, and was thus stressed as the wind caused a higher rate of evapotranspiration. Blocks one and two were more sheltered and therefore experienced less water stress, resulting in higher yields.

The results of the means separation analysis are presented in Table 4.3. Several significant differences were observed.

Table 4.3 Results of the means separation test for corn grain weight.					
Treatment	Treatment 2	Standard Error	DF	t Value	P value
Agrotain	No fertilizer	8.95	6.00	1.98	0.10
Agrotain	N-Yield	8.95	6.00	-3.67	0.01*
Agrotain	Urea	8.95	6.00	-0.27	0.80
No Fertilizer	N-Yield	8.95	6.00	-5.65	$\leq 0.01^*$
No Fertilizer	Urea	8.95	6.00	-2.25	0.07
N-Yield	Urea	8.95	6.00	3.41	0.01*

*Treatment means are significantly different ($p \leq 0.05$).

The N-Yield treatment yielded 26% more grain by weight than the next highest treatment, Urea Only. Based on a significance level of 0.05, the No Fertilizer, Urea only, and Agrotain treatments were not significantly different from each other. A larger study could help better eliminate variables such as edge effect. This would hopefully reduce some of the variability within treatments.

Grain protein analysis

Results for the 100% dry matter crude protein are presented in Figure 4.8. Statistical analysis was not performed on this data as grain samples were composited across block prior to lab analysis.

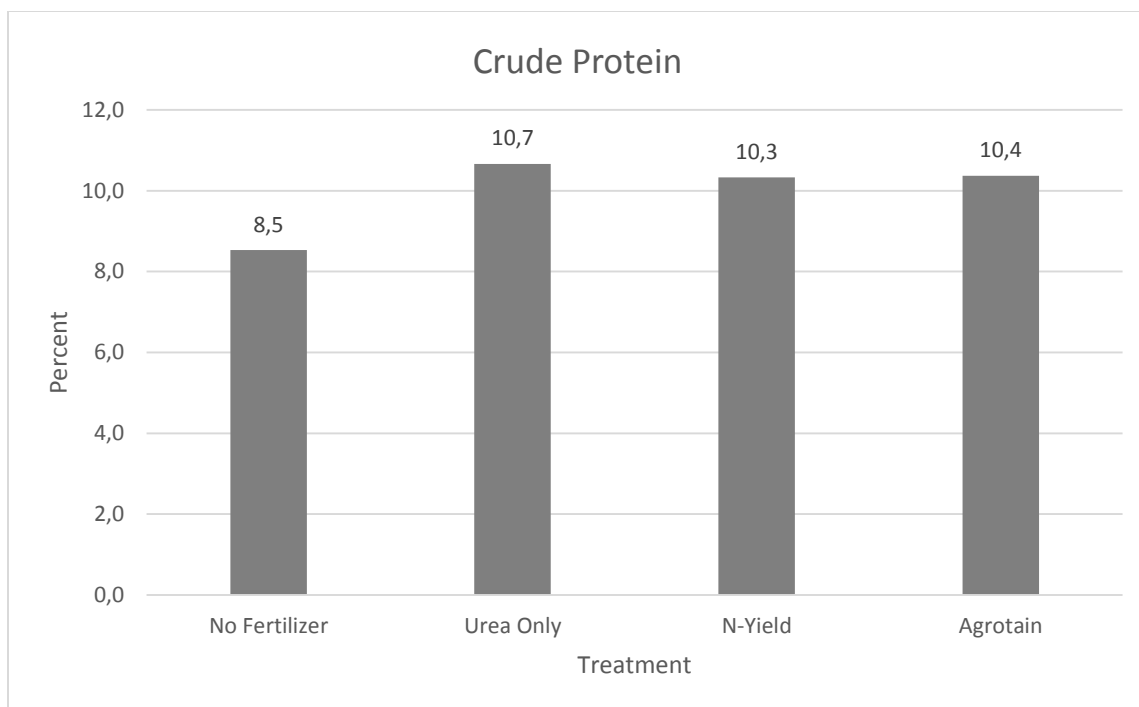


Figure 4.9 Corn grain crude protein analysis.

The no fertilizer treatment appeared lower than the fertilized treatments, testing at 8.5% crude protein. Only slight differences appear among the urea only and the NBPT coated urea treatments. The Urea Only treatment had a protein content of 10.7%, while the Agrotain treated grain had 10.4% protein. The grain from the N-Yield treatment yielded 10.3% crude protein. More research needs to be conducted in order to determine the significance of urease inhibitors on corn protein content.

SUMMARY AND CONCLUSION

The objectives of this study were to evaluate the effect that urea fertilizer coated with the urease inhibitor NBPT in a DMSO/PG solution (N-Yield) had on the grain yield

and protein content of dent corn, when compared to untreated urea. Additionally we wanted to evaluate how N-Yield performed next to a similar urease inhibitor product.

A significant difference was found between treatment and grain weight, with the N-Yield treatment yielding the highest. N-Yield performed significantly higher than No Fertilizer, Urea Only, and the Agrotain treatments. At a 95% significance level, no other treatments were significantly different from one another. The protein content analysis suggested that while applying urea fertilizer improved the protein content in the grain, the addition of a urease inhibitor had no additional benefit.

From these results, it can be concluded that applying N-Yield with urea fertilizer improves the grain yield of dent corn in the field, and N-Yield performed better than another NBPT product. Furthermore, the addition of a urease inhibitor with urea fertilizer had no added grain protein benefit over urea fertilizer alone.

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APPENDIX A

WHEAT RESPONSE DATA

SAS code for the Olton Clay Loam wheat study grain protein content and above ground biomass.

```
ods html close;  
ods html;
```

```
data ocwheat;  
input trt$ protein agb;  
cards;  
nf          10.163    1.39  
nf          10.513    1.16  
nf          10.577    2.036  
u50         11.780    1.114  
u50         15.297    1.519  
u50         13.257    2.337  
u75         11.005    1.478  
u75         11.320    1.581  
u75         13.380    2.394  
u100        12.063    1.29  
u100        11.960    1.277  
u100        12.087    1.383  
ny50        12.537    1.434  
ny50        13.350    2.204  
ny50        11.133    0.999  
ny75        15.183    1.608  
ny75         9.550    0.953  
ny75        10.887    1.449  
ny100       15.050    1.417  
ny100       14.147    1.043  
ny100       13.095    1.709  
at50        13.177    2.417  
at50        11.890    2.713  
at50        11.863    2.355  
at75        14.713    2.373  
at75        13.493    4.116  
at75        13.137    1.541  
at100       12.450     2.1  
at100       12.203    2.428  
at100       12.520    2.308  
  
;  
proc univariate normal all;
```

```

proc glm
alpha=0.1;
class trt;
model protein=trt;
means trt/lsd
alpha=0.1;
run;
proc glm
alpha=0.1;
class trt;
model
agb=trt;
Means
trt/lsd
alpha=0.1;

run;

```

SAS code for the Amarillo Fine Sandy Loam soil type wheat study grain protein and above ground biomass results.

```

ods html close;
ods html;

```

```

data aswheat;
input trt$ protein agb;
cards;
nf          5.543      1.44
nf          6.457      1.275
nf          6.77       2.222
u50         10.36      2.048
u50         10.703     3.402
u50         10.287      2.2
u75         10.06      2.018
u75         10.657     2.352
u75         11.193     2.315
u100        10.06      1.889
u100        11.163     2.785
u100        9.727      1.637
ny50        10.763      2.34
ny50        11.203     3.057

```

ny50	11.543	2.947
ny75	11.643	2.114
ny75	11.837	2.589
ny75	11.56	3.096
ny100	12.633	1.574
ny100	12.393	2.51
ny100	12.907	1.654
at50	11.09	2.058
at50	11.42	2.257
at50	10.92	1.85
at75	11.897	3.015
at75	12.433	2.695
at75	12.483	2.159
at100	12.88	1.5131
at100	11.917	2.619
at100	12.543	2.084

```

;
proc univariate normal all;
proc glm
alpha=0.1;
class trt;
model protein=trt;
means trt/lsd;
run;
proc glm alpha=0.1;
class trt;
model
agb=trt;
means
trt/lsd;
run;

```

Olton Clay Loam wheat response data

Collected Data for the Olton Clay Loam Wheat Study						
Sample	NIR Protein %	# Spikelets	Weight Head (g)	Weight Stalk (g)	Total Weight (g)	Pot Sum (g)
oc_nf_A0_1_1		8	0.23	0.180	0.410	
oc_nf_0_1_2		8	0.28	0.190	0.47	
oc_nf_0_1_3	10.163	7	0.28	0.230	0.51	1.390
oc_nf_0_2_1		8	0.075	0.294	0.369	
oc_nf_0_2_2		9	0.108	0.285	0.393	
oc_nf_0_2_3	10.513	9	0.094	0.304	0.398	1.160
oc_nf_0_3_1		7	0.214	0.27	0.484	
oc_nf_0_3_2		9	0.411	0.394	0.805	
oc_nf_0_3_3	10.577	6	0.385	0.362	0.747	2.036
oc_ur_100_1_1		9	0.128	0.44	0.568	
oc_ur_100_1_2		0	0	0.249	0.249	
os_ur_100_1_3	11.780	0	0	0.297	0.297	1.114
os_ur_100_2_1		6	0.201	0.208	0.409	
oc_ur_100_2_2		7	0.332	0.323	0.655	
oc_ur_100_2_3	15.297	8	0.168	0.287	0.455	1.519
oc_ur_100_3_1		7	0.331	0.306	0.637	
oc_ur_100_3_2		9	0.597	0.412	1.009	
oc_ur_100_3_3	13.257	8	0.331	0.36	0.691	2.337
oc_ur_75_1_1		7	0.147	0.272	0.419	
oc_ur_75_1_2		6	0.365	0.378	0.743	
oc_ur_75_1_3	11.005	7	0.133	0.183	0.316	1.478
oc_ur_75_2_1		8	0.262	0.257	0.519	
oc_ur_75_2_2		7	0.333	0.244	0.577	
oc_ur_75_2_3	11.320	7	0.256	0.229	0.485	1.581
oc_ur_75_3_1		5	0.406	0.288	0.694	
oc_ur_75_3_2		9	0.355	0.534	0.889	
oc_ur_75_3_3	13.380	8	0.5	0.311	0.811	2.394
oc_ur_50_1_1		7	0.08	0.245	0.325	
oc_ur_50_1_2		9	0.133	0.347	0.48	
oc_ur_50_1_3	12.063	8	0.208	0.277	0.485	1.290
oc_ur_50_2_1		0	0	0.3	0.3	
oc_ur_50_2_2		7	0.174	0.337	0.511	
oc_ur_50_2_3	11.960	7	0.124	0.342	0.466	1.277
oc_ur_50_3_1		9	0.157	0.335	0.492	
oc_ur_50_3_2		8	0.066	0.307	0.373	
oc_ur_50_3_3	12.087	8	0.108	0.41	0.518	1.383

oc_tp_100_1_1		6	0.04	0.263	0.303	
oc_tp_100_1_2		12	0.257	0.517	0.774	
oc_tp_100_1_3	12.537	9	0.078	0.279	0.357	1.434
oc_tp_100_2_1		10	0.391	0.429	0.82	
oc_tp_100_2_2		7,7	0.184	0.702	0.886	
oc_tp_100_2_3	13.350	7	0.275	0.223	0.498	2.204
oc_tp_100_3_1		6	0.266	0.222	0.488	
oc_tp_100_3_2		7	0.187	0.231	0.418	
oc_tp_100_3_3	11.133	0	0	0.093	0.093	0.999
oc_tp_75_1_1		10	0.141	0.375	0.516	
oc_tp_75_1_2		9	0.158	0.434	0.592	
oc_tp_75_1_3	15.183	8	0.129	0.371	0.5	1.608
oc_tp_75_2_1		0	0	0	0	
oc_tp_75_2_2		7	0.13	0.407	0.537	
oc_tp_75_2_3	9.550	7	0.131	0.285	0.416	0.953
oc_tp_75_3_1		0	0	0.254	0.254	
oc_tp_75_3_2		10	0.266	0.378	0.644	
oc_tp_75_3_3	10.887	9	0.188	0.363	0.551	1.449
oc_tp_50_1_1		0	0	0.218	0.218	
oc_tp_50_1_2		0	0	0.18	0.18	
oc_tp_50_1_3	15.050	9	0.468	0.551	1.019	1.417
oc_tp_50_2_1		0	0	0.082	0.082	
oc_tp_50_2_2		0	0	0.076	0.076	
oc_tp_50_2_3	14.147	10	0.413	0.472	0.885	1.043
oc_tp_50_3_1		7	0.294	0.228	0.522	
oc_tp_50_3_2		7	0.284	0.233	0.517	
oc_tp_50_3_3	13.095	7	0.372	0.298	0.67	1.709
oc_cp_100_1_1		8	0.527	0.351	0.878	
oc_cp_100_1_2		7	0.462	0.307	0.769	
oc_cp_100_1_3	13.177	7	0.41	0.36	0.77	2.417
oc_cp_100_2_1		10	0.837	0.516	1.353	
oc_cp_100_2_2		8	0.37	0.362	0.732	
oc_cp_100_2_3	11.890	6	0.315	0.313	0.628	2.713
oc_cp_100_3_1		10	0.136	0.377	0.513	
oc_cp_100_3_2		9	0.526	0.398	0.924	
oc_cp_100_3_3	11.863	8	0.458	0.46	0.918	2.355
oc_cp_75_1_1		9	0.219	0.61	0.829	
oc_cp_75_1_2		8	0.374	0.391	0.765	
oc_cp_75_1_3	14.713	8	0.355	0.424	0.779	2.373
oc_cp_75_2_1		10	0.815	0.592	1.407	
oc_cp_75_2_2		10	0.788	0.489	1.277	

oc_cp_75_2_3	13.493	7	0.868	0.564	1.432	4.116
oc_cp_75_3_1		8	0.091	0.337	0.428	
oc_cp_75_3_2		10	0.159	0.419	0.578	
oc_cp_75_3_3	13.137	10	0.154	0.381	0.535	1.541
oc_cp_50_1_1		8	0.423	0.374	0.797	
oc_cp_50_1_2		8	0.281	0.266	0.547	
oc_cp_50_1_3	12.450	7	0.329	0.427	0.756	2.100
oc_cp_50_2_1		9	0.529	0.395	0.924	
oc_cp_50_2_2		6	0.131	0.238	0.369	
oc_cp_50_2_3	12.203	10	0.647	0.488	1.135	2.428
oc_cp_50_3_1		7	0.512	0.452	0.964	
oc_cp_50_3_2		7	0.293	0.344	0.637	
oc_cp_50_3_3	12.520	7	0.342	0.365	0.707	2.308

Amarillo Fine Sandy Loam wheat response data

Collected Data for the Amarillo Fine Sandy Loam Wheat Study						
Sample	NIR Protein	# Spikelets	Weight Head (g)	Weight Stalk (g)	Total Weight (g)	Pot Sum (g)
as_nf_0_1_1		3	0.209	0.174	0.383	
as_nf_0_1_2		5	0.378	0.314	0.692	
as_nf_0_1_3	5.543	3	0.183	0.182	0.365	1.440
as_nf_0_2_1		4	0.248	0.248	0.496	
as_nf_0_2_2		3	0.278	0.271	0.549	
as_nf_0_2_3	6.457	2	0.093	0.137	0.230	1.275
as_nf_0_3_1		5	0.466	0.377	0.843	
as_nf_0_3_2		5	0.431	0.313	0.744	
as_nf_0_3_3	6.770	4	0.323	0.312	0.635	2.222
as_ur_50_1_1		4	0.387	0.284	0.671	
as_ur_50_1_2		5	0.532	0.299	0.831	
as_ur_50_1_3	10.360	4	0.339	0.207	0.546	2.048
as_ur_50_2_1		7	0.814	0.545	1.359	
as_ur_50_2_2		5	0.602	0.457	1.059	
as_ur_50_2_3	10.703	5	0.605	0.379	0.984	3.402
as_ur_50_3_1		6	0.560	0.363	0.923	
as_ur_50_3_2		4	0.311	0.248	0.559	
as_ur_50_3_3	10.287	5	0.383	0.335	0.718	2.200
as_ur_75_1_1		4	0.318	0.309	0.627	
as_ur_75_1_2		6	0.395	0.315	0.710	
as_ur_75_1_3	10.060	6	0.363	0.318	0.681	2.018

as_ur_75_2_1		7	0.754	0.394	1.148	
as_ur_75_2_2		4	0.393	0.257	0.650	
as_ur_75_2_3	10.657	4	0.311	0.243	0.554	2.352
as_ur_75_3_1		7	0.692	0.455	1.147	
as_ur_75_3_2		4	0.343	0.229	0.572	
as_ur_75_3_3	11.193	4	0.277	0.319	0.596	2.315
as_ur_100_1_1		5	0.432	0.242	0.674	
as_ur_100_1_2		6	0.338	0.318	0.656	
as_ur_100_1_3	10.060	4	0.330	0.229	0.559	1.889
as_ur_100_2_1		7	0.739	0.374	1.113	
as_ur_100_2_2		7	0.496	0.346	0.842	
as_ur_100_2_3	11.163	6	0.551	0.279	0.830	2.785
as_ur_100_3_1		3	0.249	0.242	0.491	
as_ur_100_3_2		3	0.190	0.173	0.363	
as_ur_100_3_3	9.727	4	0.445	0.338	0.783	1.637
as_tp_50_1_1		6	0.592	0.330	0.922	
as_tp_50_1_2		8	0.598	0.383	0.981	
as_tp_50_1_3	10.763	4	0.253	0.184	0.437	2.340
as_tp_50_2_1		7	0.733	0.576	1.309	
as_tp_50_2_2		7	0.632	0.419	1.051	
as_tp_50_2_3	11.203	5	0.393	0.304	0.697	3.057
as_tp_50_3_1		5	0.628	0.527	1.155	
as_tp_50_3_2		5	0.451	0.344	0.795	
as_tp_50_3_3	11.543	5	0.564	0.433	0.997	2.947
as_tp_75_1_1		3	0.207	0.228	0.435	
as_tp_75_1_2		7	0.280	0.428	0.708	
as_tp_75_1_3	11.643	5	0.513	0.458	0.971	2.114
as_tp_75_2_1		7	0.846	0.523	1.369	
as_tp_75_2_2		3	0.204	0.203	0.407	
as_tp_75_2_3	11.837	6	0.470	0.343	0.813	2.589
as_tp_75_3_1		5	0.524	0.366	0.890	
as_tp_75_3_2		5	0.558	0.399	0.957	
as_tp_75_3_3	11.560	6	0.738	0.511	1.249	3.096
as_tp_100_1_1		5	0.200	0.292	0.492	
as_tp_100_1_2		5	0.202	0.265	0.467	
as_tp_100_1_3	12.633	5	0.324	0.291	0.615	1.574
as_tp_100_2_1		7	0.489	0.417	0.906	
as_tp_100_2_2		4	0.333	0.244	0.577	
as_tp_100_2_3	12.393	6	0.589	0.438	1.027	2.510
as_tp_100_3_1		6	0.331	0.316	0.647	
as_tp_100_3_2		3	0.055	0.154	0.209	

as_tp_100_3_3	12.907	6	0.445	0.353	0.798	1.654
as_cp_50_1_1		4	0.330	0.302	0.632	
as_cp_50_1_2		5	0.312	0.358	0.670	
as_cp_50_1_3	11.090	5	0.365	0.391	0.756	2.058
as_cp_50_2_1		5	0.475	0.290	0.765	
as_cp_50_2_2		4	0.443	0.282	0.725	
as_cp_50_2_3	11.420	4	0.346	0.421	0.767	2.257
as_cp_50_3_1		5	0.367	0.200	0.567	
as_cp_50_3_2		6	0.505	0.240	0.745	
as_cp_50_3_3	10.920	4	0.350	0.188	0.538	1.850
as_cp_75_1_1		4	0.508	0.339	0.847	
as_cp_75_1_2		5	0.572	0.569	1.141	
as_cp_75_1_3	11.897	5	0.610	0.417	1.027	3.015
as_cp_75_2_1		4	0.541	0.337	0.878	
as_cp_75_2_2		11	0.599	0.474	1.073	
as_cp_75_2_3	12.433	5	0.464	0.280	0.744	2.695
as_cp_75_3_1		6	0.366	0.421	0.787	
as_cp_75_3_2		4	0.305	0.256	0.561	
as_cp_75_3_3	12.483	5	0.418	0.393	0.811	2.159
as_cp_100_1_1		4	0.177	0.250	0.427	
as_cp_100_1_2		4	0.290	0.297	0.587	
as_cp_100_1_3	12.880	4	0.251	0.248	0.499	1.513
as_cp_100_2_1		9	0.640	0.468	1.108	
as_cp_100_2_2		9	0.422	0.493	0.915	
as_cp_100_2_3	11.917	4	0.362	0.234	0.596	2.619
as_cp_100_3_1		3	0.258	0.198	0.456	
as_cp_100_3_2		5	0.405	0.323	0.728	
as_cp_100_3_3	12.543	6	0.578	0.322	0.900	2.084

APPENDIX B

RICE RESPONSE DATA

SAS code for the rice study above ground biomass and grain weight.

```
ods html close ;
ods html ;
data rice grain;
input trt$ rep wt agb;
cards;

nf 1 20.383 108
nf 2 22.612 98
nf 3 25.54 119
nf 4 21.51 98
u20 1 13.575 110
u20 2 16.734 135
u20 3 8.719 135
u20 4 21.336 115
u40 1 38.659 118
u40 2 14.331 143
u40 3 7.019 134
u40 4 31.527 128
ny20 1 24.163 145
ny20 2 18.946 123
ny20 3 21.464 114
ny20 4 22.117 140
ny40 1 20.028 139
ny40 2 30.849 115
ny40 3 36.581 133
ny40 4 32.336 138
at20 1 21.218 116
at20 2 27.166 132
at20 3 34.848 107
at20 4 15.417 133
at40 1 31.843 106
at40 2 35.6979 126
at40 3 35.7 139
at40 4 27.277 114
;
Proc univariate normal all;
proc glm alpha=0.1;
class trt;
model wt=trt;
means trt/lsd alpha=0.1;
```

```

run;
proc glm alpha=0.1;
class trt;
model agb=trt;
means trt/lsd
alpha=0.1;
run;

```

Rice response data

Grain Weight Data for Rice Study						
Fertilizer	treatment	plant #	weight	pot sum	pot average	trt average
no fert.	1	nf_1_1	2.358			
no fert.	1	nf_1_2	3.201			
no fert.	1	nf_1_3	5.864			
no fert.	1	nf_1_4	4.454			
no fert.	1	nf_1_5	4.506	20.383	4.0766	
no fert.	1	nf_2_1	5.45			
no fert.	1	nf_2_2	4.485			
no fert.	1	nf_2_3	3.827			
no fert.	1	nf_2_4	5.023			
no fert.	1	nf_2_5	3.827	22.612	4.5224	
no fert.	1	nf_3_1	6.046			
no fert.	1	nf_3_2	8.798			
no fert.	1	nf_3_3	1.245			
no fert.	1	nf_3_4	0.83			
no fert.	1	nf_3_5	8.621	25.54	5.108	
no fert.	1	nf_4_1	3.693			
no fert.	1	nf_4_2	6.134			
no fert.	1	nf_4_3	5.992			
no fert.	1	nf_4_4	2.67			
no fert.	1	nf_4_5	3.021	21.51	4.302	4.50225
urea 20	2	u20_1_1	4.522			
urea 20	2	u20_1_2	2.285			
urea 20	2	u20_1_3	4.13			
urea 20	2	u20_1_4	1.684			
urea 20	2	u20_1_5	0.954	13.575	2.715	
urea 20	2	u20_2_1	5.894			
urea 20	2	u20_2_2	0.143			

urea 20	2	u20_2_3	4.169			
urea 20	2	u20_2_4	3.403			
urea 20	2	u20_2_5	3.125	16.734	3.3468	
urea 20	2	u20_3_1	0.474			
urea 20	2	u20_3_2	1.704			
urea 20	2	u20_3_3	0.917			
urea 20	2	u20_3_4	1.067			
urea 20	2	u20_3_5	4.557	8.719	1.7438	
urea 20	2	u20_4_1	7.212			
urea 20	2	u20_4_2	3.289			
urea 20	2	u20_4_3	0.143			
urea 20	2	u20_4_4	6.063			
urea 20	2	u20_4_5	4.659	21.366	4.2732	3.0197
urea 40	3	u40_1_1	8.438			
urea 40	3	u40_1_2	10.405			
urea 40	3	u40_1_3	7.034			
urea 40	3	u40_1_4	7.528			
urea 40	3	u40_1_5	5.254	38.659	7.7318	
urea 40	3	u40_2_1	2.821			
urea 40	3	u40_2_2	3.41			
urea 40	3	u40_2_3	1.794			
urea 40	3	u40_2_4	2.488			
urea 40	3	u40_2_5	3.818	14.331	2.8662	
urea 40	3	u40_3_1	1.828			
urea 40	3	u40_3_2	0.366			
urea 40	3	u40_3_3	0.812			
urea 40	3	u40_3_4	3.678			
urea 40	3	u40_3_5	0.335	7.019	1.4038	
urea 40	3	u40_4_1	5.258			
urea 40	3	u40_4_2	7.127			
urea 40	3	u40_4_3	1.672			
urea 40	3	u40_4_4	8.622			
urea 40	3	u40_4_5	8.848	31.527	6.3054	4.5768
nyield 20	4	ny20_1_1	0.721			
nyield 20	4	ny20_1_2	3.605			
nyield 20	4	ny20_1_3	6.894			
nyield 20	4	ny20_1_4	6.349			
nyield 20	4	ny20_1_5	6.594	24.163	4.8326	
nyield 20	4	ny20_2_1	3.139			
nyield 20	4	ny20_2_2	5.415			
nyield 20	4	ny20_2_3	3.05			

nyield 20	4	ny20_2_4	6.964			
nyield 20	4	ny20_2_5	0.378	18.946	3.7892	
nyield 20	4	ny20_3_1	0.588			
nyield 20	4	ny20_3_2	7.915			
nyield 20	4	ny20_3_3	4.017			
nyield 20	4	ny20_3_4	4.285			
nyield 20	4	ny20_3_5	4.659	21.464	4.2928	
nyield 20	4	ny20_4_1	8.613			
nyield 20	4	ny20_4_2	2.637			
nyield 20	4	ny20_4_3	4.943			
nyield 20	4	ny20_4_4	0.922			
nyield 20	4	ny20_4_5	5.002	22.117	4.4234	4.3345
nyield 40	5	ny40_1_1	8.353			
nyield 40	5	ny40_1_2	0.622			
nyield 40	5	ny40_1_3	4.042			
nyield 40	5	ny40_1_4	4.079			
nyield 40	5	ny40_1_5	2.932	20.028	4.0056	
nyield 40	5	ny40_2_1	5.582			
nyield 40	5	ny40_2_2	4.295			
nyield 40	5	ny40_2_3	6.663			
nyield 40	5	ny40_2_4	8.183			
nyield 40	5	ny40_2_5	6.126	30.849	6.1698	
nyield 40	5	ny40_3_1	9.552			
nyield 40	5	ny40_3_2	9.212			
nyield 40	5	ny40_3_3	5.875			
nyield 40	5	ny40_3_4	5.543			
nyield 40	5	ny40_3_5	6.399	36.581	7.3162	
nyield 40	5	ny40_4_1	4.205			
nyield 40	5	ny40_4_2	2.548			
nyield 40	5	ny40_4_3	8.188			
nyield 40	5	ny40_4_4	5.178			
nyield 40	5	ny40_4_5	12.247	32.366	6.4732	5.9912
agrotain 20	6	at20_1_1	5.056			
agrotain 20	6	at20_1_2	2.403			
agrotain 20	6	at20_1_3	4.336			
agrotain 20	6	at20_1_4	6.134			
agrotain 20	6	at20_1_5	3.289	21.218	4.2436	

agrotain 20	6	at20_2_1	3.663			
agrotain 20	6	at20_2_2	8.628			
agrotain 20	6	at20_2_3	4.254			
agrotain 20	6	at20_2_4	5.293			
agrotain 20	6	at20_2_5	5.328	27.166	5.4332	
agrotain 20	6	at20_3_1	6.459			
agrotain 20	6	at20_3_2	9.904			
agrotain 20	6	at20_3_3	4.937			
agrotain 20	6	at20_3_4	4.758			
agrotain 20	6	at20_3_5	8.79	34.848	6.9696	
agrotain 20	6	at20_4_1	3.804			
agrotain 20	6	at20_4_2	3.124			
agrotain 20	6	at20_4_3	2.686			
agrotain 20	6	at20_4_4	2.404			
agrotain 20	6	at20_4_5	3.399	15.417	3.0834	4.93245
agrotain 40	7	at40_1_1	5.294			
agrotain 40	7	at40_1_2	8.924			
agrotain 40	7	at40_1_3	3.145			
agrotain 40	7	at40_1_4	7.324			
agrotain 40	7	at40_1_5	7.156	31.843	6.3686	
agrotain 40	7	at40_2_1	6.407			
agrotain 40	7	at40_2_2	6.022			
agrotain	7	at40_2_3	7.821			

40						
agrotain 40	7	at40_2_4	6.9819			
agrotain 40	7	at40_2_5	8.466	35.6979	7.13958	
agrotain 40	7	at40_3_1	7.189			
agrotain 40	7	at40_3_2	7.288			
agrotain 40	7	at40_3_3	6.608			
agrotain 40	7	at40_3_4	6.82			
agrotain 40	7	at40_3_5	7.795	35.7	7.14	
agrotain 40	7	at40_4_1	4.768			
agrotain 40	7	at40_4_2	6.21			
agrotain 40	7	at40_4_3	7.655			
agrotain 40	7	at40_4_4	3.909			
agrotain 40	7	at40_4_5	4.735	27.277	5.4554	6.525895

Above Ground Biomass Data for the Rice Study						
Fertilizer	treatment	plant #	above ground biomass (g)	pot average	pot sum	trt average
no fert.	1	nf_1_1	16			
no fert.	1	nf_1_2	26			
no fert.	1	nf_1_3	17			
no fert.	1	nf_1_4	24			
no fert.	1	nf_1_5	25	21.6	108	
no fert.	1	nf_2_1	23			
no fert.	1	nf_2_2	14			
no fert.	1	nf_2_3	24			
no fert.	1	nf_2_4	19			
no fert.	1	nf_2_5	18	19.6	98	
no fert.	1	nf_3_1	25			
no fert.	1	nf_3_2	32			

no fert.	1	nf_3_3	18			
no fert.	1	nf_3_4	15			
no fert.	1	nf_3_5	29	23.8	119	
no fert.	1	nf_4_1	20			
no fert.	1	nf_4_2	23			
no fert.	1	nf_4_3	14			
no fert.	1	nf_4_4	21			
no fert.	1	nf_4_5	20	19.6	98	21.15
urea 20	2	u20_1_1	22			
urea 20	2	u20_1_2	24			
urea 20	2	u20_1_3	23			
urea 20	2	u20_1_4	21			
urea 20	2	u20_1_5	20	22	110	
urea 20	2	u20_2_1	21			
urea 20	2	u20_2_2	28			
urea 20	2	u20_2_3	24			
urea 20	2	u20_2_4	32			
urea 20	2	u20_2_5	30	27.75	135	
urea 20	2	u20_3_1	31			
urea 20	2	u20_3_2	20			
urea 20	2	u20_3_3	28			
urea 20	2	u20_3_4	35			
urea 20	2	u20_3_5	21	27	135	
urea 20	2	u20_4_1	23			
urea 20	2	u20_4_2	23			
urea 20	2	u20_4_3	16			
urea 20	2	u20_4_4	30			
urea 20	2	u20_4_5	23	23	115	24.78947368
urea 40	3	u40_1_1	21			
urea 40	3	u40_1_2	27			
urea 40	3	u40_1_3	20			
urea 40	3	u40_1_4	29			
urea 40	3	u40_1_5	21	23.6	118	
urea 40	3	u40_2_1	24			
urea 40	3	u40_2_2	30			
urea 40	3	u40_2_3	32			
urea 40	3	u40_2_4	22			
urea 40	3	u40_2_5	35	28.6	143	
urea 40	3	u40_3_1	28			
urea 40	3	u40_3_2	32			
urea 40	3	u40_3_3	23			

urea 40	3	u40_3_4	25			
urea 40	3	u40_3_5	26	26.8	134	
urea 40	3	u40_4_1	31			
urea 40	3	u40_4_2	20			
urea 40	3	u40_4_3	29			
urea 40	3	u40_4_4	20			
urea 40	3	u40_4_5	28	25.6	128	26.15
nyield 20	4	ny20_1_1	10			
nyield 20	4	ny20_1_2	28			
nyield 20	4	ny20_1_3	33			
nyield 20	4	ny20_1_4	42			
nyield 20	4	ny20_1_5	32	29	145	
nyield 20	4	ny20_2_1	17			
nyield 20	4	ny20_2_2	26			
nyield 20	4	ny20_2_3	25			
nyield 20	4	ny20_2_4	26			
nyield 20	4	ny20_2_5	29	24.6	123	
nyield 20	4	ny20_3_1	16			
nyield 20	4	ny20_3_2	23			
nyield 20	4	ny20_3_3	29			
nyield 20	4	ny20_3_4	16			
nyield 20	4	ny20_3_5	30	22.8	114	
nyield 20	4	ny20_4_1	30			
nyield 20	4	ny20_4_2	27			
nyield 20	4	ny20_4_3	42			
nyield	4	ny20_4_4	23			

20						
nyield 20	4	ny20_4_5	18	28	140	26.1
nyield 40	5	ny40_1_1	33			
nyield 40	5	ny40_1_2	24			
nyield 40	5	ny40_1_3	25			
nyield 40	5	ny40_1_4	33			
nyield 40	5	ny40_1_5	24	27.8	139	
nyield 40	5	ny40_2_1	30			
nyield 40	5	ny40_2_2	21			
nyield 40	5	ny40_2_3	26			
nyield 40	5	ny40_2_4	24			
nyield 40	5	ny40_2_5	14	23	115	
nyield 40	5	ny40_3_1	28			
nyield 40	5	ny40_3_2	29			
nyield 40	5	ny40_3_3	31			
nyield 40	5	ny40_3_4	20			
nyield 40	5	ny40_3_5	25	26.6	133	
nyield 40	5	ny40_4_1	28			
nyield 40	5	ny40_4_2	24			
nyield 40	5	ny40_4_3	26			
nyield 40	5	ny40_4_4	23			
nyield 40	5	ny40_4_5	37	27.6	138	26.25
agrotain 20	6	at20_1_1	24			

agrotain 20	6	at20_1_2	24			
agrotain 20	6	at20_1_3	21			
agrotain 20	6	at20_1_4	23			
agrotain 20	6	at20_1_5	24	23.2	116	
agrotain 20	6	at20_2_1	19			
agrotain 20	6	at20_2_2	32			
agrotain 20	6	at20_2_3	27			
agrotain 20	6	at20_2_4	30			
agrotain 20	6	at20_2_5	24	26.4	132	
agrotain 20	6	at20_3_1	19			
agrotain 20	6	at20_3_2	25			
agrotain 20	6	at20_3_3	12			
agrotain 20	6	at20_3_4	22			
agrotain 20	6	at20_3_5	29	21.4	107	
agrotain 20	6	at20_4_1	28			
agrotain 20	6	at20_4_2	24			
agrotain 20	6	at20_4_3	28			
agrotain 20	6	at20_4_4	28			
agrotain 20	6	at20_4_5	25	26.6	133	24.4
agrotain 40	7	at40_1_1	21			
agrotain 40	7	at40_1_2	23			
agrotain 40	7	at40_1_3	21			
agrotain	7	at40_1_4	23			

40						
agrotain 40	7	at40_1_5	18	21.2	106	
agrotain 40	7	at40_2_1	25			
agrotain 40	7	at40_2_2	24			
agrotain 40	7	at40_2_3	23			
agrotain 40	7	at40_2_4	25			
agrotain 40	7	at40_2_5	29	25.2	126	
agrotain 40	7	at40_3_1	25			
agrotain 40	7	at40_3_2	30			
agrotain 40	7	at40_3_3	25			
agrotain 40	7	at40_3_4	26			
agrotain 40	7	at40_3_5	33	27.8	139	
agrotain 40	7	at40_4_1	12			
agrotain 40	7	at40_4_2	28			
agrotain 40	7	at40_4_3	25			
agrotain 40	7	at40_4_4	19			
agrotain 40	7	at40_4_5	30	22.8	114	24.25

Laboratory results for the 100% dry matter crude protein for the rice study

Servi-Tech analysis for rice crude protein	
Sample	100% dry matter crude protein
Nf	7.1
U20	7.9
U40	7.7
Ny20	7.6
Ny40	7.6
At20	7.2
At40	7.1

APPENDIX C

CORN RESPONSE DATA

SAS code for corn test grain weight.

```
ods html close ;
ods html ;
data cornyield;
input block trt$ wt;
cards;

1          nofert    1309
1          urea      2051
1          nyield    2702
1          agrotain  2142
2          nofert    1563
2          urea      2129
2          nyield    2342
2          agrotain  1730
3          nofert    671
3          urea      888
3          nyield    1996
3          agrotain  896
;
proc univariate normal all;

proc mixed;
class trt;
model wt=trt block;
random block;
lsmeans trt/pdiff;
proc plot;
run;
```

Corn response data

Ear Data for the Corn Study										
TRT	Plant ID	Ear WT (g)	Rows	Columns	Est. # of Kernels	# Kernels Avg.	WT of Kernels (g)	WT of Cob (g)	Row Avg. (g)	TRT Avg. (g)
nyield	1_2_1	151.00	33	16	528		127.00	24.00		
nyield	1_2_2	132.00	34	16	544		110.00	22.00		
nyield	1_2_3	135.00	32	16	512		112.00	23.00		
nyield	1_2_4	223.00	38	16	608		187.00	36.00		

nyield	1_2_5	135.00	34	12	408		113.00	22.00	
nyield	1_2_6	161.00	27	16	432		127.00	34.00	
nyield	1_2_7	184.00	28	14	392		150.00	34.00	
nyield	1_2_8	232.00	41	18	738		197.00	35.00	
nyield	1_2_9	104.00	28	12	336		87.00	17.00	
nyield	1_2_10	127.00	29	16	464	496.20	106.00	21.00	131.60
nyield	1_3_1	134.00	34	14	476		114.00	20.00	
nyield	1_3_2	239.00	34	18	612		206.00	33.00	
nyield	1_3_3	210.00	40	16	640		180.00	30.00	
nyield	1_3_4	136.00	34	14	476		113.00	23.00	
nyield	1_3_5	211.00	37	18	666		179.00	32.00	
nyield	1_3_6	127.00	32	16	512		107.00	20.00	
nyield	1_3_7	116.00	33	16	528		95.00	21.00	
nyield	1_3_8	209.00	39	16	624		176.00	33.00	
nyield	1_3_9	123.00	25	16	400		105.00	18.00	
nyield	1_3_10	132.00	30	16	480	541.40	111.00	21.00	138.60 135.10
urea	2_2_1	218.00	44	14	616		185.00	33.00	
urea	2_2_2	94.00	18	14	252		78.00	16.00	
urea	2_2_3	142.00	37	12	444		118.00	24.00	
urea	2_2_4	171.00	35	14	490		147.00	24.00	
urea	2_2_5	79.00	23	13	299		66.00	13.00	
urea	2_2_6	179.00	34	16	544		152.00	27.00	
urea	2_2_7	87.00	20	12	240		74.00	13.00	
urea	2_2_8	163.00	36	16	576		136.00	27.00	
urea	2_2_9	213.00	42	14	588		180.00	33.00	
urea	2_2_10	104.00	22	14	308	435.70	87.00	17.00	122.30
urea	2_3_1	101.00	19	14	266		82.00	19.00	
urea	2_3_2	95.00	28	14	392		78.00	17.00	
urea	2_3_3	104.00	35	16	560		83.00	21.00	
urea	2_3_4	119.00	29	16	464		97.00	22.00	
urea	2_3_5	132.00	36	16	576		109.00	23.00	
urea	2_3_6	108.00	23	15	345		88.00	20.00	
urea	2_3_7	73.00	14	16	224		60.00	13.00	
urea	2_3_8	105.00	34	14	476		82.00	23.00	
urea	2_3_9	90.00	29	15	435		70.00	20.00	
urea	2_3_10	94.00	25	16	400	413.80	79.00	15.00	82.80 102.55
agrotain	3_2_1	150.00	37	18	666		122.00	28.00	
agrotain	3_2_2	82.00	27	14	378		67.00	15.00	
agrotain	3_2_3	101.00	21	16	336		82.00	19.00	
agrotain	3_2_4	79.00	24	14	336		66.00	13.00	
agrotain	3_2_5	96.00	31	15	465		79.00	17.00	

agrotain	3_2_6	108.00	23	15	345		88.00	20.00	
agrotain	3_2_7	74.00	27	13	351		58.00	16.00	
agrotain	3_2_8	114.00	28	18	504		93.00	21.00	
agrotain	3_2_9	74.00	18	14	252		60.00	14.00	
agrotain	3_2_10	80.00	28	13	364	399.70	67.00	13.00	78.20
agrotain	3_3_1	220.00	36	16	576		187.00	33.00	
agrotain	3_3_2	209.00	39	16	624		179.00	30.00	
agrotain	3_3_3	153.00	31	16	496		128.00	25.00	
agrotain	3_3_4	41.00	7	10	70		29.00	12.00	
agrotain	3_3_5	211.00	35	16	560		179.00	32.00	
agrotain	3_3_6	203.00	40	12	480		170.00	33.00	
agrotain	3_3_7	35.00	6	12	72		25.00	10.00	
agrotain	3_3_8	160.00	30	18	540		136.00	24.00	
agrotain	3_3_9	202.00	38	14	532		171.00	31.00	
agrotain	3_3_10	186.00	37	14	518	446.80	156.00	30.00	136.00 107.10
no fert	4_2_1	46.00	14	13	182		37.00	9.00	
no fert	4_2_2	15.00	6	8	48		12.00	3.00	
no fert	4_2_3	139.00	32	14	448		115.00	24.00	
no fert	4_2_4	27.00	14	12	168		19.00	8.00	
no fert	4_2_5	95.00	22	14	308		78.00	17.00	
no fert	4_2_6	58.00	22	12	264		48.00	10.00	
no fert	4_2_7	190.00	33, 14	14, 6	#VALUE!		153.00	37.00	
no fert	4_2_8	54.00	21	10	210		44.00	10.00	
no fert	4_2_9	150.00	36	16	576		128.00	22.00	
no fert	4_2_10	76.00	20	14	280	#VALUE!	62.00	14.00	69.60
no fert	4_3_1	140.00	35	12	420		118.00	22.00	
no fert	4_3_2	5.00	.	.	#VALUE!		4.00	1.00	
no fert	4_3_3	.	.	.	#VALUE!		.	#VALUE!	
no fert	4_3_4	20.00	.	.	26		6.00	14.00	
no fert	4_3_5	41.00	16	10	160		35.00	6.00	
no fert	4_3_6	60.00	21	12	252		49.00	11.00	
no fert	4_3_7	157.00	36	14	504		135.00	22.00	
no fert	4_3_8	24.00	9	13	117		21.00	3.00	
no fert	4_3_9	166.00	32	16	512		140.00	26.00	
no fert	4_3_10	121.00	30	16	480	#VALUE!	105.00	16.00	68.11 68.86
urea	5_2_1	193.00	35	14	490		163.00	30.00	
urea	5_2_2	84.00	29	16	464		66.00	18.00	
urea	5_2_3	167.00	33	14	462		142.00	25.00	
urea	5_2_4	181.00	37	16	592		152.00	29.00	
urea	5_2_5	128.00	29	14	406		106.00	22.00	
urea	5_2_6	125.00	27	16	432		103.00	22.00	

urea	5_2_7	81.00	29	16	464		65.00	16.00	
urea	5_2_8	119.00	37	16	592		99.00	20.00	
urea	5_2_9	109.00	32	16	512		91.00	18.00	
urea	5_2_10	112.00	37	16	592	500.60	88.00	24.00	107.50
urea	5_3_1	169.00	35	14	490		141.00	28.00	
urea	5_3_2	58.00	20	10	200		45.00	13.00	
urea	5_3_3	95.00	23	15	345		80.00	15.00	
urea	5_3_4	234.00	38, 4	16, 4	#VALUE!		192.00	42.00	
urea	5_3_5	132.00	38	16	608		108.00	24.00	
urea	5_3_6	84.00	17	18	306		68.00	16.00	
urea	5_3_7	138.00	36	18	648		113.00	25.00	
urea	5_3_8	91.00	30	14	420		74.00	17.00	
urea	5_3_9	78.00	22	13	286		65.00	13.00	
urea	5_3_10	198.00	37	18	666	#VALUE!	168.00	30.00	105.40 106.45
agrotain	6_2_1	38.00	.	.	#VALUE!		.	#VALUE!	
agrotain	6_2_2	114.00	28	14	392		95.00	19.00	
agrotain	6_2_3	111.00	24	16	384		92.00	19.00	
agrotain	6_2_4	97.00	25	16	400		81.00	16.00	
agrotain	6_2_5	87.00	17	14	238		70.00	17.00	
agrotain	6_2_6	61.00	18	17	306		51.00	10.00	
agrotain	6_2_7	58.00	19	13	247		47.00	11.00	
agrotain	6_2_8	81.00	30	16	480		66.00	15.00	
agrotain	6_2_9	91.00	36	16	576		71.00	20.00	
agrotain	6_2_10	124.00	32	16	512	#VALUE!	106.00	18.00	75.44
agrotain	6_3_1	126.00	36	18	648		103.00	23.00	
agrotain	6_3_2	64.00	15	11	165		49.00	15.00	
agrotain	6_3_3	94.00	26	14	364		80.00	14.00	
agrotain	6_3_4	87.00	28	14	392		69.00	18.00	
agrotain	6_3_5	153.00	34	18	612		126.00	27.00	
agrotain	6_3_6	109.00	28	14	392		92.00	17.00	
agrotain	6_3_7	144.00	35	16	560		122.00	22.00	
agrotain	6_3_8	202.00	37	18	666		169.00	33.00	
agrotain	6_3_9	139.00	39	12	468		122.00	17.00	
agrotain	6_3_10	150.00	34, 22	16, 8	#VALUE!	#VALUE!	119.00	31.00	105.10 90.27
no fert	7_2_1	78.00	25	14	350		64.00	14.00	
no fert	7_2_2	112.00	26	14	364		98.00	14.00	
no fert	7_2_3	54.00	17	13	221		43.00	11.00	
no fert	7_2_4	181.00	38	12	456		151.00	30.00	
no fert	7_2_5	185.00	38	14	532		154.00	31.00	
no fert	7_2_6	30.00	.	.	#VALUE!		.	#VALUE!	
no fert	7_2_7	225.00	41	18	738		189.00	36.00	

no fert	7_2_8	76.00	14, 10	14, 9	#VALUE!		48.00	28.00	
no fert	7_2_9	168.00	34	16	544		142.00	26.00	
no fert	7_2_10	6.00	.	.	#VALUE!	#VALUE!	1.00	5.00	98.89
no fert	7_3_1	62.00	19	16	304		51.00	11.00	
no fert	7_3_2	124.00	33	12	396		104.00	20.00	
no fert	7_3_3	124.00	33	12	396		104.00	20.00	
no fert	7_3_4	68.00	20	11	220		47.00	21.00	
no fert	7_3_5	.	.	.	#VALUE!		.	#VALUE!	
no fert	7_3_6	123.00	33	14	462		103.00	20.00	
no fert	7_3_7	143.00	36	17	612		120.00	23.00	
no fert	7_3_8	90.00	22	16	352		76.00	14.00	
no fert	7_3_9	31.00	.	.	#VALUE!		18.00	13.00	
no fert	7_3_10	58.00	16	16	256	#VALUE!	50.00	8.00	74.78 86.83
nyield	8_2_1	127.00	37	16	592		106.00	21.00	
nyield	8_2_2	147.00	35	14	490		125.00	22.00	
nyield	8_2_3	99.00	26	16	416		84.00	15.00	
nyield	8_2_4	61.00	24	14	336		51.00	10.00	
nyield	8_2_5	96.00	30	15	450		81.00	15.00	
nyield	8_2_6	141.00	36	18	648		116.00	25.00	
nyield	8_2_7	78.00	24	16	384		68.00	10.00	
nyield	8_2_8	144.00	34	16	544		123.00	21.00	
nyield	8_2_9	89.00	31	16	496		74.00	15.00	
nyield	8_2_10	127.00	34	16	544	490.00	107.00	20.00	93.50
nyield	8_3_1	123.00	32	16	512		103.00	20.00	
nyield	8_3_2	105.00	31	15	465		86.00	19.00	
nyield	8_3_3	250.00	37	18	666		212.00	38.00	
nyield	8_3_4	150.00	30	18	540		130.00	20.00	
nyield	8_3_5	112.00	30	16	480		92.00	20.00	
nyield	8_3_6	135.00	40	14	560		108.00	27.00	
nyield	8_3_7	171.00	34	16	544		141.00	30.00	
nyield	8_3_8	226.00	37	16	592		190.00	36.00	
nyield	8_3_9	217.00	36	14	504		183.00	34.00	
nyield	8_3_10	189.00	36	16	576	543.90	162.00	27.00	140.70 117.10
nyield	9_2_1	111.00	32	16	512		88.00	23.00	
nyield	9_2_2	116.00	30	14	420		94.00	22.00	
nyield	9_2_3	96.00	27	16	432		78.00	18.00	
nyield	9_2_4	110.00	19, 18	14, 6	#VALUE!		94.00	16.00	
nyield	9_2_5	157.00	40	16	640		129.00	28.00	
nyield	9_2_6	130.00	34	14	476		108.00	22.00	
nyield	9_2_7	87.00	32	14	448		70.00	17.00	

nyield	9_2_8	130.00	38	14	532		109.00	21.00		
nyield	9_2_9	172.00	29	14	406		140.00	32.00		
nyield	9_2_10	138.00	30	16	480	#VALUE!	114.00	24.00	102.40	
nyield	9_3_1	254.00	42	18	756		212.00	42.00		
nyield	9_3_2	76.00	22	18	396		60.00	16.00		
nyield	9_3_3	97.00	34	16	544		75.00	22.00		
nyield	9_3_4	95.00	28	14	392		78.00	17.00		
nyield	9_3_5	100.00	34	16	544		81.00	19.00		
nyield	9_3_6	104.00	30	18	540		85.00	19.00		
nyield	9_3_7	117.00	38	18	684		93.00	24.00		
nyield	9_3_8	188.00	39	16	624		160.00	28.00		
nyield	9_3_9	74.00	33	15	495		58.00	16.00		
nyield	9_3_10	84.00	23	14	322	529.70	70.00	14.00	97.20	99.80
urea	10_2_1	73.00	21	16	336		59.00	14.00		
urea	10_2_2	93.00	24	14	336		74.00	19.00		
urea	10_2_3	110.00	34	16	544		89.00	21.00		
urea	10_2_4	98.00	32	14	448		80.00	18.00		
urea	10_2_5	63.00	17	15	255		53.00	10.00		
urea	10_2_6	72.00	17	14	238		55.00	17.00		
urea	10_2_7	48.00	17	13	221		41.00	7.00		
urea	10_2_8	102.00	32	16	512		84.00	18.00		
urea	10_2_9	92.00	30	16	480		72.00	20.00		
urea	10_2_10	83.00	28	16	448	381.80	68.00	15.00	67.50	
urea	10_3_1	30.00	12	12	144		25.00	5.00		
urea	10_3_2	19.00	8	7	56		14.00	5.00		
urea	10_3_3	14.00	.	.	#VALUE!		10.00	4.00		
urea	10_3_4	.	.	.	#VALUE!		.	#VALUE!		
urea	10_3_5	.	.	.	#VALUE!		.	#VALUE!		
urea	10_3_6	54.00	20	16	320		43.00	11.00		
urea	10_3_7				0			0.00		
urea	10_3_8	.	.	.	#VALUE!		.	#VALUE!		
urea	10_3_9	66.00	22	15	330		53.00	13.00		
urea	10_3_10	85.00	24	18	432	#VALUE!	68.00	17.00	35.50	51.50
agrotain	11_2_1	140.00	35	18	630		112.00	28.00		
agrotain	11_2_2	72.00	24	15	360		58.00	14.00		
agrotain	11_2_3	109.00	36	16	576		85.00	24.00		
agrotain	11_2_4	36.00	15	12	180		30.00	6.00		
agrotain	11_2_5	48.00	20	12	240		38.00	10.00		
agrotain	11_2_6	.	.	.	#VALUE!		.	#VALUE!		
agrotain	11_2_7	109.00	34	16	544		87.00	22.00		
agrotain	11_2_8	21.00	15	5	75		16.00	5.00		

agrotain	11_2_9	29.00	12	9	108		22.00	7.00		
agrotain	11_2_10	74.00	27	16	432	#VALUE!	57.00	17.00	56.11	
agrotain	11_3_1	49.00	23	13	299		39.00	10.00		
agrotain	11_3_2	75.00	28	16	448		57.00	18.00		
agrotain	11_3_3	.	.	.	#VALUE!		.	#VALUE!		
agrotain	11_3_4	.	.	.	#VALUE!		.	#VALUE!		
agrotain	11_3_5	42.00	18	9	162		33.00	9.00		
agrotain	11_3_6	100.00	30	16	480		81.00	19.00		
agrotain	11_3_7	63.00	24	14	336		51.00	12.00		
agrotain	11_3_8	71.00	29	16	464		56.00	15.00		
agrotain	11_3_9	94.00	34	18	612		74.00	20.00		
agrotain	11_3_10	.	.	.	#VALUE!	#VALUE!	.	#VALUE!	55.86	55.98
no fert	12_2_1	.	.	.	#VALUE!		.	#VALUE!		
no fert	12_2_2	66.00	21	16	336		59.00	7.00		
no fert	12_2_3	40.00	16	12	192		35.00	5.00		
no fert	12_2_4	48.00	16	11	176		42.00	6.00		
no fert	12_2_5	93.00	25	16	400		80.00	13.00		
no fert	12_2_6	43.00	17	14	238		36.00	7.00		
no fert	12_2_7	64.00	28	16	448		52.00	12.00		
no fert	12_2_8	.	.	.	#VALUE!		.	#VALUE!		
no fert	12_2_9	44.00	24	10	240		37.00	7.00		
no fert	12_2_10	49.00	21	16	336	#VALUE!	40.00	9.00	47.63	
no fert	12_3_1	142.00	34	14	476		119.00	23.00		
no fert	12_3_2	26.00	.	.	#VALUE!		10.00	16.00		
no fert	12_3_3	20.00	15	5	75		15.00	5.00		
no fert	12_3_4	25.00	11	7	77		19.00	6.00		
no fert	12_3_5	.	.	.	#VALUE!		.	#VALUE!		
no fert	12_3_6	113.00	32	16	512		91.00	22.00		
no fert	12_3_7	.	.	.	#VALUE!		.	#VALUE!		
no fert	12_3_8	21.00	15	6	90		18.00	3.00		
no fert	12_3_9	21.00	14	11	154		18.00	3.00		
no fert	12_3_10	.	.	.	#VALUE!	#VALUE!	.	#VALUE!	41.43	44.53

Corn crude protein data

Servi-Tech Crude Protein Results			
Sample ID	Block	treatment	100% Dry Matter Crude Protein
1	1	N-Yield	10.2
2	1	Urea	10.6
3	1	Agrotain	9.3
4	1	No Fertilier	7.1
5	2	Urea	10.1
6	2	Agrotain	10.4
7	2	No Fertilizer	7.9
8	2	N-Yield	10.4
9	3	N-Yield	10.4
10	3	Urea	11.3
11	3	Agrotain	11.4
12	3	No Fertilier	10.6