

**EVALUATION OF NITROGEN SOURCES FOR ALABAMA COTTON PRODUCTION
SYSTEMS**

by

Evan Rose

A Thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Degree of Master of Science

Auburn, Alabama
August 6, 2022

Copyright 2022 by Evan Rose

Approved by

Dr. Audrey Gamble, Chair, Assistant Professor of Crop, Soil and Environmental Sciences,
Auburn University
Dr. Elizabeth Guertal, Committee Member, Professor of Crop, Soil and Environmental Sciences,
Auburn University
Dr. Steve Brown, Committee Member, Assistant Professor of Crop, Soil and Environmental
Sciences, Auburn University

Abstract

Cotton is the most-widely produced cash crop in Alabama, with an average of 415,000 acres per year planted during the 2011-2020 growing seasons. Nitrogen (N) is often the most limiting nutrient in cotton production systems and must be supplemented with fertilizer applications annually. Loss pathways such as leaching and volatilization can lead to significant losses of applied N fertilizer, particularly in coarse-textured, highly weathered soils of the Coastal Plain. Stabilized and controlled-released N fertilizers have been documented to reduce N losses and increase efficiency and profitability for farmers. However, these products lack evaluation in cotton (*Gossypium hirsutum* L.) production systems of the Coastal Plain. The objective of this experiment was to 1) evaluate N fertilizer source impact on N uptake and cotton yield and 2) evaluate urease inhibitors for their ability to reduce N volatilization. Field experiments were established at the Wiregrass Research Extension Center (WREC) in Headland, AL, and E.V. Smith Research Center (EVS) in Shorter, AL. Fertilizer treatments were organized in a randomized complete block design and included: 1) urea, 2) urea + NBPT, 3) urea + duromide/NBPT, 4) polymer-coated urea, 5) urea ammonium nitrate (UAN), 6) ammonium nitrate / ammonium sulfate blend, 7) urea / ammonium sulfate blend, 8) polymer-coated urea / ammonium sulfate blend, and 9) an untreated control. Data collected included leaf N, petiole N, soil N, cotton yield, and fiber quality. Leaf and petiole N were measured to assess uptake of N according to N source treatment. Leaf N for WREC 2021 showed treatment differences at early bloom, where 28-0-0-(5) had a greater leaf N concentration than all other treatments. Similarly, 28-0-0-(5) had a greater leaf N than all treatments at peak bloom for WREC in 2021 and was the only treatment with greater leaf N content than the control. However, no differences between

treatments in leaf N were observed in 2020 at WREC or EVS. When evaluating petiole data, it was observed that 39-0-0 PC and 44-0-0 PC had greater petiole N content than all other treatments at WREC during first square. There were limited treatment differences in petiole N at early bloom and peak bloom at WREC or EVS. Soil N for both years and locations were variable and showed limited differences between treatments. Cotton lint yield for WREC showed 28-0-0-5 (1381 kg ha⁻¹) as the greatest yielding treatment. At WREC, 28-0-0-(5), 46-0-0 +NBPT, 46-0-0 + NBPTD, 40-0-0, and 39-0-0 PC produced 400-600 kg ha⁻¹ greater lint yield than the control. Other N source treatments including the 46-0-0, 44-0-0 PC, and 32-0-0, were not different than the control. Results indicate that urease inhibitor products 46-0-0 + NBPT (1216 kg ha⁻¹) and 46-0-0 + NBPTD (1255 kg ha⁻¹) were more likely to increase yields above the control treatment than base 46-0-0 (1185 kg ha⁻¹). The polymer coated products did not provide differences compared to the untreated base products. However, these data suggest a possible reduction in production costs associated with polymer coated products since they require only one pass through the field.

Laboratory incubation experiments were established to measure nitrogen volatilization for three conventional fertilizer sources: 1) urea, 2) UAN 3) homogenized urea + ammonium sulfate blend. Each fertilizer was evaluated with 1) NBPT 2) NBPT/Duromide (NBPTD) and 3) untreated control and replicated four times for the Coastal Plain (Shorter, Al) soil type. Ammonia volatilization studies were performed using a laboratory incubation method. Results from the Coastal Plain soils showed untreated urea having almost 40% of total N lost as NH₃ compared to only 25% with urea was applied with NBPT products. NBPT and NBPTD applications significantly minimized NH₃ losses by 10-15% within the first 4 days of treatment compared to the untreated urea. For urea, NBPT and NBPTD reduced N volatilization from 5% cumulative N loss to less than 1% three days after fertilizer application. At 4 days after application NBPT and

NBPTD reduced cumulative N loss from 21% to less than 3%. For Amidas[®], NBPT and NBPTD reduced nitrogen volatilization from 6% cumulative N loss to less than 1% cumulative N loss at day 4 compared to untreated Amidas[®]. The utilization of volatilization inhibitors proved to be effective at reducing volatilization up to four days, which urea would allow producers the time for a rain/irrigation event to occur.

Acknowledgments

I owe a special thanks to my Chair, Dr. Audrey Gamble, without her guidance and support this research would have not been possible. My utmost gratitude goes out to Anna Johnson, Hannah Decker, and all student workers in the department of Crop Soil and Environmental Sciences.

Thank you to the Alabama Cotton Commission, Alabama Agriculture Experiment Station, and the staff of Alabama Extension Research Centers for providing the funding and full research support. Finally, I would like to thank my family and friends for the continued support along the way.

Table of Contents

Abstract.....	1
Acknowledgments.....	4
List of Tables	6
List of Figures.....	7
List of Abbreviations	10
I. Chapter 1 LITERATURE REVIEW.....	11
Introduction.....	11
Nitrogen Cycle.....	12
Nitrogen Cycle Additions	12
Nitrogen Cycle Losses	15
Nitrogen Loss Inhibition.....	21
Nitrogen Use Efficiency in Cotton	26
II. ENHANCING NITROGEN USE EFFICIENCY IN ALABAMA COTTON PRODUCTION SYSTEMS	
Methods and Material	35
Results and Discussion	41
Conclusions.....	51
Literature Cited.....	68
III. APPENDIX.....	83

List of Tables

Table 1. Nitrogen (N) fertilizer chart for reference to fertilizers source, Rating, and active ingredient or mechanism of slow release	53
Table 2. Field trial Fertilizer application and soil and leaf nitrogen (N) sample date.....	54
Table 3. Summary of analysis of variance (ANOVA) for Petiole Nitrogen (N), Leaf N, and Cotton Yield. All variables were measured by Year (Y), Treatment (T), and Year*Treatment (YxT). Sample dates for Petiole and Leaf N are denoted by first square (T1), early bloom(T2), and peak bloom(T3)	55
Table 4. Summary of analysis of variance (ANOVA) for soil Nitrogen (N) for ammonium and nitrate. Soil samples were evaluated at (0-6”) and (6-12”) depths. All variables were measured by Year (Y), Treatment (T), and Year*Treatment (YxT). Sample dates for soil N are denoted by first square(T1), early bloom(T2), and peak bloom(T3).....	56
Table 5. Summary of soil nitrogen (N) data All variables were measured by Year (Y) and Treatment (T) where location and soil sample depth were evaluated for soil ammonium and nitrate. Sample dates for soil N are denoted by first square (T1), early bloom(T2), and peak bloom(T3).	57
Table 6. Lint quality data was evaluated by treatment at Wiregrass Research and Extension Center for the year 2021. Data was evaluated for lint length, strength, micronaire, and uniformity. Data showed no significant difference between treatments	58
Table 7. Soil characteristics of a course textured Bama sandy loam (Fine-loamy, siliceous, subactive, thermic Typic Paleudults)	59

List of Figures

- Figure 1 Diagram of the laboratory system used to measure ammonia volatilization, with jars, pump, and air scrubbers, following the procedure of O'Halloran (1993). The glass manifold is connected to an opening in each jar with silicon tubing 60
- Figure 2 Leaf Nitrogen (N) for Wiregrass Research and Extension Center (WREC) was measured by Year (Y), Treatment (T), and Year*Treatment (YxT). Sample dates are denoted by A. first square, B. early bloom, and C. peak bloom. Leaf N showed significant differences between treatments for B. ($P < 0.0001$) and C. ($P < 0.0001$) respectively. All sample dates showed differences between years ($P < 0.0001$). Error bars indicate the standard error about the mean..... 61
- Figure 3 Leaf Nitrogen (N) for E.V. Smith research center (EVS) was measured by Year (Y), Treatment (T), and Year*Treatment (YxT). Sample dates are denoted by A. first square, B. early bloom, and C. peak bloom. Field Data from 2021 was highly variable and was not presented. 2020 Leaf N showed no significant differences between treatments A. ($P = 0.2141$) and B. ($P = 0.4545$) respectively. Error bars indicate the standard error about the mean..... 62
- Figure 4 Petiole Nitrogen (N) for Wiregrass Research and Extension Center (WREC) was measured by Year (Y), Treatment (T), and Year*Treatment (YxT). Sample dates are denoted by A. first square, B. early bloom, and C. peak bloom. Petiole N showed

significant differences between treatments for A. ($P < 0.0001$) and B. ($P < 0.0001$) respectively. All sample dates showed differences between years ($P < 0.0001$). Error bars indicate the standard error about the mean..... 63

Figure 5 Petiole Nitrogen (N) for E.V. Smith research center (EVS) was measured by Year (Y), Treatment (T), and Year*Treatment (YxT). Sample dates are denoted by A. first square, B. early bloom, and C. peak bloom. Field Data from 2021 was highly variable and was not presented. 2020 Petiole N showed no significant differences between treatments A. ($P = 0.0836$) and B. ($P = 0.5005$) respectively 64

Figure 6 WREC Cotton Yield 2020-2021 Differences between treatments at Wiregrass Research and Extension Center (WREC) for cotton lint yield for 2020 and 2021. Year (Y), Treatment (T), and Year*Treatment (YxT) were evaluated for WREC lint yield. Significant differences between treatments were shown at ($P = 0.0032$). Columns with the same letter do not differ between cover crop treatments ($\alpha = 0.05$). Error bars indicate the standard error about the mean..... 65

Figure 7. Differences between treatments at E.V. Smith Research Center (EVS) for cotton lint yield for 2020. Year (Y), Treatment (T), and Year*Treatment (YxT) were evaluated for EVS lint yield. No significant differences between treatments were shown at ($P = 0.4134$). Field Data from 2021 was highly variable and was not presented. Error bars indicate the standard error about the mean..... 66

Figure 8. Cumulative percent nitrogen volatilized for (A) untreated urea, urea+NBPT,(N-(n-butyl) thiophosphoric triamide) and Urea+ NBPTD(NBPT + Duromide)(B) untreated Amidas, Amidas+NBPT, and Amidas+NBPTD (C) untreated UAN, UAN+NBPT, and UAN+NBPTD for the Coastal Plain soil type across a 10-day experiment. * Indicates

significantly higher nitrogen volatilization for untreated fertilizer compared to treated with NBPT within a day at $\alpha=0.05$. **indicates significantly higher nitrogen volatilization for untreated fertilizer compared to NBPT or NBPTD within a day at ($\alpha=0.05$) 67

ABBREVIATIONS

CEC	Cation Exchange Capacity
CRF	Control Release Fertilizer
N	Nitrogen
NUE	Nitrogen Use Efficiency
SRF	Slow-Release Fertilizer
WREC	Wiregrass Research and Extension Center
EVS	E.V. Smith Research and Extension Center

I. LITERATURE REVIEW

INTRODUCTION

Nitrogen (N) is an essential nutrient needed for plant growth as a component of molecules such as DNA, proteins, and chlorophyll. Nitrogen is often the most limiting nutrient to plant growth due to the quantity of N needed and the lack of naturally occurring N in the soil. The N cycle consists of various additions and losses which are controlled by environmental conditions and biological forces. Nitrogen is added to the N cycle by processes including: 1) biological fixation, 2) mineralization, 3) lightning fixation, and 4) fertilization. Nitrogen is also lost from the N cycle through: 1) leaching, 2) volatilization, 3) denitrification, 4) runoff, and 5) crop removal. Additions and losses are affected by soil type, temperature, soil pH, soil moisture, rainfall, and microbial activity.

Efforts to improve N fertilizer management have been at the forefront of research to minimize N losses and increase yields. Nitrogen sources such as urea are effective for supplying plant-available N but are susceptible to volatilization. Stabilized fertilizers treated with urease inhibitors have been documented to reduce losses from volatilization. Other fertilizers which have potential to reduce N loss include controlled release fertilizers (CRF) such as polymer coated fertilizers. CRF have been documented to reduce environmental impacts while maintaining yield, but more research is needed in row crop production systems.

Cotton (*Gossypium hirsutum*) is the most-widely produced cash crop in Alabama, with an average of 415,000 acres per year planted during the 2011-2020 growing seasons (National Agricultural Statistics Service, 2021). Nitrogen use efficiency (NUE) in cotton production has been a priority for researchers and producers over the recent decades to improve economic

returns and reduce N loss. Factors that influence fertilizer management for production include rate, time, source, and placement. The combination of correct fertilizer management and enhanced efficiency fertilizers can aid to increase yield effectively. Research is needed in cotton for products such as volatilization inhibitors and CRF in coarse textured highly weathered soils of the Coastal Plain.

THE NITROGEN CYCLE

Nitrogen undergoes constant transformations because of chemical, biological, and physical processes. The series of these transformations is referred to as the N cycle, which consists of N additions and losses to the soil. Nitrogen is added to the soil N pool through biological fixation, mineralization, lightning fixation, and fertilization. N is lost from the soil N pool through leaching, volatilization, denitrification, runoff, and crop removal. Additions and losses are affected by soil type, temperature, soil pH, moisture, and microbial activity. Nitrogen forms can be broadly divided into two categories: inorganic N (e.g., nitrate, ammonia, N₂ gas) and organic N (e.g., amino acids, proteins, urea). Nitrogen is primarily taken up by plants as ammonium (NH₄⁺) and nitrate (NO₃⁻). The most abundant form of N is N₂ gas or atmospheric N. Although abundant, N₂ is not readily available for most organism to use. Converted forms of N are needed for plants to reach optimum growth habits.

Soil Nitrogen Cycle Additions

Nitrogen gas (N₂) can be fixed most efficiently through biological fixation or industrial fixation. Industrial fixation was accomplished by a process called the Haber-Bosch method in which atmospheric N and H₂ are converted to ammonia (NH₃) by extreme heat and pressure.

When cooled, NH_3 gas is transformed into anhydrous NH_3 (Canfield et al., 2010). This process led to the development and synthesis of other fertilizer products that have allowed agriculture to advance. Another form of N fixation is biological fixation which can occur through symbiotic relationships between host plants and bacteria or through non-symbiotic fixation. *Rhizobia* is responsible for about 50% of the of biological N_2 fixed which estimates to be 130 to 180 x 10^6 Mg annually (Havling et al 2013). The primary plant family which forms symbiotic relationships with N-fixing bacteria is the legume plant family, *Fabaceae*. In traditional agricultural systems, common leguminous crops include peanuts, soybeans, clovers, vetches, alfalfa, and dry beans (Herridge et al., 2008).

Biological Fixation

Biological fixation consists of symbiotic and non-symbiotic N fixation. In symbiotic fixation, an association between roots and *Rhizobia* occurs (Herridge et al., 2008). Non-symbiotic fixation is carried out by free-living organisms. Both symbiotic and non-symbiotic fixing organisms consist of an enzyme called nitrogenase, which converts N_2 gas into ammonia. Symbiotic organisms use these nitrogenases to create ureides (i.e., allantoin and allantoic acid) which are transported via xylem to meet N demand of the plant. Root nodulation is initiated by bacteria (*Rhizobia*) and flavonoids which initiate node formation. Approximately 2.4×10^{12} mol N year^{-1} is fixed in agricultural systems. This N fixation is predominantly contributed by legumes cultivated for forage and feed production (Canfield et al, 2010). Free living or non-symbiotic fixing organisms consist of three main categories: anaerobic (e.g. Clostridium), facultative aerobic (e.g. Klebsiella, Enterobacter, Bacillus) and aerobic organisms (e.g. Azotobacter) (Keuter et al. 2014). Data have shown that free living N fixing organisms can be

the dominant force in temperate grassland systems from 0.1 to 21 kg N ha⁻¹ yr⁻¹ with a mean of 4.7 kg N ha⁻¹ yr⁻¹ (Reed et al., 2011).

Mineralization

Mineralization is defined as the conversion of organic N by microorganisms into inorganic forms (Hart et al., 1994). Unlike other additions of N which are external N inputs, mineralization can be thought of as an internal addition to the plant-available N pool. The internal N cycle consists of processes that convert N from one chemical form to another. For example, mineralization converts organic N sources to inorganic sources i.e. ammonium (NH₄⁺) and nitrate (NO₃⁻) (Hart et al., 1994). Mineralization is a two-step process which includes ammonification and nitrification. Ammonification is the breakdown of organic N by a variety of microorganisms into ammonium (NH₄⁺). Heterotrophic microorganisms convert proteins to amino acids and urea via aminization (Havling et al., 2014). These organic N compounds are then converted to NH₄⁺ via ammonification. Nitrification is the conversion of ammonium to nitrite (NO₂⁻) and then nitrate (NO₃⁻), which is performed by *Nitrosomonas* and then *Nitrobacter* bacteria.

Lightning Deposition

Atmospheric N deposition can also be added by lightning. Lightning strikes the soil surface and atmospheric N (N₂) reacts with oxygen (O₂) to form nitric oxide (NO). Nitric oxide combines with O₂ to form N dioxide (NO₂). NO₂ can then be solubilized and converted to nitric acid (HNO₃) and nitrous acid (HNO₂) (Noxon, 1976). These forms are then converted to nitrite (NO₂⁻) and nitrate (NO₃⁻) and are made available by dissolution. The estimated N fixated per year is 14.4×10⁶ metric tons of NO₂ (Hill et al., 1980).

Fertilizer Additions

The most notable addition to the N cycle is the application of organic or inorganic fertilizers. Fertilizer is the largest addition of N due to high nutrient requirements in most agricultural systems. Fertilizer was made readily available by the Haber-Bosch process which allowed inorganic fertilizers to be produced industrially (Canfield et al., 2010). The Haber-Bosch process starts where with the conversion of N_2 and H_2 to NH_3 under extreme heat and pressure. Global N usage from 1960 – 2000 has increased greater than 800% because of available N sources. This has increased agricultural productivity but decreased N use efficiency (NUE). The estimated NUE of plants globally is around 40% (Canfield et al., 2010). The losses of N that can occur from the remaining 60% such as leaching or runoff, can have hazardous environmental and mammalian health effects. Thus, practices must be used to increase N use efficiency as more N is being added into ecosystems.

Agricultural professionals are consistently working to improve N use efficiency, which improves profitability for farmers and reduces the waste of N inputs. Various products have been introduced to producers in recent decades to help manage N waste as well as maintain consistent crop production. Fertilizer products such as slow or CRF are being introduced to reduce N loss to the environment via gaseous N and leachate. Volatilization (urease) inhibitors are also important for controlling the loss of volatile gaseous ammonia (NH_3).

Soil Nitrogen Cycle Losses

Leaching

Leaching is a major loss pathway in the N cycle. Leaching is the movement of a material through the soil profile with water. Nitrate is readily leached from the soil due to the anionic charge of the molecule. Since soil colloids have a net negative charge, NO_3^- leaches through the

soil profile readily. Leaching of nitrate can cause an economic loss to producers as well as ground or surface water contamination. The United States public health service states that “10 ppm nitrate is the maximum amount allowed in drinking water” (Timmons and Dylla, 1981). Nitrate accumulation in drinking water has also been shown to cause human health problems such as methemoglobinemia (Golden et al., 1999).

Leaching studies have been conducted in agricultural settings to better evaluate N loss and its effect on agriculture. Smika et al. (1977) used vacuum extractors to collect percolating water under irrigated corn in Colorado on a loamy fine sand. It was estimated that the annual nitrate (NO_3^-) leaching ranged from about 19 to 60 kg ha⁻¹ depending on the percolation rate. In the southeastern cropping systems, nitrate is more readily leached due to the coarse texture of the soils in the Coastal Plain. Leaching is influenced by rate and timing of N application as well as rainfall and irrigation timing and amount. The probability of NO_3^- leaching increases when irrigation is applied and with single fertilizer applications. Nakamura et al., (2004) reported that split application compared to single fertilizer application reduced nitrate leaching by approximately one-third.

Runoff

Runoff is another factor which can cause the loss of soluble N. Runoff is defined as that portion of precipitation or irrigation that does not infiltrate in each area, but instead is discharged. Runoff contributes to the pollution of surface water such as rivers, streams, and lakes, when chemicals and nutrients are carried to water sources through runoff. For example, in many agricultural watersheds over application of fertilizers has become a problem due to excesses of nutrients such as N and phosphorous (P). These nutrients can pollute water systems and cause eutrophication (Shuman, 2002). Irrigation is also a problem that producers face when

controlling runoff from agricultural fields. After fertilization, especially with urea-based products, producers with irrigation will water in the fertilizer to prevent losses. Although this is beneficial, loss of nutrients can occur especially with over application of N (Chichester, 1977).

Denitrification

Denitrification is another pathway in which N is lost from the N cycle. The process of denitrification consists of the breakdown or reduction of NO_3^- to NO_x . The intermediate of the reaction which has been documented as a pollutant is N_2O . The pathway of denitrification is largely influenced by the presence of soil bacteria such as *Pseudomonas*, *Bacillus*, *Thiobacillus*, and *Nitrobacter* (Coyne, 2008). The physiological trait that all enables these soil bacteria to cause denitrification is the ability to produce N gas by respiratory nitrate reduction (Delwiche, 1976). Factors that influence activity of microorganisms and therefore the denitrification process include oxygen availability, pH, growth requirements, and temperature. Denitrification is an anaerobic process, and can cause increased rates of denitrification (Delwiche, 1976; Tiedje, 1998). A study in lower Coastal Plain soils noted that denitrification was also increased in highly acidic pH values, those less than 4.0, and at values above 7.0 (Waring et al, 1983).

Crop Removal

Crop removal is another form of N loss present in modern agriculture. In general, production agriculture of row crops removes substantial N from the soil which raises the need for consistent N fertilization per growing season. Various studies indicate crop rotations can help reduce N losses. For example, in the Corn Belt, yields with a continuous corn system produced 5-15% less yield than corn following soybean, even after the contribution of N had been considered (Benson, 1985; Crookston et al., 1991). Other studies have examined historical dry

matter and nutrient partitioning in cotton cultivars developed over the past 30 years (Pabuayon et al., 2020). With increasing yields over this 30-year period, boll production has increased and the partitioning of essential nutrients (N, P, K, Ca, Mg) has moved towards reproductive (seed and fiber). Although yield has increased, nutrient loss has increased from nutrient removal during harvest.

Volatilization

Volatilization is another major pathway of loss present in the N cycle in which NH_4^+ is converted to NH_3 and subsequently lost to the atmosphere (Frame et al., 2013; Hargrove, 1988). This conversion process occurs through hydrolysis of the urea molecule into NH_4^+ via the urease enzyme. The effects of high (?) pH combined with the presence of bicarbonate allows for NH_4^+ conversion to NH_3 (Ciurli et al., 1999). Source of N fertilizer dictates the susceptibility to volatilization. Urea based fertilizers such as granular urea (46-0-0) and UAN (28-0-0) are more often subject to higher volatilization rates due to hydrolysis. As hydrolysis occurs, H^+ ions are consumed causing an increase in pH and NH_4^+ decomposition. Other N fertilizer sources such as ammonium sulfate and ammonium nitrate are less susceptible to volatilization because they avoid the pH increase that accompanies urea hydrolysis (Del Moro et al., 2017; Kissel et al., 2008). Volatilization is influenced by several factors including: 1) soil texture, 2) cation exchange capacity (CEC), 3) soil organic matter, 4) pH, 5) soil moisture, and 6) temperature. These factors have been studied to show an effect on volatilization percentage of N-based fertilizers (Ernst et al., 1960).

Soil Texture and Cation Exchange Capacity

Soil texture and CEC play a significant role in N volatilization. Coarse textured soil types are prone to higher volatilization rates (McCarty et al., 1989; Silva et al., 2017). Ammonia losses are negatively correlated with silt, clay, and SOM content thus clay content has been noted to decrease volatilization losses while soils with high sand content increase volatilization rates (Francisco et al., 2011). The effect of soil texture on N volatilization is closely tied to soil CEC. Clay minerals and organic matter have negatively charged sites on their surfaces which adsorb and hold positively charged ions, like NH_4^+ , by electrostatic force. Clays such as kaolinite have a CEC of about 10 meq/100 g, while illite and smectite have CECs ranging from 25 to 100 meq/100 g. Organic matter has a very high CEC, ranging from 250 to 400 meq/100 g (CUCE, 2007). The amount of NH_4^+ adsorbed to the soils is related to the clay content and the type of clay minerals. For example, with Kaolinites have less NH_4^+ adsorption capacity than smectites. Volatilization tends to decrease with increasing CEC (Hargrove 1988). In a study conducted by Fenn and Kissel, N loss from volatilization ranged from 14% to 90% and volatilization decreased as CEC increased (Fenn and Kissel., 1976).

Soil organic matter can reduce volatilization rates due to its high CEC (O'Toole et al., 1985). However, urease activity has also been negatively associated with decomposing organic matter and soil organic C (Rochette et al., 2009). The presence of crop residue or OM on soil surfaces has been shown to also increase volatilization with urea based products due to the lack of contact with the soil, thus limiting NH_4^+ adsorption at the cation exchange sites (Silva et al., 2017; Francisco et al., 2011).

Soil pH

Soil pH is another major factor influencing ammonia volatilization in production systems, and volatilization tends to increase with increasing soil pH. Ernst et al., (1960) used a lab incubation method and determined that as pH increased, NH_3^g emissions increased with granular urea for any soil pH above 6.0. Another soil property related to soil pH is the H^+ buffering capacity. Work reported by Ferguson (1984) and Hargrove (1988) demonstrated that NH_3 release decreased with increasing H^+ buffering capacity. Other results showed that for a soil the amount of H^+ buffering capacity between the initial pH and a pH of about 7.5 would be more directly related to NH_3 loss potential rather than just the initial soil pH (Ferguson et al., 1984).

Soil Moisture

Soil moisture also plays a role in NH_3^g retention or release. Studies have noted that air dry soil allows for maximum volatilization (Fenn and Kissel., 1976). However, moisture is needed from humidity for the urea hydrolysis to occur. When applying urea-based products, follow-up rainfall or irrigation are of utmost importance in minimizing NH_3 losses. Adequate moisture and precipitation events following urea application can decrease volatilization losses to less than 10% N applied (Engel et al., 2011). In a study conducted by Burch and Fox (1989), soil moisture contents of 0.15 kg kg^{-1} resulted in 30% NH_3 volatilization from surface applied urea for a silt loam soil. Nitrogen loss was reduced to 18.3% for the same soil at 0.22 kg kg^{-1} moisture. These data demonstrated that adequate soil moisture can reduce volatilization rates. However, temperature fluctuations can induce variable effects.

Temperature

Temperature is one of the most important factors to affect NH_3 volatilization from surface applications of N fertilizer (Fan et al., 2011). The loss of NH_3 following soil application of urea increases with an increase in temperature up to 45 °C. Volatilization rate increases because soil temperature influences ammonium NH_4^+ absorption and NH_3 diffusion rate (Avnimelech and Laher 1977, Fenn and Hossner 1985; He et al. 1999). A study by He et al. (1999) reported the potential maximum NH_3 volatilization increased two and threefold with an increase in the incubation temperature.

Nitrogen Loss Inhibition

Volatilization Inhibitors

Nitrogen losses come in various forms, and many efforts have been made to limit those losses in the environment. Urease inhibitors are an important tool for reducing N loss from volatilization. Various types of volatilization inhibitors are commercially available for slowing and preventing N release. The active ingredients in these product block specific enzymes, thereby preventing or slowing the transformation of NH_4^+ to volatile NH_3 . The urease enzyme is responsible for the hydrolysis reaction present in the urea transformation process. In general, the goal of the urease-inhibiting products is to delay this reaction and allow time for rainfall, irrigation, or incorporation after fertilizer application. Once an adequate rainfall or irrigation event occurs, urea-based fertilizers move into the soil profile, thus preventing losses by volatilization. Urease inhibitors delay the conversion of NH_4^+ to volatile NH_3 by blocking the urease enzyme, therefore giving producers more time to receive additional rainfall, irrigate, or

incorporate moving the fertilizer down within the soil profile, (Grant et al., 1996). The use of these inhibitors is common in row crop production in the southeast United States, especially in no till or conservation tillage systems due to lack of possibility for incorporation. Application of urease inhibitors to urea-based fertilizers has been studied to reduce NH_3 losses from surface applied fertilizers (Clay et al. 1990; Bremner et al. 1991).

Various forms of urease inhibitors are present in commercial markets such as 1) N-(n-butyl) thiophosphoric triamide (NBPT), 2) N-(n-propyl) thiophosphoric triamide (NPPT), 3) phenylphosphorodiamidate (PPD), 4) thiophosphoryl triamide (TPT), and 5) ammonium thiosulfate (ATS) (Franzen et al., 2011). Urease inhibitors work by forming a nickel-dependent chelated complex within the active site of the urease enzyme. This then causes the enzyme to be inactive, limiting the breakdown of the urea molecule (Clay et al. 1990; Bremner et al. 1991; Mazzei et al. 2020). The most frequently used volatilization inhibitor in today's agricultural market is N-(n-butyl) thiophosphoric triamide (NBPT). This product was first trademarked as Agrotain® (Koch Agronomic Services, Wichita, KS). When fertilizer is applied to the soil surface, NBPT converts to its oxon analog N-(n-butyl) phosphoric tri-amide (NBPTO), which is the actual mode of inhibition on urease activity (McCarty et al. 1989; Rawluk et al. 2001). The delay in hydrolysis reaction reduces the concentration of NH_3 present near the soil surface, which decreases the potential for volatilization and improves the opportunity for rainfall or irrigation to move urea deeper into the soil.

Field and lab studies have shown reduced NH_3 losses from surface applied urea fertilizers. A study conducted by Frame et al. (2012) was aimed to “quantify in vitro N loss from surface-applied urea; and measure the rate and total N volatilization loss from urea coated with

calcium sulfate, potassium sulfate, alone and in combination with the urease inhibitor, (NBPT).” After 96 h, NH₃ volatilization of uncontrolled urea exceeded 70% N loss whereas NBPT reduced cumulative NH₃ losses to 25% of applied N. Others have compared rates of NBPT at 0.05, 0.10, and 0.15% wt/wt, and showed that all rates decreased volatilization (Rawluk et al. 2001). This study also confirmed previous research that sandy loam soils had higher NH₃ losses than clay loam soils. One other lab study conducted by Goos and Guertal (2019) aimed to compare the effects of Agrotain® (NBPT), Nutrisphere®-N (maleic-itaconic polymer (MIP)), and NZone® (Ca-aminoethylpiperazine and Ca-heteropolysaccharides) on urea hydrolysis and ammonia volatilization, when used with granular urea. Cumulative ammonia loss was reduced by 6 to 25% with the addition of NBPT compared to untreated urea 4 days after application. Nutrisphere-N and NZone not reduce volatilization. A field study assessed physiological and yield responses of cotton to urea with and without urease inhibitor NBPT and nitrification inhibitor DCD applied to urea (Kawakami et al., 2012). The urea treatment with NBPT improved cotton N uptake by 17% and N use efficiency by 41% when compared to the urea alone. The addition of NBPT to urea also positively affected leaf chlorophyll content, plant growth and fiber quality. The use of DCD resulted in a decrease in N uptake, N use efficiency, plant growth, and yields.

Controlled Release Fertilizers

Another product aimed to reduce environmental risk as well as increase N use efficiency is controlled release fertilizers (CRF), a concept new in commercial crop agriculture. The goal of these fertilizers is to provide N to the crop over an extended period, especially when N needs are greatest. Another intent of CRF is to prevent N losses associated with volatilization and denitrification. Various forms of CRF are present within agriculture, but most have been studied

in horticultural settings. The main forms of CRF consist of methylene urea's, sulfur coated fertilizers, and polymer coated fertilizers.

Two extended-release fertilizers forms include CRF or slow-release fertilizers (SRF). Slow-release fertilizers represent products for which N release is reduced but not controlled, while CRF products control the rate and duration of N release (Hayling et al., 2013). Examples of SRF fertilizers consist of products such as biological decomposition (urea formaldehyde or methylene urea's) and chemical decomposition (isobutyridene diurea) (Carrow et al., 2001). These products act by slowly dissolving as NO_3^- during the growing season at amounts that coincide with crop utilization rate and reduce potential N losses (Hayling et al., 2013; Olson, 1971). CRF fertilizer products consist of polymer coated and sulfur coated ureas in which N release rate depends primarily on microbial activity and hydrolysis as well as temperature (Hayling et al., 2013).

Over the last decade the use of CRF fertilizers has doubled in North America with uses primarily in horticulture and turf markets (Hayling et al., 2013; Peacock and DiPaola, 1992; Shaviv, 1999). Recent increases in production agriculture have led to further interest and research in row crops. Technologies include resin coated ureas, which are polymer coated products using alkyd polyester, polyurethane, or polyolefin coatings. For these products, N release begins to occur as water moves in and out of the prill (Hayling et al., 2013). The N release occurs as the water diffuses back into the soil (Hummel, 1989; Guertal, 2009) and release is dependent on the coating characteristics, soil temperature, and moisture levels (Christianson, 1988). Studies suggest that CRF fertilizers maintain crop yields while reducing the rates of

leaching, volatilization, and denitrification (Hayling et al., 2013). The biggest problem the CRF fertilizer sources is high product costs.

Various studies have been conducted, mostly in horticulture and turf, showing the benefits of the CRF products. In western Canada, fall application of polymer-coated urea on barley resulted in decreased nitrate accumulation and fertilizer-N loss, while spring application of polymer-coated urea increased crop N uptake (Nyborg et al., 1993). Research on potatoes (*Solanum tuberosum* L.) and onions (*Allium cepa* L.) also showed an increase in yield and quality with CRN (Tindall and Detrick, 1999). A study that evaluated CRN in bell pepper production systems also observed few effects on bell pepper yield or quality in which peppers harvested from the sulfur coated urea treatment had a lower total marketable yield with sulfur coated ureas than peppers from the resin coated urea or drip applied ammonium nitrate (Guertal, 2009). These results support the use of CRN fertilizer products in bell pepper production.

Although horticulture and turf systems have benefited from CRF fertilizers, there is limited research on these products for row crop production systems. One experiment by Howard and Oosterhuis (2008) showed that N application rates on cotton may be reduced by 40% when CRN is used. Another potential CRN use is in the U.S. Corn Belt, since much of the required N is applied in advance of crop uptake. “Winter and spring precipitation in the U.S. Corn Belt often exceeds evapotranspiration, and N loss potential is high” (Balkcom et al., 2003). CRN has potential to improve N use-efficiency in these production systems (Blaylock et al, 2005).

NITROGEN USE EFFICIENCY IN COTTON

Cotton (*Gossypium hirsutum*) is the most-widely produced cash crop in Alabama, with an average of 415,000 acres per year planted during the 2011-2020 growing seasons (Nation Agricultural Statistics Service). Cotton acreage has increased in the Southeast since the eradication of the boll weevil in the 1990s (Mitchell et al., 2010). With increases in acreage, cultivars, production practices, and nutrient management have been studied to improve cotton yield potential. Nitrogen is the element that is most commonly limiting to cotton production, particularly in Coastal Plain soils due to the lack of organic matter and clay content. Deficiencies of N can result in reduction of lint yield and lint quality. Oversupply of N can also be detrimental to yield due to excessive vegetative growth and associated problems of boll rot, defoliation challenges, etc. (Gardner et al., 1967). The main physiological function of N is a central role in plant metabolism. It is a constituent of proteins, nucleic acids, chlorophyll, coenzymes, phytohormones, and secondary metabolites (Hawkesford et al. 2012). Improvements in cotton cultivars and pest control have increased interest in improving N use efficiency to maximize yield and reduce environmental impact. To manage cotton nutrient deficiencies, proper fertilizer use must be incorporated. Factors that influence fertilizer management for production include rate, time, source, and placement (Reiter et al., 2008).

Rate

Testing for optimum N rates for cotton has been performed in Alabama since the 1950s (Scarsbrook and Cope, 1957; Cope, 1970, 1984; Touchton et al., 1981). The optimum N rate for cotton grown on Coastal Plain soils is approximately 100kg ha⁻¹ (Mitchell et al., 2010). Cotton plants take up 90 kg N ha⁻¹ to produce one bale of harvested cotton. Of the 90 kg N ha⁻¹, around

42 kg N ha⁻¹ is removed in the harvested crop, which consists of seed and fiber (Mitchell et al., 2010). Most of the N information gathered for dryland cotton production in the southeast was established prior to the 1940's where cotton cultivars and yield potential were less productive than present day (Fraps 1919, McHargue 1926). However, recent investigators have updated N requirements with current yield potential and cultivars (Teague et al, 2016; Main et al., 2013). Main et al. (2013) reported the optimal N requirement of cotton to be 23 kg ha⁻¹ per 218 kg bale of lint. A study conducted by Boquet and Breitenbeck (2000) aimed to better understand the fate of N in cotton with respect to fertilization rates of 0, 84, and 168 kg ha⁻¹ and quantified the effects of N fertilization rate on seasonal uptake and partitioning of N and dry matter. By the end of effective bloom, plants receiving 168 kg N ha⁻¹ assimilated 15 to 40% more N, primarily in leaves and lower bolls, than plants receiving 84 kg N ha⁻¹. Results showed that increasing the fertilizer N rate to an above-optimal rate of 168 kg ha⁻¹ increased total N uptake to 242 kg ha⁻¹ and decreased lint yield and fertilizer efficiency. Another study by McConnell et al. (1993) illustrates that N rates beyond the N recommendations (e.g., 112 kg N per hectare) delayed harvest without an increase in yield. Various studies have shown the effects of N deficiency evident in reduced vegetative growth, early cutout, and reduced fruiting index, thus demonstrating the importance of N application rates (Gardner 1967; Guinn, 1982; Radin 1986; Gerik et al., 1989). Tissue testing in cotton has been studied to be one of the more effective ways to evaluate N use in cotton production. Plant tissue analysis is the sampling of a diagnostic plant parts with measurement of the nutrient concentration in the tissue or the sap from the tissue collected. Nutrient deficiencies can be evaluated from these tests to apply mid-season or plan for further crops (Mitchell et al., 2010, Touchton et al., 1981).

Timing

Timing of N application in cotton plays a significant role in plant nutrient uptake. Nitrogen is frequently applied in split applications. In the Coastal Plain region, it is common to apply approximately one-third at plant and two-thirds at side dress. Application of fertilizers at these separate intervals gives cotton plants nitrogen when it needs it most. For example, previous studies evaluated cotton growth stages which can be characterized by five growth stages that are interdependent and overlap (Mauney, 1986; Oosterhuis, 1990). These growth stages are emergence, first square (floral bud), first flower, first open boll, and harvest. Timing or interval of these stages is influenced by climate, most notably temperature (Mauney, 1986). Nitrogen availability also influences these growth stages. Therefore, the timing of N fertilizer application is essential to the critical fruiting period. The critical fruiting is approximately from first square (>2 mm) to peak flowering (40-85 days after planting) in which N plays an important role in producing lint yield and lint quality (Gerik et al, 1998). Before flowering, cotton leaves contain 60-85% of the total N. However, N content declines after flowering. Nitrogen translocates from leaves to developing bolls (Gerik et al, 1998). Cotton bolls have a high N requirement, and over 50% of N is concentrated in lint and seed at harvest (Mitchell, 2010). Therefore, cotton N requirements are highest during the latter growth stages, when N supplies typically diminish, and root activity is less. Applying fertilizer at optimum growth stages such as pre-plant and first square aid in advancing cotton boll development therefore maximizing yield.

Placement

Fertilizer placement in cotton production is another factor that influences plant growth. Various N placement strategies exist in cotton production including injection, broadcast,

broadcast incorporated, surface banded, sub-surface banded, foliar application, and seed placement. Studies have been performed to evaluate different placement methods effects on yield. McClanahan et al., (2020) used three placement methods of banding (urea ammonium nitrate [UAN] + ammonium thiosulfate), broadcast (urea + ammonium sulfate) and injected (UAN + ammonium thiosulfate) with five total N rates (0, 45, 90, 135, and 180 kg N ha⁻¹). Nitrogen rate and placement had a significant effect on lint yield, with rates of 133, 128, and 180 kg N ha⁻¹ were the optimum N rates for injected, surface banded, and broadcast systems, respectively, in sandy loam and loamy fine sand soils of Virginia and North Carolina (McClanahan et al., 2020). Another study by Guthrie (1991) showed that lint yield was increased 9% by side-banded starter fertilizer placement compared to broadcast. Foliar application is another placement method that is not as common but has been evaluated across the U.S. Cotton Belt to supplement plant N requirements (Gerik et al, 1998). Studies have suggested that foliar applied N may serve as an N supplement to reduce N deficiency caused by low soil N availability, and to provide cotton plants with the N required by the critical fruiting period (Hake and Kerby, 1988; Miley, 1988).

Source

Fertilizer source also plays a role in maximizing cotton producers yield potentials. The two forms of fertilizer N accessible to plants are ammonium (NH₄⁺) and nitrate (NO₃⁻). Some common forms of N fertilizer include anhydrous ammonia, ammonium sulfate, ammonium phosphate, urea, ammonium nitrate, and potassium nitrate (Jones, 1982). Urea is an inexpensive source of N but is susceptible to volatilization. Organic fertilizer sources are less frequently used for cotton production because organic N must be mineralized before it can be taken up by the

plant (Stevenson, 1982). Legume cover crops are another N source that can be added to the soil (Reeves, 1994). Legume species can fix atmospheric N, by means of a symbiotic relationship with soil microorganisms. Leguminous N fixation can provide greater than a hundred pounds depending on the cropping system, but this N is not always available to the plant (Burton, 1972; Reeves, 1994; Guldan et al., 1996; Gerik et al., 1998; Frankow-Lindberg and Dahlin, 2013). Other products such as enhanced efficiency fertilizers (EENF's) are being introduced into cotton production systems. One study by (Watts et al., 2015) used a closed chamber method to compare greenhouse gas emissions between standard fertilizers and CRF. Another study by Kawakami et al., (2012) showed the volatilization inhibitor NBPT improved cotton uptake and NUE by 17% and 41%, respectively, when compared to urea. Various EENF products are being evaluated in row crops to better understand their potential in in those systems.

Work with volatilization inhibitors (VI) and CRF in cotton production has been limited over the years. Evidence that VI and CRF have aided in various production efforts throughout the southeast lead to the furthering of this research. The problems presented with cotton production and overall N use efficiency should be addressed within agricultural research. Additionally, work with newer forms of CRF and VI should be pursued to better evaluate production benefits and environmental management. This information is the purpose of this research.

RESEARCH OBJECTIVES

It is important to improve N management in agricultural systems to improve profitability for producers and reduces the risk of N loss to the environment. The use of stabilized and controlled release N products has potential to increase NUE in the coastal plain soils of Alabama, where coarse textured soils and the hot humid climate increase risk of N loss. More research is needed to better understand the efficiency of stabilized and controlled release products for cotton production systems. Thus, the objective of this experiment is to assess N use efficiency for various N fertilizers and stabilizers through field and laboratory experiments and to evaluate N fertilizer source impact on cotton yield and fiber quality.

II. ENHANCING NITROGEN USE EFFICIENCY IN ALABAMA COTTON PRODUCTION SYSTEMS

ABSTRACT

Cotton is the most-widely produced cash crop in Alabama, and N is often the most limiting nutrient in cotton production systems. Loss pathways such as leaching and volatilization can lead to significant losses of applied N fertilizer, particularly in coarse-textured, highly weathered soils of the Coastal Plain. Stabilized and controlled-released N fertilizers have been documented to reduce N losses and increase efficiency and profitability for farmers. However, these products lack evaluation in cotton (*Gossypium hirsutum* L.) production systems. The objective of this experiment was to 1) evaluate N fertilizer source impact on N uptake and cotton yield and 2) evaluate urease inhibitors for their ability to reduce N volatilization. Field experiments were established at the Wiregrass Research Extension Center (WREC) in Headland, AL and E.V. Smith Research Center (EVS) near Shorter, AL. Nine fertilizer treatments were organized in a randomized complete block design and replicated four times. Data collection included leaf N, petiole N, soil N, cotton yield, and fiber quality. Leaf N for WREC 2021 at early bloom and peak bloom showed that 28-0-0-(5) had a greater leaf N concentration than all other treatments, while few other treatments were different than the untreated control for leaf N. For WREC, 39-0-0 PC and 44-0-0 PC had higher petiole N than all other treatments at first square, indicating that polymer coated products released N too early in the growing season. All early bloom treatments except for 39-0-0 PC and 44-0-0 PC were higher than the control in 2020. Soil N for both years and locations were variable and showed limited differences between treatments. At WREC, 28-0-0-(5), 46-0-0 +NBPT, 46-0-0 + NBPTD, 40-0-0, and 39-0-0 PC produced 400-

600 kg ha⁻¹ greater lint yield than the control. Other N source treatments including the 46-0-0, 44-0-0 PC, and 32-0-0, were not different than the control. Data indicated that urease inhibitor products 46-0-0 + NBPT (1216 kg ha⁻¹) and 46-0-0 + NBPTD (1255 kg Ha⁻¹) show effectiveness at increasing yield when compared to base 46-0-0 (1185 kg ha⁻¹). The polymer coated products did not indicate differences when compared to the untreated base products. However, this data suggests the opportunity to apply polymer coated N products in only a single pass through the field rather than in multiple applications, thus saving some costs.

Laboratory incubation experiments were established to measure N volatilization for three conventional fertilizer sources: 1) urea, 2) UAN 3) Amidas[®]. Each fertilizer was evaluated with 1) NBPT 2) NBPT/Duromide (NBPTD) and 3) untreated control and replicated four times for the Coastal Plain soil type. The ammonia volatilization studies were performed using a laboratory incubation method. Results showed untreated urea having almost 40% of total N lost as NH₃, compared to 25% with NBPT products applied. NBPT and NBPTD applications significantly minimized NH₃ losses by 10-15% within the first 4 days of treatment compared to the untreated urea. For Amidas[®], NBPT and NBPTD reduced nitrogen volatilization from 5.9% cumulative N loss to less than 1% cumulative N loss at day 4 compared to untreated Amidas[®]. The utilization of volatilization inhibitors proved to be effective at reducing volatilization up to four days which urea would allow producers the time for a rain/irrigation event to occur.

INTRODUCTION

Cotton (*Gossypium hirsutum*) is the most-widely produced cash crop in Alabama. Nutrient management is an important consideration in cotton production in order to optimize yield and reduce off-site movement of nutrients and thus minimizing environmental pollution. Nitrogen is the most limiting nutrient to cotton production. In the Coastal Plain, soils are coarse textured and low in organic matter, leading to high N loss potential. Loss pathways such as leaching have been reported to reduce yields by the leaching of NO_3 through the soil profile (Nakamura et al., 2004; Smika et al., 1977). Other loss pathways that negatively affect N use efficiency are denitrification and volatilization. Denitrification is the process by which NO_3^- is converted to NO_x . Volatilization is the loss of gaseous NH_3 which is common in urea base fertilizers (Frame et al., 2013; Hargrove, 1988). Various factors which influence urea-based fertilizer volatilization include 1) soil texture, 2) CEC, 3) soil organic matter, 4) pH, 5) soil moisture, and 6) temperature (Ernst et al., 1960). Practices such as improving fertilizer placement and timing of application can help prevent N loss (Reiter et al., 2008), but enhanced efficiency fertilizer products can further increase N use efficiency. Urease inhibitors reduce volatilization and thereby reduce N losses. The active ingredients in urease inhibitors block specific enzymes to slow the transformation of NH_4^+ to volatile NH_3 . In a field study examining physiological and yield responses of cotton to urea with and without N stabilizers, Kawakami et al. (2012) found that urea with the urease inhibitor NBPT improved N uptake 17% compared to urea alone. NBPT addition to urea also positively affected leaf chlorophyll content, plant growth and fiber quality. Other lab studies have shown that NH_3 volatilization of urea can exceed 70% N loss over a 96 h incubation, whereas NBPT reduced cumulative NH_3 losses to 24.7% of applied N (Frame et al. 2012). Controlled release fertilizers (CRF) are another enhanced efficiency

fertilizer product intended to provide N to the crop over an extended period when the crop needs it most. Another intention of the CRF is to prevent N losses by limiting N release over time, which can prevent volatilization and denitrification. Over the last decade use of controlled release products has doubled in North America, but uses have largely been in turf, ornamental, and vegetable production systems. As production of CRF increases and the price of N rises, it may be more economically feasible to use CRF products in row crop production. Studies have shown that CRF N use in the U.S. Corn Belt has potential to improve N use-efficiency (Blaylock et al, 2005). More information on these products in row crops such as cotton.

Enhanced efficiency fertilizers including stabilized and CRF products aim to reduce N losses in addition to NUE practices such as rate, time, place, and source. Information regarding the efficacy of enhanced efficiency fertilizers is needed to help producers reduce N losses and improve profitability. The objective of this study was to first evaluate N fertilizer source effects on soil inorganic N, plant nitrogen uptake, and cotton yield and fiber quality. The second objective was to evaluate urease inhibitors for reducing N loss from urea-based products.

MATERIALS AND METHODS

Experimental Design

Two Alabama locations within the Coastal Plain were selected to evaluate nitrogen sources for cotton production: Wiregrass Research and Extension Center (WREC) and E.V. Smith Research Center (EVS). The WREC soil was a Lucy loamy sand (Loamy, kaolinitic, thermic Arenic Kandiudults), while the EVS soil type was a Bama sandy loam (Fine-loamy, siliceous, subactive, thermic Typic Paleudults). Nine treatments were organized in randomized

complete block design with 4 replications. Nitrogen treatments included: 1) Urea, 2) Urea+Agrotain® or (26.7%) (N-(n-butyl)-thiophosphoric triamide (NBPT)), 3) Urea+Anvol™ or (16%) +Duromide (Duromide (27%) (NBPTD), 4) ESN® (polymer coated urea), 5) Polymer coated Amidas® (urea/ammonium sulfate blend), 6) Amidas® (urea/ammonium sulfate blend), 7) ammonium nitrate/ammonium sulfate blend, 8) urea ammonium nitrate (UAN), and 9) an untreated control (Table 1). Treatments will be referred to by their fertilizer rating ??? throughout this chapter. Each plot was 8 rows wide with a 91 cm row spacing and 10.9 m long. Nitrogen fertilizer treatments were applied at a rate of 100 kg ha⁻¹ N per acre, with one-third of total N applied at plant and two-thirds applied at side dress. Polymer-coated products ESN® and polymer-coated Amidas® were applied entirely at plant. For products which do not contain sulfur (S), 15 kg ha⁻¹ S per acre was applied at plant as MgSO₄. Granular fertilizer was spread by a First Products stainless steel in row banded spreader for pre plant and side dress application dates. Liquid products were surface banded approximately 10 cm from the cotton row.

The cotton variety (Deltapine) DP 1646 B2XF was planted at both locations. Tillage was performed prior to plant with a KMC strip till. Crop management followed general recommendations from the Alabama Cooperative Extension System. Cotton was machine harvested with a two-row John Deere 9910 picker, hand ginned for lint turnout, with samples sent to the USDA Cotton Classing Office in Memphis, TN, for fiber analysis.

Soil and Plant Material Collection

Soil, leaf, and petiole samples were taken according to plot at the following cotton growth stages: first squaring, early-bloom, and mid-bloom (Table 2). In 2021, WREC mid-bloom soil and leaf samples were collected at late bloom due to rainfall and field conditions. Soil

samples were collected by push probe from the 0-15 cm and 15-30 cm depths, for which each sample was composited from 10 to 12 subsamples per plot. Composite samples from each plot were sieved to 2mm and stored at 4°C . Inorganic N was then measured for field moist soil samples within 48 hours of sample collection. A 5-g sample of field moist soil was weighed, and 20 mL of 2M potassium chloride (KCl) was added and shaken in centrifuge tubes for 60min. Centrifuge tubes were then allowed to settle and soil solution was extracted through Whatman™ 40mm filter paper. Potassium chloride extractions were kept at 4°C until the calorimetric analysis was performed (Keeney and Nelson 1982). Nitrate-N (NO₃-N) at 695nm and ammonium-N (NH₄-N) 542nm were measured by colorimetric determination with a BioTek® uQuant™ microplate spectrophotometer (Keeney and Nelson 1982). Dry weight was calculated by weighing approximately 5g of moist soil and drying at 105°C for 48h, the reweighing. Dry weight was divided by wet weight to obtain % moisture.

Leaf and petiole samples were collected from the uppermost fully expanded leaves from 10 to 12 plants per plot. Composited samples were measured for total N content in leaf and petiole tissue samples at Waters Agricultural Lab in Camilla, GA. Leaf and petiole tissue were measured separately by a combustion analysis to determine N concentration on a per plot basis. Leaf and petiole analysis for the 2020 EVS mid-bloom sampling date were lost in shipment to Waters Agricultural Lab.

EVS data were measured and evaluated for the 2021 growing season, but due to variability in field and environmental conditions, data are not presented in this thesis. The trial location was previously half in pine forest and other in fallow land, which resulted in extremely variable growth and masked any treatment effects

Polymer Coated Fertilizer Release

A buried bag technique was used to evaluate the N release of the polymer coated N fertilizers (Carson, 2014). Polymer coated fertilizers were weighed out to 10g and placed in mesh bags. The bags were then buried in corresponding plots and collected at weekly intervals for ten weeks after application at each location. The bags were then dried at 40°C overnight and weighed to measure total N loss by weight.

Ammonia Volatilization:

Soil samples of a course textured Bama sandy loam (Fine-loamy, siliceous, subactive, thermic Typic Paleudults) were collected to approximately 0-15 cm depth. Soil was sieved and air dried before bringing to field capacity. Dry weight was calculated by weighing approximately 5g of moist soil and drying at 105°C for 48h, the reweighed dry weight was divided by wet weight to obtain % moisture. Percent moisture and water holding capacity at 1/3 Bar were then used to calculate water per weight of soil to reach field capacity. Field capacity soil was then placed in into the volatilization system jars. To begin each experiment, fertilizers and inhibitors were added to the soil surface and the jars sealed. N volatilization rates were measured for three conventional fertilizer sources: 1) urea, 2) 32% urea ammonium nitrate (UAN), and 3) homogenized urea + ammonium sulfate blend (Amidas®). Each fertilizer was evaluated with 1) NBPT (26.7%)(N-(n-butyl)-thiophosphoric triamide (NBPT)), 2) NBPT (16%) +Duromide (Duromide (27%) (CAS RN 94317-64-3) (NBPTD), and 3) untreated control. Each treatment was replicated four times for the Coastal Plain soil type. All the fertilizers were applied at a rate

of 100 kg ha⁻¹ to the surface of the soil Samples. The ammonia volatilization studies were performed using a laboratory incubation method (Figure 1). The volatilization system consisted of a series of 16 jars where ammonia loss was measured for 10 consecutive days via 0.01 N boric acid trap. To do this ammonia was collected via an ammonia trap system, following the method of O'Halloran (1993) (Figure 1). Air flow was generated by passing 100 mL min⁻¹ air stream through a 5N sulfuric acid air scrubber and across each jar, with resultant NH₃ trapped in 100 mL of 0.01 N boric acid. The boric acid trap was changed every day for 10 days, with collected samples titrated to the original pH of the boric acid using 0.01 N sulfuric acid. The mg of N and percent N volatilized were measured by the formulas below.

Equation 1:
$$mg - N = (mL\ acid \times N\ acid \times 14)$$

(here 14 is the equivalent weight of N)

Equation 2
$$\% N\ Volatilized = \frac{mgN}{179}$$

(179 is the mg-N per jar.)

Statistical Analysis:

For the volatilization incubation study daily and cumulative N volatilization, data were subjected to mixed model repeated measures analysis of covariance using PROC GLIMMIX in SAS Version 9.4 (SAS Institute Inc., Cary, NC). The volatilization trend was modeled using

splines (piecewise second-degree polynomials). The first order autoregressive covariance structure AR (1) was used to account for repeated measures among days. Treatment and the daily trend were used as fixed effects and test was used as random effect. N volatilization differences due to treatment effect was evaluated at each day. For all analysis, degrees of freedom were calculated using the Kenward-Rodger method and the adjust=simulate option was used to adjust mean differences for multiplicity at $\alpha=0.05$ (Littell et al., 2006).

All data were subjected to mixed model analysis of variance using PROC GLIMMIX in SAS Version 9.4 (SAS Institute Inc., Cary, NC). For cotton yield, data were analyzed separately by location. For leaf and petiole N content, data were analyzed by location and time. For ammonium and nitrate content, data were analyzed by location, time, and depth. In all analyses, treatment, and year (for WREC) were used as fixed effects and replication was used as random effect. Degrees of freedom were calculated using the Kenward-Rodger method and the Tukey adjustment was used to adjust mean differences for multiplicity $\alpha= 0.1$ (Littell et al., 2006).

RESULTS AND DISCUSSION

Leaf N

Leaf N was measured to assess uptake of N according to N source treatment. Treatments were analyzed by sampling date (first square, early bloom, and peak bloom), in which differences were compared between treatments. Treatments for 2020 and 2021 at first square showed no significant differences between treatments (Fig. 1A). However, there were differences between years for the first square sampling time. Across treatments, leaf N was higher for 2021 compared to 2020. All treatments were numerically greater than the control at first square in 2020 and 2021. At WREC, an interaction of year by treatment was observed for early bloom and

peak bloom (Table 3). At the early bloom sampling date, leaf N for WREC did not show statistical differences in 2020 (Figure 2B). However, differences did occur in 2021. The 28-0-0-(5) treatment had greater leaf N concentration than all other treatments, and 46-0-0 and 28-0-0-(5) had greater leaf N than the control. All treatments had numerically greater leaf N than the control. At the peak bloom sampling date, WREC leaf N data (Figure 2C) did not show statistical differences for 2020. However, similar to early bloom, treatment differences did occur in 2021. The treatment 28-0-0-5 was the only treatment with greater leaf N than the control. The similar trend between early bloom and peak bloom for WREC in 2021 showed that leaf N content was greatest for 28-0-0-(5). Other studies have also shown plant N accumulation for 28-0-0-(5) to be higher than other N sources (Gagnon et al., 2012, Cahill et al 2007). The increased leaf N uptake for the 28-0-0-(5) treatment may be due to the precise placement of fertilizer with the liquid applicator approximately 10 cm from the plant row. Interestingly, leaf N for the control was statistically similar to most N source treatments for both years and sample dates despite visual deficiency symptoms being observed in the field.

Leaf N for EVS was only analyzed for 2020, due to high field variability in 2021. Nitrogen source did not affect leaf N content at EVS (Figure 3). The 44-0-0 PC, 39-0-0 PC, and the untreated control treatments were numerically the highest at first square, demonstrating that leaf N data can be highly variable. All treatments had greater than 5.5% leaf N (Figure 3A). Leaf N content for early bloom was numerically lower than first square, and all treatments had less than 4.5% leaf N (Figure 3B). There were no significant differences between treatments at early bloom, but 46-0-0 + NBPT and 28-0-0-5 had numerically higher leaf N content than other treatments. However, unlike first square, early bloom data showed the control to be numerically the lowest leaf N content.

Leaf N data was compared to reference sufficiency ranges for cotton in the southern region (Campbell and Plank 2011). Sufficiency ranges for cotton are based upon observations for plant tissue analysis from normal, healthy cotton crops. The critical range for cotton leaf N concentration from early bloom to late bloom is 3 to 4.5% N (Campbell and Plank 2011). In the current study, all measurements for leaf N at each sampling time showed concentrations within or above the reference sufficiency range. This was unexpected, since visual N deficiency symptoms were observed in the field, especially in the untreated control. A dilution effect may have masked deficiency symptoms in stunted plants observed in untreated control plots in the current study. Nitrogen concentrations associated with deficiencies are not absolute and factors such as water availability, node position, date of sampling, and leaf position can also influence leaf N concentration (Oosterhuis et al., 2002).

Petiole N

Petiole N was measured to assess uptake of N according to N source treatment and to compare with leaf N content. Treatments were measured by sampling date (i.e. first square, early bloom, and peak bloom) in which differences were compared between treatments (Table 3). There was no interaction of year by treatment for the first square sampling date, but there was a treatment effect (Table 3). All treatments had greater petiole N than the control. The two polymer coated treatments, 44-0-0 PC and 39-0-0 PC, both had greater petiole N content than the control treatment, 28-0-0-(5), 32-0-0, and 40-0-0 (data not shown). The higher petiole N contents for the polymer-coated treatments at first square are likely due to the higher N rates applied to

these treatments at planting. These products are intended to provide a slow-release form of N throughout the growing season. However, greater than 70% of applied N was released by six weeks after application (Fig, A1).

For the early bloom sampling time at WREC, an interaction of year by treatment was observed for the early bloom sample date (Table 3). There were no treatment differences in 2021, but differences did occur in 2020 (Figure 4B). All treatments except the polymer fertilizers (i.e., 39-0-0 PC and 44-0-0 PC) had greater petiole N content than the control. This indicates that N from polymer-coated products may have been released prior to peak N demand by the cotton crop. Overall, petiole N was numerically lower for early bloom (200-4200 mg NO₃-N kg⁻¹) which is a decrease from the first square sampling date (800-8000 mg NO₃-N kg⁻¹). Peak bloom petiole N data (Figure 4C) showed limited differences between treatments for both years. Peak bloom data for 2020 showed the urea treatment to be the greatest numerically with the control being the next greatest. However, most treatments showed no statistical differences. Peak bloom data for 2021 was similar to 2020 where most treatments were not different except for 28-0-0-5. Treatments for 2021 were all below 700 mg NO₃-N kg⁻¹ except for 28-0-0-5, which was 5300 mg NO₃-N kg⁻¹.

Petiole N for EVS (Figure 5) showed no differences between treatments in 2020 for both sampling dates. First square petiole N data was extremely high, with all values above 19,000 mg NO₃-N kg⁻¹. First square petiole N concentrations showed 44-0-0 PC and the control to be the highest numerically. Early bloom data showed a similar pattern where there were no treatment differences. However, petiole N content was significantly lower where values were no

greater than 1400 mg NO₃-N kg⁻¹. All treatments were greater than the control numerically. EVS data for peak bloom data was lost during the shipment process and could not be evaluated.

Petiole N data were compared to reference sufficiency ranges for cotton in the southern region (Campbell and Plank 2011). Sufficiency ranges for cotton are based upon observations for plant tissue analysis from healthy or normal cotton crops. Results were compared to the Georgia interpretation of petiole analysis (Lutrick et al., 1986; Plank 1988), since soil types are similar to those in the current study. It is important to note that factors such as water availability and node position can affect the petiole NO₃⁻ readings (Oosterhuis et al., 2002). The sensitivity of petiole tissue sampling can be beneficial, but correct sampling methods, analysis readings, and data interpretation are very important to effectively interpret results and maximize production potential (Livingston et al., 1996). Recommended sufficiency ranges at the week before first bloom range from 7,000 to 13,000. In the current study, not all treatments were within or above the reference sufficiency ranges for this sampling interval. WREC first square (Figure 4A) was outside the sufficiency range where treatments were between 1000 (control) and 6000 except for 39-0-0 PC. WREC Early bloom data for 2021 were observed to be mostly within sufficiency ranges except for the control (Figure 4B). Studies have noted that petiole NO₃⁻ levels decrease from the first week before flowering (squaring) until the third week after first flower (Mozaffari et al., 2004). Redistribution within the plant from day 40 to 60 was consistent with the results. In some instances, petiole N was more reflective of treatment differences than leaf N. For example, at first square for WREC, petiole N was much lower for the control treatment compared to other N treatments, which corresponded to visual deficiencies observed in the field.

Soil Nitrogen

Soil inorganic N (i.e., NO_3^- and NH_4^+) was measured to assess plant-available soil N content for the 0-15 and 15-30 cm depths at first square, early bloom, and peak bloom (Table 4). Ammonium data for WREC were measured for 2020 and 2021 (Table 4). First square data for 0-15 cm and 15-30 cm showed no differences for year by treatment. Most treatments were below detection levels for 2021. First square data from 2020 did show higher levels of NH_4 N where values were between 1.4 and 3.2 mg N kg^{-1} (Table 5). Early bloom data showed differences between year and year by treatment for both depths. Most treatments were not different except for 46-0-0 + NBPT and 28-0-0-5 which were greater than all other treatments for 2021 at 0-15 cm and 15-30 cm. Early bloom 46-0-0 + NBPT for 2020 0-15 cm was numerically greater than all treatments at 12.52 mg N kg^{-1} . Peak bloom was assessed for 2020 and showed no treatment differences.

Nitrate data from the WREC location showed differences in the first square sampling date where year by treatment for 0-15 cm was significant but treatment was not (Table 4). First square sample date for 0-15 cm showed 40-0-0 in 2020 to be the greatest NO_3 concentration where in 2021 it was below detection levels with 46-0-0 + NBPT being the greatest. Early bloom data showed no differences between treatments for both years and sample depths, however there was some numerical differences between treatments. Data from the 2020 0-15 cm depth showed high variability in treatments where 46-0-0 + NBPT and the control were greater than 20 mg N kg^{-1} . The equivalent of this NO_3 value is approximately 60 lbs of N. Data from 2021 were not as variable; however, 28-0-0-5 and 46-0-0 + NBPT had the greatest NO_3 concentrations. Peak bloom data showed no significant differences between any effect and were only measured for 2020 due

to late sampling conditions for 2021. Overall, very few meaningful treatment differences were observed.

Ammonium data for EVS in 2020 showed differences among treatments for first square and peak bloom for both 0-6" and 6-12" depths (Table 4). Polymer coated products (44-0-0 PC and 39-0-0 PC) had numerically higher NH_4 than all other treatments for first square at 0-6" and 6-12". At early bloom for both depths there were no significant differences among treatments. For the 0-6" depth, NH_4 N ranged from 1- 2.5 mg N kg^{-1} soil for all treatments. At early bloom at the 6-12" depth, 40-0-0 and 28-0-0-5 had numerically greater NH_4 (Table 5). There were slight numerical differences in NH_4 at peak bloom; however, most treatments were significantly lower than previous sample dates possibly due to the time between fertilizer application date and sample date. All NH_4 N values for peak bloom were below 1 mg N kg^{-1} except for the 46-0-0 + NBPT (Table 5). These data showed minimal treatment difference for soil NH_4 N. However, the data suggest that soil NH_4 decreases with later sampling date. Data for 2021 were not included due to high variability in the field.

Nitrate N was also evaluated for EVS but there were no differences among treatments for 0-6" and 6-12" depths (Table 4). Nitrate levels from EVS were below detection for most treatments in 2020, with all treatments below 1 mg N kg^{-1} for both depths at first square. For early bloom, there was no significant difference among samples, but there was a numerical difference where 28-0-0-5 0-6" was the highest NO_3 concentration when compared to other treatments. The early bloom 6-12" depth showed 40-0-0 to be the greatest numerically. Peak bloom sample date showed most treatments were below detection levels for both depths. These data suggest that NO_3 was removed possibly due to leaching. Soil N data can be variable in

warm, humid environments which promote continuous transformations and losses, thus the level of NO^- in the soil could change substantially between the time of sample collection and lab analysis (Raper and Duncan 2017). Similar to WREC, no meaningful treatment differences for EVS were observed, possibly due to the abundance of rainfall in 2021 and resulting N losses..

Cotton Yield

Cotton lint yield differences among treatments and years at WREC were evaluated in 2020 and 2021. A significant treatment effect was observed, but no interaction with year was observed (Table 3). Lint yield was lowest numerically in the control plots and greatest in 28-0-0-5 (1381 kg ha⁻¹). At WREC 28-0-0-(5), 46-0-0 +NBPT, 46-0-0 + NBPTD, 40-0-0, and 39-0-0 PC produced 400-600 kg ha⁻¹ greater lint yield than the control. Other N source treatments including the 46-0-0, 44-0-0 PC, and 32-0-0, were not different than the control. Other treatments such as urease inhibitor products 46-0-0 + NBPT (1216 kg ha⁻¹) and 46-0-0 + NBPTD (1255 kg ha⁻¹ increased yield compared to base 46-0-0 (1185 kg ha⁻¹). These data represent the ability of stabilized fertilizers to increase yields by decreasing N losses. Other treatments such as the polymer coated product 44-0-0 PC (1088 kg ha⁻¹) were not as effective and were numerically less than untreated 46-0-0. The other polymer coated product 39-0-0 PC was among the highest yielding treatments at 1325 kg ha⁻¹.

Yield differences among treatments at E.V. Smith Research Center (EVS) for 2020 are provided in Figure 7. No significant statistical yield differences were detected among treatments, though 28-0-0-5 and 32-0-0 had the greatest numerical yields at 1437 and 1432 kg ha⁻¹, respectively. Base untreated 46-0-0 was greater than both stabilized products NBPT and NBPTD. The untreated control was higher than expected which can be attributed to higher field

and soil variability in the trial location. Factors such as organic matter, crop residue, previous cropping systems, and pre-existing available soil N could be factors that led to observed variability. Data from 2021 were not presented due to extreme field variability.

Lint Quality

Lint quality, including fiber length, strength, micronaire, and uniformity, was evaluated at the WREC for 2021. Fiber length was the only variable that showed numerical differences among treatments, with 39-0-0 PC was the greatest numerically and 46-0-0 + NBPT and 44-0-0 PC were the lowest (Table 6). Strength, micronaire, and uniformity showed no statistical differences among treatments. No deductions for lint quality would have occurred based on U.S upland classification standards.

Volatilization Study

The volatilization incubation study was performed to better understand urease inhibitors on urea-based fertilizers in coastal plain soils (Table 7). Ammonia loss was analyzed as cumulative percent N loss according to sampling day. Data shown in Figure 8A indicate N loss for untreated urea compared to urea treated with NBPT and NBPTD. For urea, day 1 and day 2 showed no significant differences between treatments, and cumulative N volatilization was less than 1% NH₃ loss for all urea treatments. By day three, a significant difference between untreated urea and urea treated with NBPT and NBPTD was apparent. Both volatilization inhibitors had < 1% N volatilization compared to untreated urea at 5.3%. Day 4 was similar to day 3, where untreated urea reached 21% N volatilization. Nitrogen loss from urea was reduced to 1.3% and 2.1% when treated with NBPT and NBPTD respectively. Between day 5 and 10, there was no statistically significant differences between the untreated urea and NBPT and

NBPTD. However, day 5 and 6 did show substantial differences in cumulative N loss where day 5 untreated urea reached 29% N volatilization. Nitrogen loss from urea was reduced to 5.2% and 10.4% for NBPT and NBPTD, respectively. Total N volatilization at day 10 was relatively similar between all treatments.

Nitrogen volatilization from untreated Amidas compared to Amidas treated with NBPT and NBPTD is shown in figure 8B. Like Urea, Amidas for days 1 and 2 also showed minimal ammonia loss as well as no significant differences between treatments. At day 3, untreated Amidas had greater volatilization percentage than Amidas + NBPT. Untreated Amidas had a total ammonia loss of 1.2% compared to 0.1% for Amidas + NBPT and 0.2% for Amidas + NBPTD. Like day 3, day 4 showed a statistical difference between untreated Amidas (5.9% N loss) and Amidas + NBPT (0.60% N loss). However, there was no difference between untreated Amidas and Amidas + NBPTD. Day 5 showed no differences among treatments, but untreated Amidas did show to be numerically greater at 12.6%. Similar to urea, N loss at days 6-10 showed no significant differences among treatments by day, however there was numerical differences among treatments where untreated Amidas remained the highest cumulative N loss.

UAN was the other fertilizer source evaluated for ammonia N loss (Figure 8C). UAN had the lowest overall N loss by substantial amounts when compared to other treatments. There were no significant differences among untreated UAN, UAN + NBPT, and UAN + NBPTD over the 10-day volatilization period. No treatments exceeded 4% cumulative ammonia loss by day 10, indicating that the volatility of UAN as a fertilizer was minimal.

This current experiment suggests that the urease inhibitors NBPT and NBPTD were effective in minimizing Ammonia N loss within a 96-hour period for urea and Amidas. These

results coincide with studies performed by Frame et al. (2012). Similar research showed that when compared to untreated urea, NBPT often continued to reduce N ammonia loss throughout 7–10-day periods (Dawar et al., 2011). The newer formulation NBPTD did not show an improved ability to reduce N volatilization compared to NBPT. The data in this study suggest that urease inhibitors are most effective at approximately 3-4 days after application. This study helped to show that volatilization inhibitors afford give producers time to incorporate urea via a rainfall/irrigation event (~0.5-inch rain or irrigation). However, if rainfall or irrigation does occur within 48hrs, NBPT products are not likely to reduce N loss. There was minimal N volatilization in UAN treatments. Although other research has shown that urease inhibitors are effective when applied to UAN, these current data showed no treatment differences, possibly due to the characteristics of UAN which allow it to be more quickly incorporated into the soil than granular products.

CONCLUSION

Field and laboratory studies were used to assess the effectiveness of controlled release and stabilized N fertilizers for cotton production. Nitrogen uptake was evaluated by leaf and petiole N, but treatment differences were not indicative of cotton lint yield. Similarly, soil N data was highly variable and was not a good indicator of availability and uptake. The liquid UAN treatment was effective at increasing leaf and petiole N in some instances. Cotton lint yield showed treatment effects at WREC where stabilized urea, Amidas, polymer coated Amidas, and liquid UAN resulted in a 400 to 600 kg ha⁻¹ increase compared to the control. Polymer coated products did not differ in yield from their base products. These data suggest that controlled release fertilizers may have potential to maintain yield with lower application costs, but there is

also evidence that N release was in advance of peak crop demand. More data are needed to assess these products in Coastal Plain soils. Stabilized urea was more effective than untreated urea for improving yields and reducing volatilization losses. Stabilized urea products were most effective 3 to 4 days after application, but newer formulations of NBPT with duromide were not more effective than NBPT alone. More data in a broad range of environments and soil types are needed to better assess enhanced efficiency N products.

Table 1. Nitrogen (N) fertilizer chart for reference to fertilizers source, Rating, and active ingredient or mechanism of slow release.

Fertilizer	Rating	Active Ingredient or Mechanism of Slow-Release
Urea	46-0-0	None
Urea + Agrotain [®]	46-0-0 + NBPT	NBPT (Volatilization inhibitor)
Urea + Anvol [®]	46-0-0 + NBPTD	NBPT + Duromide (Volatilization inhibitor)
ESN [®]	44-0-0 PC	Polymer coating (ESN [®])
Ammonium Nitrate/Ammonium Sulfate Blend	32-0-0-(5) Blend	NA
Liquid Urea Ammonium Sulfate Blend (UAN)	28-0-0-(5)	NA
Amidas [®]	40-0-0-(5)	NA
Polymer-coated Amidas [®]	39-0-0 PC	Polymer coating
NA	Control	NA

Table 2. Field trial Fertilizer application and soil and leaf nitrogen (N) sample dates.

		Application Dates		
Location	Fertilizer Application	Sampling Dates		
2020	EVS	May 7 at plant	T1	June 26
		July 16 side-dress	T2	July 28
			T3	August 20
	WREC	May 14 at plant	T1	June 18th
		July 14 side dress	T2	July 14th
			T3	August 13th
2021	WREC	May 14 at plant	T1	June 14th
		July 14 side dress	T2	July 8th
			T3	September 7th

Table 3. Summary of analysis of variance (ANOVA) for Petiole Nitrogen (N), Leaf N, and Cotton Yield. Sample dates for Petiole and Leaf N are denoted by first square (T1), early bloom (T2), and peak bloom(T3).

Anova Table						
Variable	Location	Sample Date	Effect	Num DF	Type III Tests of Fixed Effects	
					F Value	Pr > F
Petiole N	EVS	T1	Treatment (T)	8	2.22	0.0836
		T2	T	8	0.95	0.5005
	WREC	T1	Year (Y)	1	2.63	0.1539
			T	8	16.78	<0.0001
		T2	Y x T	17	1.73	0.1131
			Y	1	11.75	0.0136
	T3	T	8	6.75	<0.0001	
		Y x T	17	3.21	0.0049	
Leaf N	EVS	T1	T	8	1.56	0.2141
		T2	T	8	1.02	0.4545
	WREC	T1	Y	1	128.24	<0.0001
			T	8	6.44	<0.0001
		T2	Y x T	17	0.99	0.4531
			Y	1	128.24	0.0966
	T3	T	T	8	6.44	<0.0001
			Y x T	17	0.99	0.0019
		T3	Y	1	1.94	0.2123
			T	8	3.59	0.0022
Y x T	17	2.25	0.0382			
Cotton Yield	EVS	"	T	8	10.77	0.4134
		"	Y	1	10.77	0.0169
	WREC	"	T	8	3.48	0.0032
		"	Y x T	17	0.76	0.6376

Table 4. Summary of analysis of variance (ANOVA) for soil Nitrogen (N) for ammonium and nitrate. Soil samples were evaluated at (0-6”) and (6-12”) depths. All variables were measured by sample dates for soil N: first square (T1), early bloom (T2), and peak bloom (T3).

Soil Nitrogen									
Variable	Location	Sample Date	Effect	Depth (cm)	Num DF	Type III Tests of Fixed Effects			
						F Value	Pr > F		
Ammonium	EVS	T1	Treatment (T)	0-15	8	1.63	0.1936		
		T2	T	0-15	8	0.7	0.6901		
		T3	T	0-15	8	2.1	0.0976		
		T1	T	15-30	8	5.65	0.0016		
		T2	T	15-30	8	1.07	0.4273		
		T3	T	15-30	8	2.52	0.0551		
	WREC	T1	Year (Y)	0-15	1	286.7	<.0001		
			T	0-15	8	1.68	0.1241		
			Y X T	0-15	17	1.35	0.2364		
		T2	Y	0-15	1	30.77	<.0001		
			T	0-15	8	0.89	0.528		
			Y X T	0-15	17	3.15	0.0052		
		T3	Y	0-15	1	1.58	0.1827		
			Y	15-30	1	551.81	<.0001		
			T	15-30	8	2.54	0.0199		
		T1	Y X T	15-30	17	1.19	0.3205		
			Y	15-30	1	10.61	0.0019		
			T	15-30	8	1.71	0.1179		
		T2	Y X T	15-30	17	2.44	0.0245		
			T3	Y	15-30	1	0.45	0.8774	
			T1	T	0-15	8	0.77	0.6374	
		Nitrate	EVS	T2	T	0-15	8	1.25	0.3327
				T3	T	0-15	8	"	"
				T1	T	15-30	8	1	0.469
T2	T			15-30	8	1.2	0.3576		
T3	T			15-30	8	1	0.4726		
T1	Y			0-15	1	1.04	0.3121		
	T		0-15	8	1.23	0.3005			
	Y X T		0-15	17	2.13	0.0467			
T2	Y		0-15	1	114.59	<.0001			
	T		0-15	8	1.1	0.3777			
	Y X T		0-15	17	0.87	0.548			
T3	Y		0-15	1	0.74	0.6595			
	Y		15-30	1	51.8	<.0001			
	T		15-30	8	1.3	0.2626			
T1	Y X T		15-30	17	1.67	0.1255			
	Y		15-30	1	44.54	<.0001			
	T		15-30	8	1.78	0.0991			
T2	Y X T		15-30	17	1.06	0.404			
	T3		Y	15-30	1	1.29	0.2969		

Variable	Location	Depth	Year	Sample Date	46-0-0 + NBPT	32-0-0	40-0-0	46-0-0- + NBPTD	Control	44-0-0 PC	39-0-0 PC	28-0-0-- 5	46-0-0
mg N kg ⁻¹ soil													
Ammonium	EVS	0-6"	2020	T1	3.27	5.60	2.73	2.76	3.61	7.25	5.58	4.39	3.77
				T2	1.94	1.04	1.32	1.27	1.00	1.14	1.51	1.13	2.21
				T3	0.16 AB	0.33 AB	0.49 A	0.17 AB	0.64 A	0.35 AB	0.01 B	0.17 AB	0.18 AB
		6-12"		T1	6.59 ABC	5.35 ABC	3.15 C	3.15 ABC	4.02 BC	12.18 A	8.17 AB	5.89 ABC	5.84 ABC
				T2	2.58	0.67	3.39	0.78	0.84	1.83	1.07	3.50	1.67
				T3	2.35 A	0.14 AB	0.36 AB	1.00 AB	0.25 AB	0.22 AB	0.01 B	0.18 AB	0.01 B
	WREC	0-6"	2020	T1	2.17	1.46	1.55	1.47	1.52	2.04	3.14	1.57	1.35
				T2	12.52	1.64	0.85	1.08	0.80	0.65	0.75	0.09	3.14
				T3	0.43	0.73	0.57	0.76	0.23	0.34	0.46	0.71	0.57
				T1	0.79	1.66	nd	nd	nd	nd	nd	nd	nd
		6-12"		T2	0.04 AB	0.01 B	0.07 AB	0.09 AB	0.02 B	0.07 AB	0.01 B	4.51 A	0.04 AB
				T1	0.15	0.15	0.17	0.16	0.11	0.22	0.48	0.17	0.07
				T2	0.80	0.10	0.10	0.56	0.16	0.11	0.03	0.03	0.60
				T3	0.62	0.43	0.73	0.92	0.60	0.63	0.49	0.90	0.55
WREC	6-12"	T1	0.01	0.03	0.13	0.06	nd	0.08	0.18	0.06	nd		
		T2	0.01 B	0.01 B	0.01 B	0.14 AB	0.02 B	0.01 B	0.01 B	1.74 A	0.03 AB		
		T1	nd	0.10	0.19	nd	0.73	nd	nd	nd	nd		
		T2	0.64	0.66	0.73	0.64	1.55	1.52	0.53	2.02	0.98		
Nitrate	EVS	0-6"	2020	T3	nd	nd	nd	nd	nd	nd	nd	nd	nd
				T1	nd	nd	nd	nd	nd	nd	nd	nd	0.19
				T2	0.96	1.11	8.76	1.18	0.96	0.98	0.88	2.12	1.64
		6-12"		T3	0.51	0.00	0.88	nd	nd	nd	nd	nd	nd
				T1	0.04	0.00	nd	3.54	nd	0.23	1.95	0.01	nd
				T2	25.47	2.13	9.06	3.73	20.53	2.16	2.04	1.41	4.93
	WREC	0-6"	T3	nd	0.48	0.21	0.91	nd	nd	0.23	nd	nd	
			T1	1.67	0.90	0.31	nd	0.25	nd	0.30	0.17	nd	
			T2	2.17	0.92	0.37	0.14	nd	nd	nd	5.60	nd	
			T1	nd	0.02	nd	0.23	0.14	0.07	nd	0.05	nd	
		6-12"	T2	1.49	1.48	1.49	1.41	1.71	1.00	1.68	1.78	1.74	
			T3	0.95	nd	0.20	0.12	0.18	nd	nd	nd	0.15	
			T1	4.23	0.96	2.29	1.49	0.84	5.88	8.07	1.24	0.25	
			T2	1.68	0.05	4.27	0.54	nd	1.07	nd	5.41	nd	

Table 5. Summary of soil nitrogen (N) data All variables were measured where location and soil sample depth were evaluated for soil ammonium and nitrate for first square (T1), early bloom(T2), and peak bloom(T3).

Table 6. Lint quality data were evaluated by treatment at Wiregrass Research and Extension Center for the year 2021. Data were evaluated for lint length, strength, micronaire, and uniformity. Data showed no significant difference among treatments.

		Treatment								
Location	Variable	46-0-0	46-0-0 + NBPTD	46-0-0 + NBPT	44-0-0 PC	40-0-0	39-0-0 PC	32-0-0	28-0-0	Control
WREC	Length	1.23	1.24	1.21	1.20	1.22	1.25	1.22	1.22	1.24
	Strength	29.50	29.65	29.75	29.88	29.48	29.58	30.23	28.88	30.50
	Micronaire	4.3	4.3	4.3	4.4	4.3	4.5	4.3	4.1	4.5
	Uniformity	82.0	82.8	82.9	82.3	83.0	83.2	82.5	82.1	81.9

Table 7. Soil characteristics of a course textured Bama sandy loam (Fine-loamy, siliceous, subactive, thermic Typic Paleudults).

Soil Characteristics								
Value	Organic Matter	CEC	pH	Phosphorous (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Texture
	0.70%	5.5 meq/100g	5	124 lbs/A	108 Lbs/A	857 lbs/A	63 lbs/A	Loamy Sand

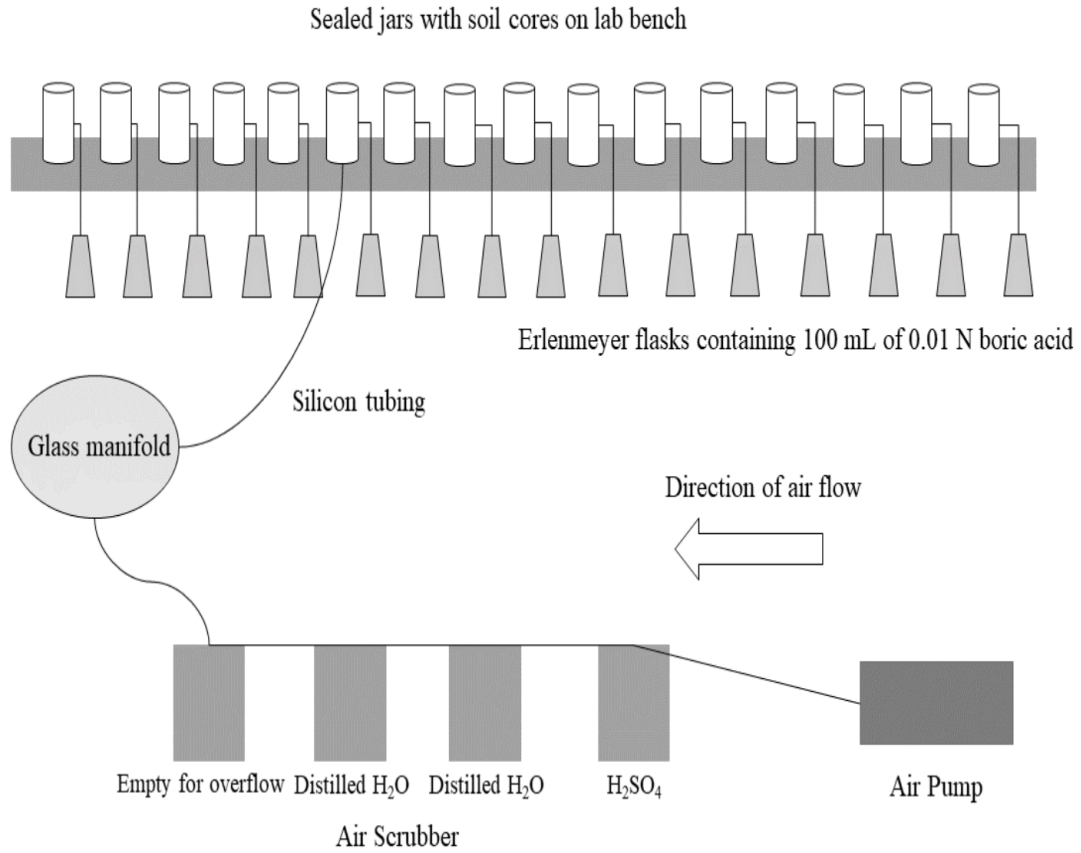


Figure 1. Diagram of the laboratory system used to measure ammonia volatilization, with jars, pump, and air scrubbers, following the procedure of O'Halloran (1993). The glass manifold is connected to an opening in each jar with silicon tubing.

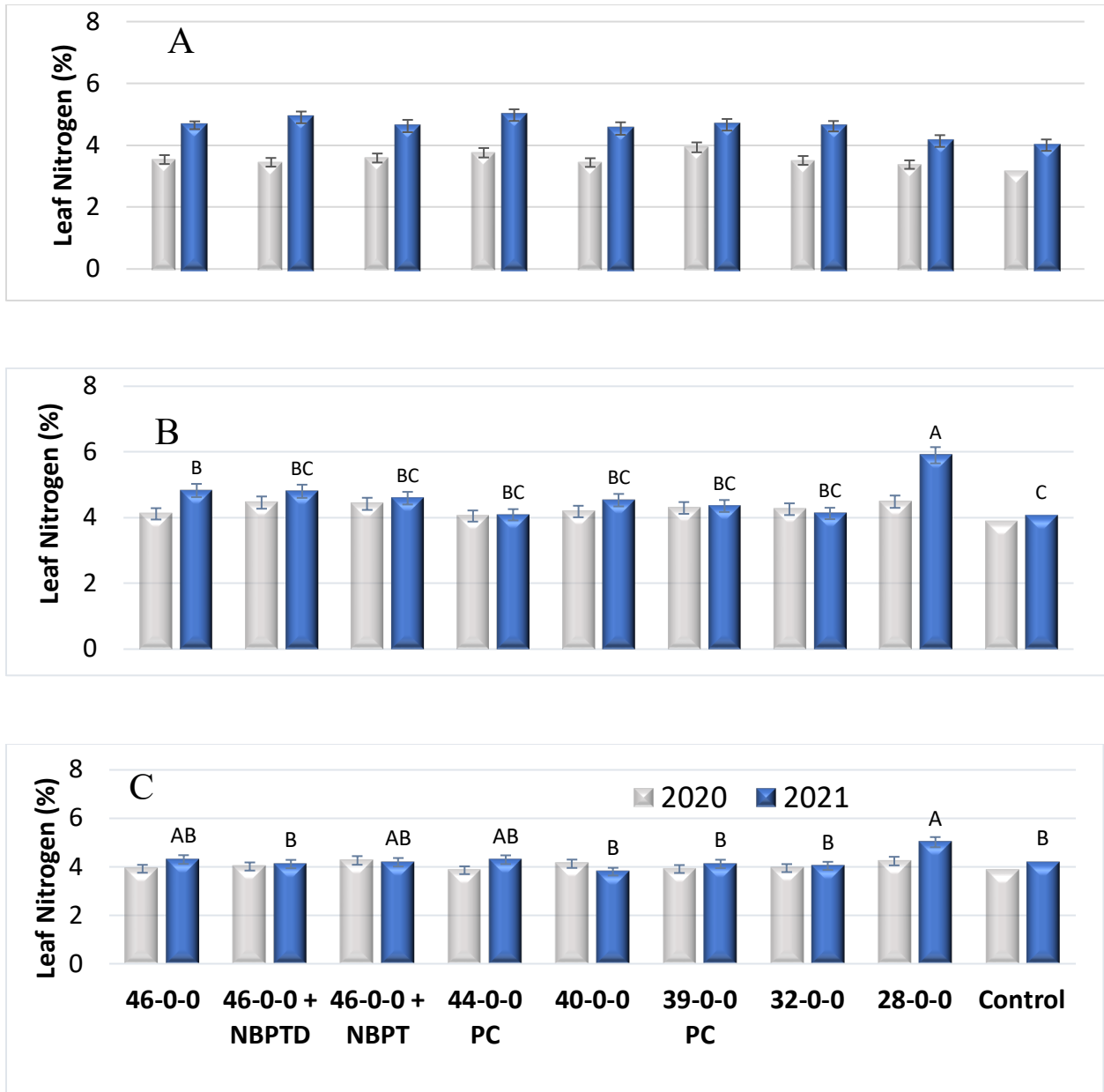


Figure 2. Leaf Nitrogen (N) for Wiregrass Research and Extension Center (WREC) for A. first square, B. early bloom, and C. peak bloom. Values followed by the same letter are not different at $\alpha=0.1$ within a sampling date and year. Error bars indicate the standard error about the mean.

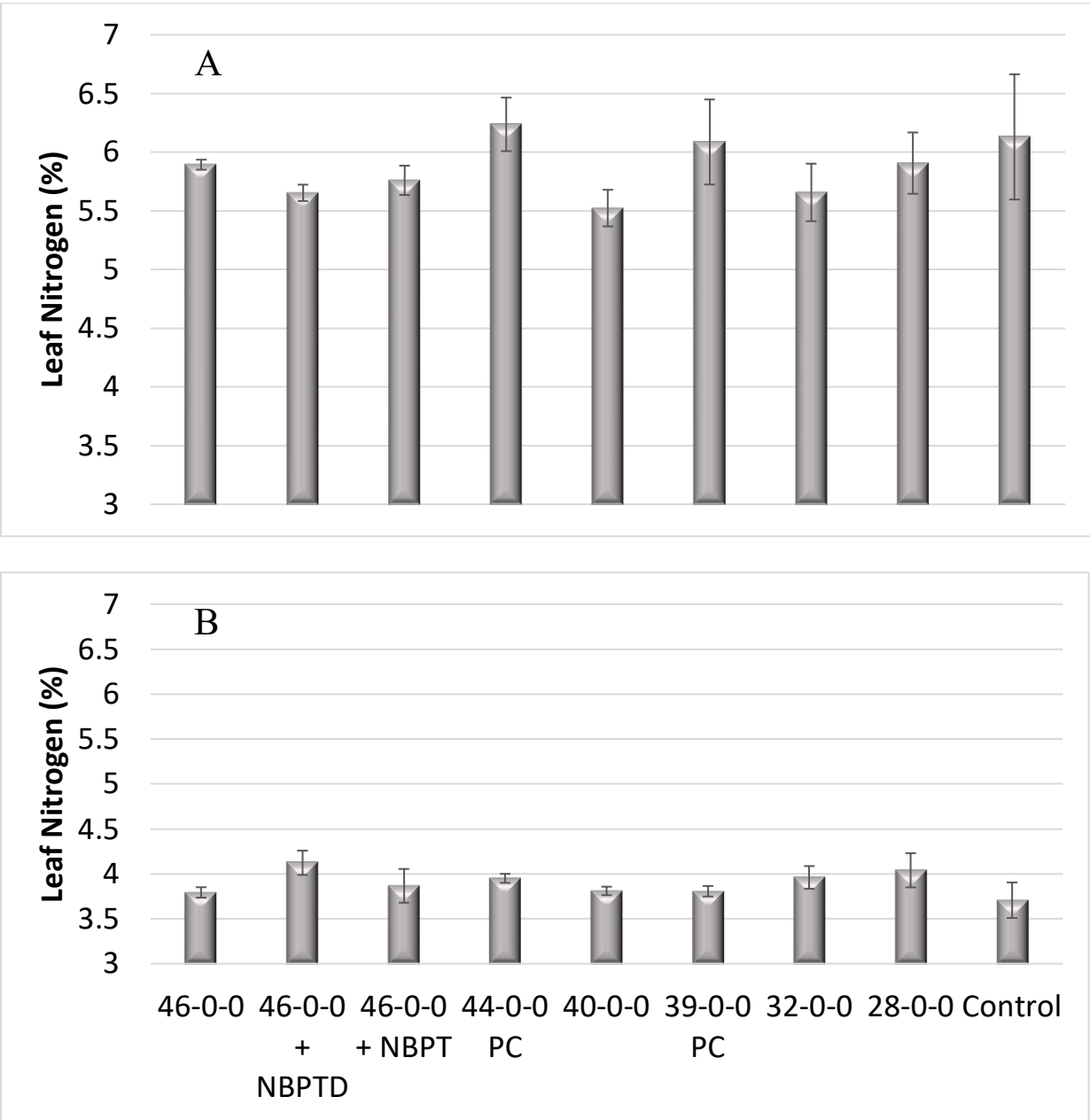


Figure 3. Leaf Nitrogen (N) for E.V. Smith research center (EVS) for A. first square, B. early bloom, and C. peak bloom. Field Data from 2021 were highly variable and were not presented. Values followed by the same letter are not different at $\alpha=0.1$ within a sampling date and year. Error bars indicate the standard error about the mean.

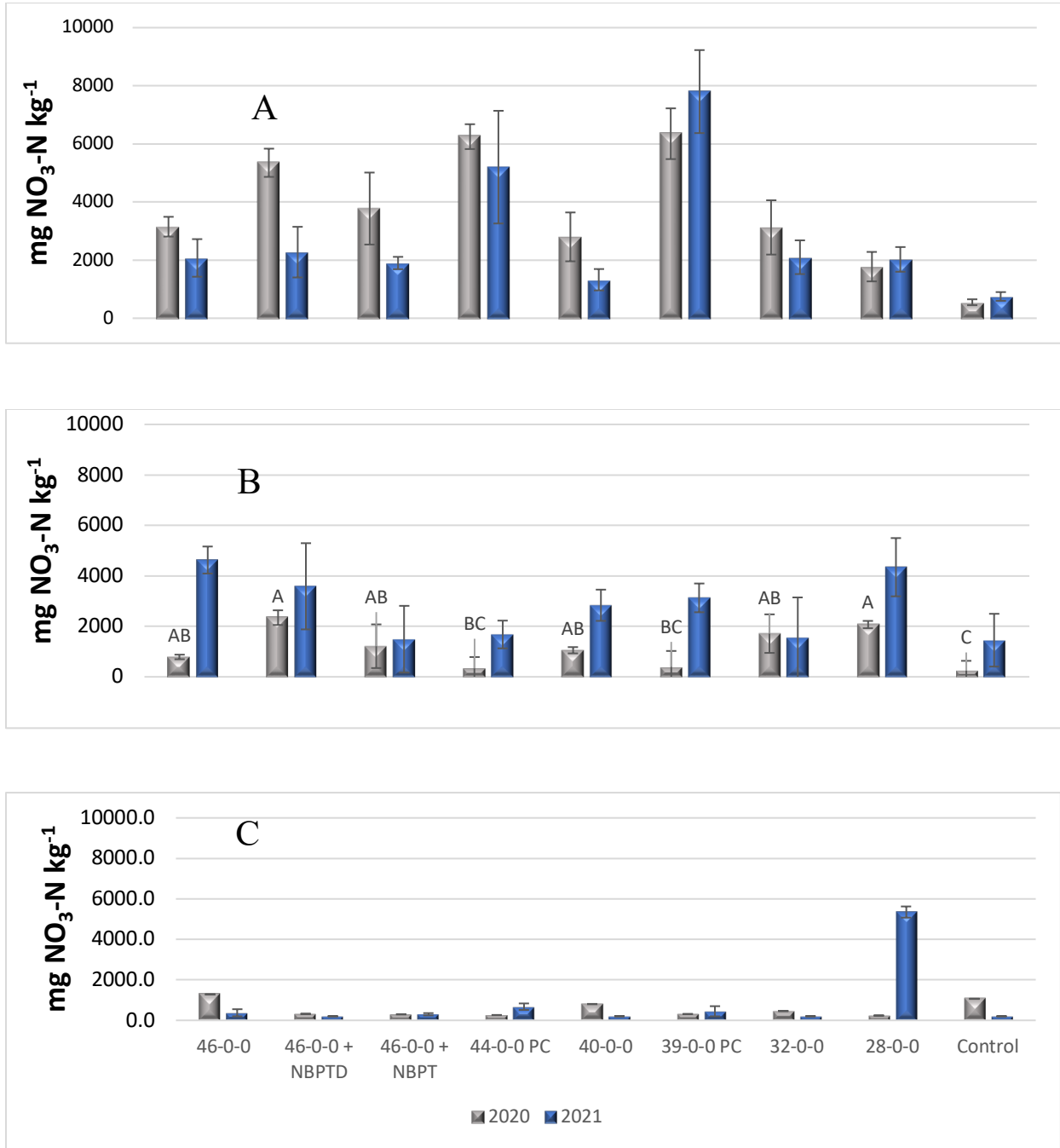


Figure 4. Petiole Nitrogen (N) for Wiregrass Research and Extension Center (WREC) for A. first square, B. early bloom, and C. peak bloom sampling dates. Values followed by the same letter are not different at $\alpha=0.1$ within a sampling date and year. Error bars indicate the standard error about the mean.

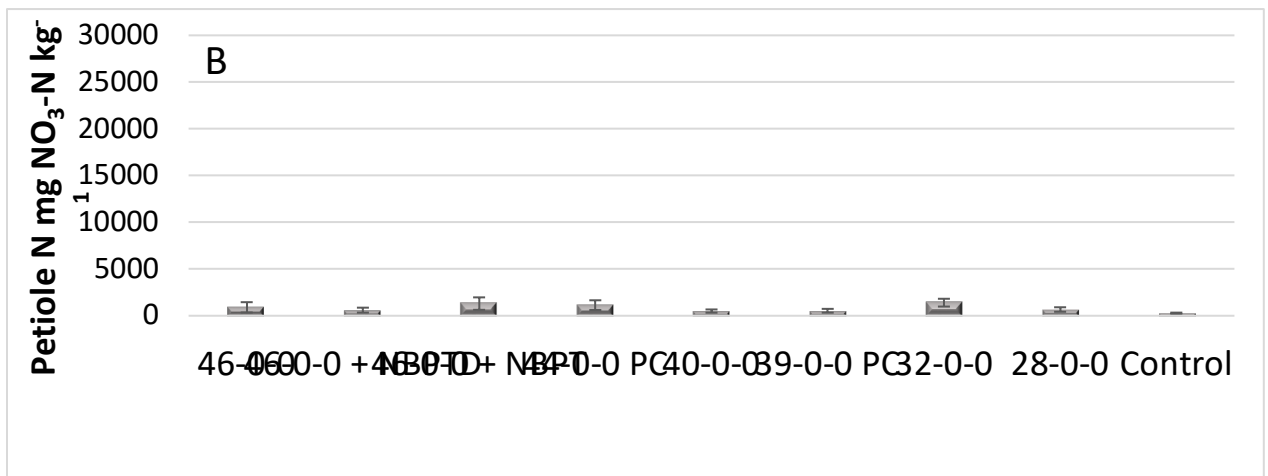
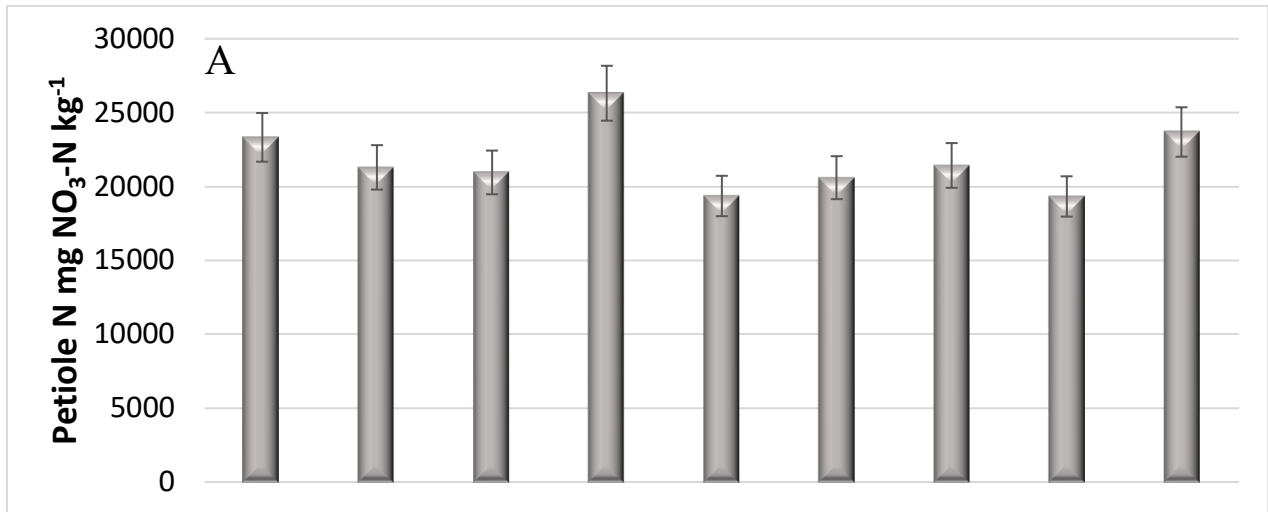


Figure 5. Petiole Nitrogen (N) for E.V. Smith research center (EVS) for A. first square, B. early bloom, and C. peak bloom. Field data from 2021 were highly variable and were not presented. Values followed by the same letter are not different at $\alpha=0.1$ within a sampling date and year. Error bars indicate the standard error about the mean.

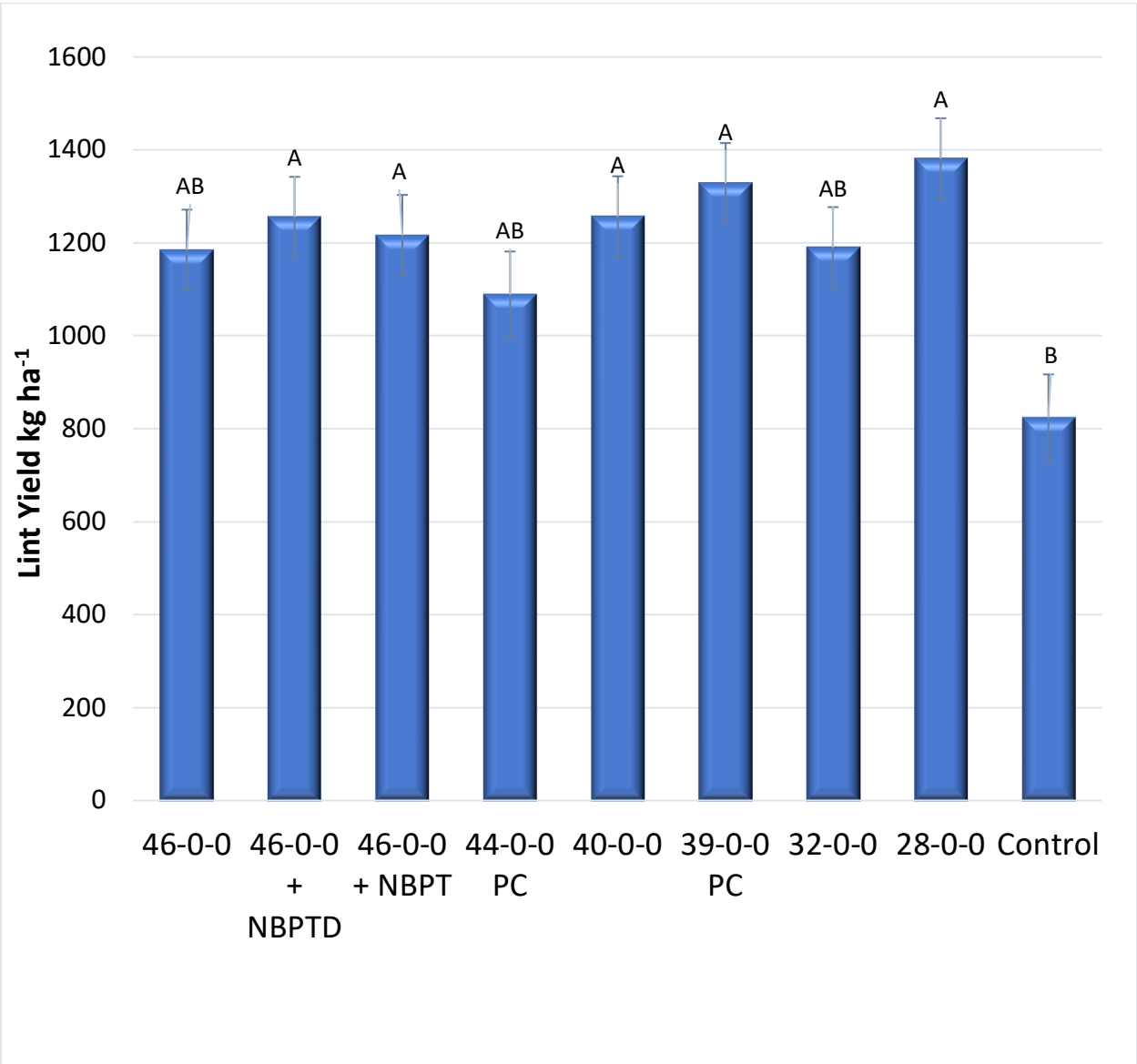


Figure 6. Cotton Yield 2020-2021 Differences between treatments at Wiregrass Research and Extension Center (WREC) for cotton lint yield. Columns with the same letter do not differ among cover crop treatments $\alpha = 0.05$. Error bars indicate the standard error about the mean.

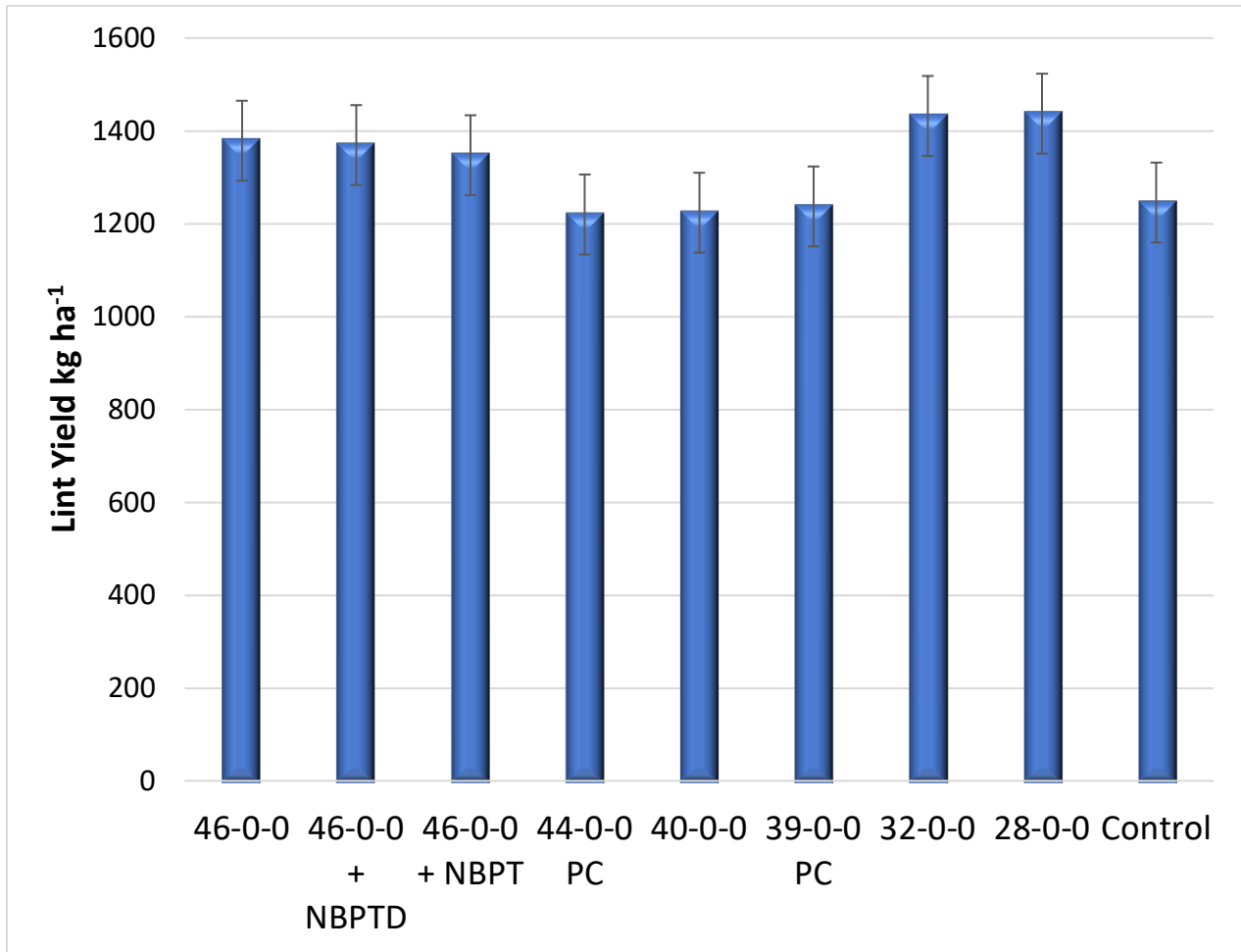


Figure 7. Differences between treatments at E.V. Smith Research Center (EVS) for cotton lint yield for 2020. Field data from 2021 were highly variable and were not presented. Values followed by the same letter are not different at $\alpha=0.1$ within a sampling date and year. Error bars indicate the standard error about the mean.

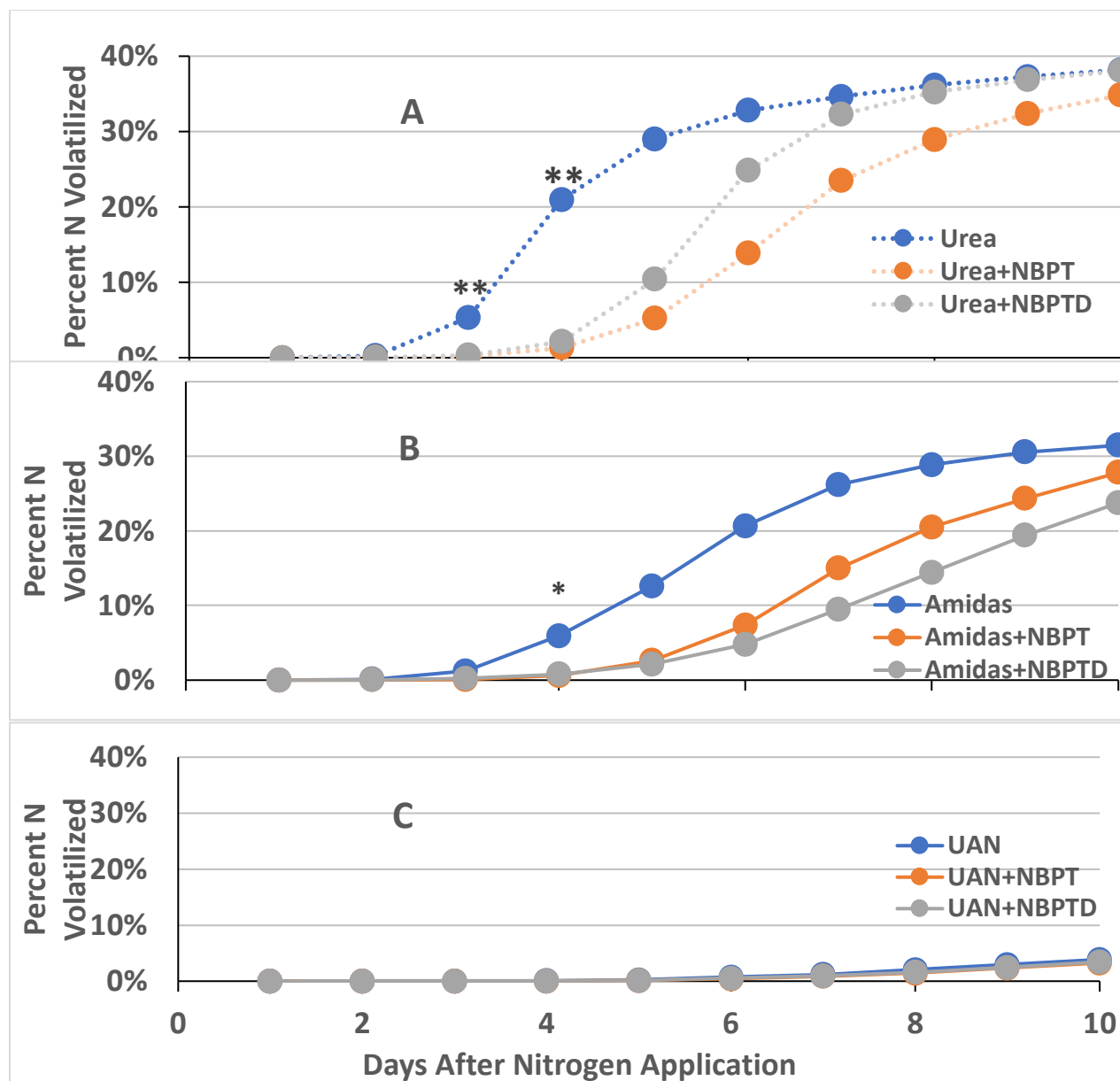


Figure 8. Cumulative percent nitrogen volatilized for (A) untreated urea, urea+NBPT, (N-(n-butyl) thiophosphoric triamide) and Urea+ NBPTD (NBPT + Duromide) (B) untreated Amidas, Amidas+NBPT, and Amidas+NBPTD (C) untreated UAN, UAN+NBPT, and UAN+NBPTD for the Coastal Plain soil type across a 10-day experiment. * Indicates significantly higher nitrogen volatilization for untreated fertilizer compared to treated with NBPT within a day at $\alpha=0.05$. ** indicates significantly higher nitrogen volatilization for untreated fertilizer compared to NBPT or NBPTD within a day at ($\alpha=0.05$).

REFERENCES

- Adams, J.F., C.C. Mitchell, and H.H. Bryant. 1994. Soil test fertilizer recommendations for Alabama crops. Agronomy & Soils Dep. Ser. No. 178. Alabama Agric. Exp. Stn., Auburn University, AL.
- Avnimelech, Y., and M. Laher. 1977. Ammonia volatilization from soils: Equilibrium considerations. *Soil Sci. Soc. Am. J.* 41:1080–1084.
- Balkcom, K., Blackmer A.M., Hansen D.J., Morris T.F., and Mallarino A.P. 2003. Testing soils and cornstalks to evaluate nitrogen management on the watershed scale. *J. Environ. Qual.* 32:1015-1024.
- Bell, P., D. Boquet, E. Millhollon, S. Moore, M.W. Ebelhar, C. Mitchell, J. Varco, E. Funderburg, C. Kennedy, G. Breitenbeck, C. Craig, and M. Holman, M. 2003. Relationships between leaf-blade nitrogen and relative seed cotton yields. *Crop Sci.* 43:1367-1374.
- Benson, G.O. 1985. Why the reduced yields when corn follows corn and possible management response. p. 161-174. *In Proc. Annu. Corn Sorghum Ind. Res. Conf.*, 40th, 11-12 Dec. 1985, Chicago, IL. Am. Seed Trade Assoc., Washington, DC.
- Blaylock, A. D., Kaufmann, J., and Dowbenko, R. D. 2005. Nitrogen fertilizer technologies. In *Western Nutrient Management Conference* (Vol. 6, pp. 8-13).
- Bremner, J. M., McCarty G.W., and Higuchi, T. 1991. Persistence of the inhibitory effects of phosphoroamides on urea hydrolysis in soils. *Soil. Sci. Plant Anal.* 22: 1519–1526.

- Burch, J.A. and Fox R.H. 1989. The effect of temperature and initial soil moisture content on the volatilization of ammonia from surface applied urea, *Soil Sci.* 147–:311-318.
- Burton, J.C. 1972. Nodulation and symbiotic nitrogen fixation. In C.H. Hanson (Ed.), *Alfalfa Science and Technology* (Monograph 15; pp. 229–246). Madison, WI: American Society of Agronomy.
- Campbell, C.R. and Plank, C.O. 2011. Cotton. In: Campbell, C.R., Ed., *Reference sufficiency ranges for plant analysis in the southern region of the United States*, Southern Cooperative Series Bulletin 394, Southern Association of Agricultural Experiment Station, Raleigh, 15-18.
- Canfield, D., Glazer, A., and Falkowski, P. 2010. The evolution and future of earth's nitrogen cycle. *Science.* 330:192-196.
- Carrow, R.N., Waddington D.V., and Rieke P.E. 2001. *Turfgrass soil fertility and chemical problems*. Sleeping Bear Press/Ann Arbor Press, Chelsea, MI.
- Cornell University Cooperative Extension, 2007. *Cation exchange capacity*. Agronomy Fact Sheet Serise # 22. Department of Crop and Soil Sciences, College of Agriculture and Life Sciences, Cornell University.
- Cope, J.T. Jr. 1970. Response of cotton, corn, bermudagrass to rates of N, P, and K. *Ala. Agric. Exp. Stn. Cir.* 181. Auburn University, AL.

- Chichester, F.W. 1977. Effects of increased fertilizer rates on nitrogen content of runoff and percolate from monolith lysimeters. *J. Environ. Qual.* 6:211-217.
- Ciurli, S., Benini, S., Rypniewski, W.R., Wilson, K.S., Miletto, S., and Mangani, S. 1999. Structural properties of the nickel ions in urease: Novel insights into the catalytic and inhibition mechanisms. *Coord. Chem. Rev.* 190-192: 331-355.
- Clay, D. E., Malzer, G. L. and Anderson, J. L. 1990. Ammonia volatilization from urea as influenced by soil temperature, soil water content, and nitrification and hydrolysis inhibitors. *Soil Sci. Soc. Am. J.* 54: 263–266.
- Coyne, M., S. J. Schepers, and W. R. Raun. 2008. Biological denitrification. Nitrogen in agricultural Systems (pp. 201– 253). Agronomy Monograph n.º 49. ASA, CSSA, SSSA.
- Crookston, R.K., Kurle J.E., Copeland P.J., Ford J.H., and Lueschen W.E. 1991. Rotational cropping sequences affects yield of corn and soybean. *Agron. J.* 83:108-113.
- Del Moro, S.K., Sullivan, D.M. and Horneck, D.A. 2017, Ammonia volatilization from broadcast urea and alternative dry nitrogen fertilizers. *Soil Science Society of America Journal*, 81: 1629-1639.
- Delwiche C.C. and Bryan B.A. 1976. Denitrification. *Annu Rev Microbiol*; 30:241-62.
- Duncan, L.A., Raper, T.B., Butler, S., Buschermohle, M., Wilkerson, J., Hart, W. and Yin, F., 2021. In-Season assessment of cotton nitrogen status from a handheld smartphone and an unmanned aerial System, *Agronomy and Soils.* 25:184–193.

- Engel, R., Jones, C., and Wallander, R. 2011. Ammonia volatilization from urea and mitigation by NBPT following surface application to cold soils. *Soil Sci Soc Am J.* 75:2348–2358.
- Ernst, J.W. and Massey, H.F. 1960. The effects of several factors on volatilization of ammonia formed from urea in the soil. *Soil Science Society of America Journal*, 24: 87-90.
- Fan, X. H., Li, Y. C., and Alva, A. K. 2011. Effects of temperature and soil type on ammonia volatilization from slow-release nitrogen fertilizers. *Communications in Soil Science and Plant Analysis*, 42:10,1111-1122.
- Fenn, L.B. and Kissel, D.E. 1976. The influence of cation exchange capacity and depth of incorporation on ammonia volatilization from ammonium compounds applied to calcareous soils. *Soil Sci Soc Am J*, 40: 394-398.
- Ferguson, R.B., Kissel, D.E., Koelliker, J.K. and Basel, W. 1984, Ammonia volatilization from surface-applied urea: effect of hydrogen ion buffering capacity. *Soil Sci Soc Am J*, 48: 578-582.
- Francisco, S. S., Urrutia, O., Martin, V., Peristeropoulos, A., and Garcia-Mina, J. M. 2011. Efficiency of urease and nitrification inhibitors in reducing ammonia volatilization from diverse nitrogen fertilizers applied to different soil types and wheat straw mulching. *J Sci Food Agr.* 91:1569-1575.
- Franzen, D., Goos, R. J., Norman, R. J., Walker, T. W., Roberts, T.L., Slaton, N. A., Endres, G., Ashley, R., Staricka, J., and Lukach, J. 2011. Field and laboratory studies comparing nitrification-inhibited urea with urea in North Dakota, Arkansas, and Mississippi. *J Plant Nutr.* 34:1198-1222.

- Frame, W. H., Alley, M. M., Thomason, W., Whitehurst, G., Whitehurst, B., and Campbell, R. 2013. Agronomic evaluation of coated urea to reduce ammonia volatilization from side-dress applications to corn. *Crop Management*. 2013;12(1):1-3.
- Frame, W. H., Alley, M. M., Whitehurst, G. B., Whitehurst, B. M., and Campbell, R. 2012. In vitro evaluation of coatings to control ammonia volatilization from surface-applied urea. *Agron J.* 104: 1201–1207.
- Frankow-Lindberg, B.E., and A.S. Dahlin. 2013. N₂ fixation, N transfer, and yield in grassland communities including a deep-rooted legume or non-legume species. *Plant and Soil*, 370, 567–581.
- Fraps, G.S. 1919. The chemical composition of the cotton plant. *Texas Agric. Exp. Stn. Bull.* 247. The chemical composition of the cotton plant. *Texas Agric. Exp. Stn. Bull.* 247.
- Gardner B. R., and Tucker T. C. 1967. Nitrogen effects on cotton: I. vegetative and fruiting characteristics. *Soil Sci Soc Am J*, 31, 780-785.
- Gerik, T.J., Rosenthal W.D., Stockle C.O., and Jackson, B.S. 1989. Analysis of cotton fruiting, boll development, and fiber properties under nitrogen stress. p. 64–65. *Cotton Physiol. Conf. Proc. Beltwide Cotton Prod. Res. Conf.*, Nashville, TN. 2–7 Jan.
- Gerik J.T., Oosterhuis M.D., and Torbert H.A., 1998. Managing cotton nitrogen, supply advances in agronomy, Academic Press, Volume 64, Pages 115-147.

- Golden, M., Leifert, C., Wilson W.S., Ball A.S., and Hinton R.H. 1999. Potential risks and benefits of dietary nitrate. In: *Managing Risks of Nitrates to Humans and the Environment*. The Royal Society of Chemistry, Cambridge, UK. Pp. 269-280.
- Goos, R. and Guertal, Elizabeth. 2019. Evaluation of commercial additives used with granular urea for nitrogen conservation. *Agron J.* 111.
- Grant, C. A., Brown, K. R., Bailey, L. D., and Jia, S. 1996. Volatile losses of NH₃ from surface-applied urea and urea ammonium nitrate with and without the urease inhibitors NBPT or ammonium thiosulphate. *Can J Soil Sci.* 76:417–419.
- Guthrie, D.S. 1991. Cotton response to starter fertilizer placement and planting dates. *Agron. J.*, 83: 836-839.
- Guinn, G. 1982. Causes of square and boll shedding in cotton. *USDA Technical Bulletins*, US Government Printing Office, Washington DC, 1-22.
- Guertal, E.A. 2009. Slow-release nitrogen fertilizers in vegetable production: A Review. *Hort-Technology*. 19. 16-19.
- Guertal, E.A. 2000. Preplant slow-release nitrogen fertilizers produces similar bell pepper yields as split applications of soluble fertilizer. *Agron. J.* 92:388–393.
- Guldan, S.J., Martin C.A., Cueto-Wong J., and Steiner R.L. 1996. Interseeding legumes into chile: Legume productivity and effect on chile yield. *Hort Sci*, 31, 1126–1128.

- Hake, K., and Kerby, T. 1988. Nitrogen fertilization in "Cotton Fertilization Guide," pp. 1-20.
Univ. of California, Bakersfield, CA.
- Hargrove, W. L. 1988. Evaluation of ammonia volatilization in the field. *J. Prod. Agri.* 1:104–111.
- Hart, S.C., Stark, J.M., Davidson, E.A., Firestone, R.W., Weaver, S., Angle, P., Bottomley, D., Bezdicsek, S., Smith, A., Tabatabai A., and Wollum M.K. 1994. Nitrogen mineralization, immobilization, and nitrification. In *Methods of Soil Analysis* 985-1018.
- Havling, S., Tisdale, S., and Nelson, W. 2013. *Soil fertility and fertilizers: An introduction to nutrient management, Eighth Edition, (8th edition) Pearson.*
- He, Z. L., Alva A. K., Calvert, D. V., and Banks, D. J. 1999. Ammonia volatilization from different fertilizer sources and effects of temperature and soil pH. *Soil Science* 164:750–758.
- Herridge, D., Peoples, M., Boddey R. 2008 Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil.* 311:1-18.
- Hill, R., Rinker, R., and Wilson, H. 1980. Atmospheric nitrogen fixation by lightning. *J Atmos. Sci.* 37:179-182.
- Hummel, N.W., Jr. 1989. Resin-coated urea evaluation for turfgrass fertilization. *Agron. J.* 81:290–294.
- Jones, U.S. 1982. *Fertilizers and Soil Fertility.* Reston Publishing., Reston, VA pg. 1-421.

- Kawakami E. M., Oosterhuis D. M., Snider J. L., and Mozaffari, M., 2012. Physiological and yield responses of field-grown cotton to application of urea with the urease inhibitor NBPT and the nitrification inhibitor DCD. *Eur Journal Agron*, Volume 43.
- Keeney, D. R., and Nelson, D. W. 1982. Nitrogen-Inorganic forms, IN: *Methods of Soil Analysis, Part 2*. Am Soc Agron, Madison, WI pp. 643- 698.
- Keuter, A., Veldkamp, E., and Corre, M. D. 2014. Asymbiotic biological nitrogen fixation in a temperate grassland as affected by management practices. *Soil Biol Biochem*. 70:38-46.
- Kissel, D.E., Cabrera M.L., Paramasivam S., Schepers J.S. and Raun W. 2008. Ammonium, ammonia, and urea reactions in soils. *Nitrogen in agricultural systems*. ASA, CSSA, and SSSA, Madison, WI. p. 101–103.
- Littell, R.C., Milliken G.A, Stroup, W.W., Wolfinger, R.D., and Schabenberger, O. 2006. *SAS for mixed models*. 2nd ed. SAS Inst., Cary, NC.
- Li, P., Dong, H., Liu, A., Liu, J., Sun, M., Li, Y., Liu, S., Zhao, X., and Mao, S., 2017. Effects of Nitrogen Rate and Split Application Ratio on Nitrogen Use and Soil Nitrogen Balance in Cotton Fields, *Pedosphere*, Volume 27, Issue 4.
- Li, Y., Hu, M., and Tenuta, M. 2020. Agronomic evaluation of polymer-coated urea and urease and nitrification inhibitors for cotton production under drip-fertigation in a dry climate. *Sci Rep* 10, 1472.
- Livingston, S., Hickey, M. G., and Stichler, C. 1996. Using petiole analysis for nitrogen management in cotton. *Tex AES Bull L-5156*.

- Lutrick MC, Peacock HA, and Cornell JA. 1986. Nitrate monitoring for cotton lint production on a Typic Paleudult. *Agron J* 78:1041–6.
- Hawkesford M., Horst W., Kichey T., Lambers H., Schjoerring J., Moller I., and White, P., 2012. Functions of macronutrients, Pages 135-189.
- Main, Chris & L., Christopher & Barber, L. & Boman, Randall & Chapman, Kent & Dodds, Darrin & Duncan, Stu & Edmisten, Keith & Horn, Patrick & Jones, Michael & Morgan, Gaylon & Norton, E. & Osborne, Shane & Whitaker, Jared & Nichols, Robert & Bronson, Kevin. 2013. Effects of Nitrogen and Planting Seed Size on Cotton Growth. *Agron J.* 105. 1853-1859. 10.2134.
- Mauney J. R. and. Stewart J. McD, 1986. Managing cotton nitrogen supply 145. Vegetative growth and development of fruiting sites. In *Cotton Physiology*. 11-28.
- Mazzei, L., Musiani, F. and Ciurli, S. 2020. The structure-based reaction mechanism of urease, a nickel dependent enzyme: tale of a long debate. *J Biol Inorg Chem* 25, 829–845.
- McCarty, G.W., Bremner, J.M. and Chai, H.S. 1989. Effect of *N*-(*n*-butyl) thiophosphoric triamide on hydrolysis of urea by plant, microbial, and soil urease. *Biol Fert Soils* 8, 123–127.

McClanahan, Sarah & Frame, William & Stewart, Ryan & Thomason, Wade. 2020. Cotton yield and lint quality responses to nitrogen rate and placement in the humid southeast. *Agron J.* 112.

McHargue, J.S. 1926. Mineral constituents of the cotton plant. *Am Soc Agron.* 18:1076-1083.

Miley, W. N. 1988. Foliar nitrogen can be good supplement. *Delra Farm Press.* 45(27), 7.

Mitchell, C.C., Phillips, S. 2010. Research-based soil testing and recommendations for cotton on coastal plain soils. *Southern Cooperative Series Bulletin no.* 410.

Moxaffari, M., McConnell, J.S., Hatenhauer, K., Slaton, N.A., Evans, E.E., Miley, N., Bourland, F. and Kennedy, C. 2004. Cotton yield and petiole N content as affected by N fertilizer application. *Summaries of Arkansas Cotton Research Fayetteville, University of Arkansas.* p.89-94.

National Agricultural Statistics Service. Year? ” USDA/Nass QuickStats AD-Hoc Query Tool,” United States Department of Agriculture.

Nakamura, K., Harter, T., Hirono, Y., Horino, H. and Mitsuno, T. 2004, Assessment of root zone nitrogen leaching as affected by irrigation and nutrient management practices. *Vadose Zone Journal*, 3: 1353-1366.

Nyborg, M., Solberg, E.D., and Zhang, M. 1993. Polymer-coated urea in the field: mineralization, and barley yield and nitrogen uptake. In *Dahlia Greidinger Memorial International Workshop on Controlled/Slow-Release Fertilizers.*

- Olson, R.A. (ed.). 1971. Fertilizer technology and use. 2nd ed. Soil Sci. Soc. Amer., Madison, WI.
- O'Toole, P., McGarry, S.J., and Morgan, M.A. 1985. Ammonia volatilization from urea-treated pasture and tillage soils: Effects of soil properties. *J. Soil Sci.* 36(4):613–620.
- Oosterhuis, D.M., Coker, D.L. and Plunkett, D.E. 2002. Field test of a new cotton petiole monitoring technique. *Summaries of Arkansas Cotton Research*. Fayetteville: University of Arkansas. p.121-128.
- Oosterhuis, D. and Howard, D. 2008. Evaluation of slow-release nitrogen and potassium fertilizers for cotton production. *Afr J Agric Res.* 3.
- Pabuayon, I. L., Lewis, K., and Ritchie, G. L., 2020. Dry matter and nutrient partitioning changes for the past 30 years of cotton production. *Agronomy Journal.* 112. 10.1002/agj2.20386.
- Peacock, C.H. and DiPaola, J.M. 1992. Bermudagrass response to reactive layer coated fertilizers. *Agron. J.* 84:946–950.
- Pitumpe A.P.S., Rosso, L.H.M., and Hansel, F.D. 2020. Temporal biological nitrogen fixation pattern in soybean inoculated with *Bradyrhizobium*. *Agrosyst Geosci Environ.*
- Plank CO. 1988. *Plant analysis handbook for Georgia*. Athens (GA): University of Georgia Cooperative Extension Service.
- Radin, J.W. and Mauney J.R. 1986. The nitrogen stress syndrome. The Cotton Foundation. Memphis, TN. In *Cotton Physiology*.

- Radin, J. W. and Sell, C. R. 1975. Some factors limiting nitrate reduction in developing ovules of cotton. *Crop Sci.* 15, 713-715.
- Rawluk, C. D. L., Grant, C.A., and Racz, G J. 2001. Ammonia volatilization from soils fertilized with urea and varying rates of urease inhibitor NBPT. *Soil Sci Soc Can J.* 81(2): 239-246.
- Reed, S.C., Cleveland, C.C., and Townsend, A.R., 2011. Functional ecology of free-living nitrogen fixation: a contemporary perspective. *Annual Review of Ecology, Evolution, and Systematics* 42, 489e512.
- Reiter M.S, Reeves D.W., and Burmester C.H. 2008. Cotton nitrogen management in a high residue conservation system: source, rate, method, and timing, *Soil Sci Soc Am J*, Pages 1330-1336.
- Rochester, I.J. 2007. Nutrient uptake and export from an Australian cotton field. *Nutrient Cycling in Agroecosystems* 77: 213-223.
- Rochette, P., Angers, D.A., Chantigny, M.H., MacDonald, J.D., Bissonnette, N., and Bertrand, N. 2009. Ammonia volatilization following surface application of urea to tilled and no-till soils: A laboratory comparison. *Soil Tillage Res.* **103**:310–315.
- Shaviv, A. 1999. Preparation methods and release mechanisms of controlled release fertilizers: Agronomic efficiency and environmental significances. *Proc. Intl. Fert. Soc.* 431:1–35.
- Schmidt, E.L., 1982. Nitrification in soil. *Nitrogen in agricultural soils*, 22, pp.253-288.

- Scarsbrook, C.E. and J.T. Cope, Jr. 1957. Sources of nitrogen for cotton and corn in Alabama. Ala. Agric. Exp. Stn. Bul. 308. Auburn, AL.
- Shuman, L.M. 2002. Phosphorus and Nitrate Nitrogen in Runoff Following Fertilizer Application to Turfgrass. *J. Environ. Qual.*, 31: 1710-1715.
- Silva, A.G.B., Sequeira, C.H., Sermarini, R.A. and Otto, R. 2017. Urease inhibitor NBPT on ammonia volatilization and crop productivity, a meta-analysis. *Agron J*, 109: 1-13.
- Smika, D.E., Heermann, D.F., Duke, H.R. and Bathchelder, A.R. 1977, Nitrate-N Percolation through irrigated sandy soil as affected by water management¹. *Agron. J.*, 69: 623-626.
- Stevenson, F. J. 1982. Organic forms of soil nitrogen. In *Nitrogen in Agricultural Soils*, Agron pp. 67-122. 22. ASA and SSSA. Madison, WI.
- Teague, T.G., Snider J., and Oosterhuis D.M. 2016. Plant-insect interactions and cotton development. In: *Linking Physiology to Management*. Pp. 27-65. Cotton Foundation Reference Book Series Number Nine. The Cotton Foundation. Cordova, TN, USA.
- Tiedje, J. 1998. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. *Environmental Microbiology of Anaerobes*. Pg. 179-224.
- Timmons, D.R. and Dylla, A.S. 1981, Nitrogen leaching as influenced by nitrogen management and supplemental irrigation level. *Journal of Environmental Quality*, 10: 421-426.
- Tindall, T.A. and Detrick, J. 1999. Controlled released fertilizer application and use in production agriculture. In *Western Canada Agronomy Workshop*, MB. pp. 93-96.

Touchton, J.T., Adams F., and Burmester C.H. 1981. Nitrogen fertilizer rates and cotton petiole analysis in Alabama field experiments. Ala. Agric. Exp. Stn. Bul. 528. Auburn University, AL.

US Upland Cotton Classification. *Cotton Incorporated*, 2020. <https://www.cottoninc.com/cotton-production/quality/classification-of-cotton/classification-of-upland-cotton/>.

Vittori A., Livia, Marzadori, C., Gioacchini, P., Ricci, S., and Gessa, C. 1996. Effects of the urease inhibitor N-(n-butyl) phosphorothioic triamide in low concentrations on ammonia volatilization and evolution of mineral nitrogen. *Biol Fert Soil*. 22. 196-201.

Waring, S.A. and Gilliam, J.W. 1983. The Effect of Acidity on Nitrate Reduction and Denitrification in Lower Coastal Plain Soils. *Soil Sci Soc Am J*, 47: 246-251.

APPENDIX

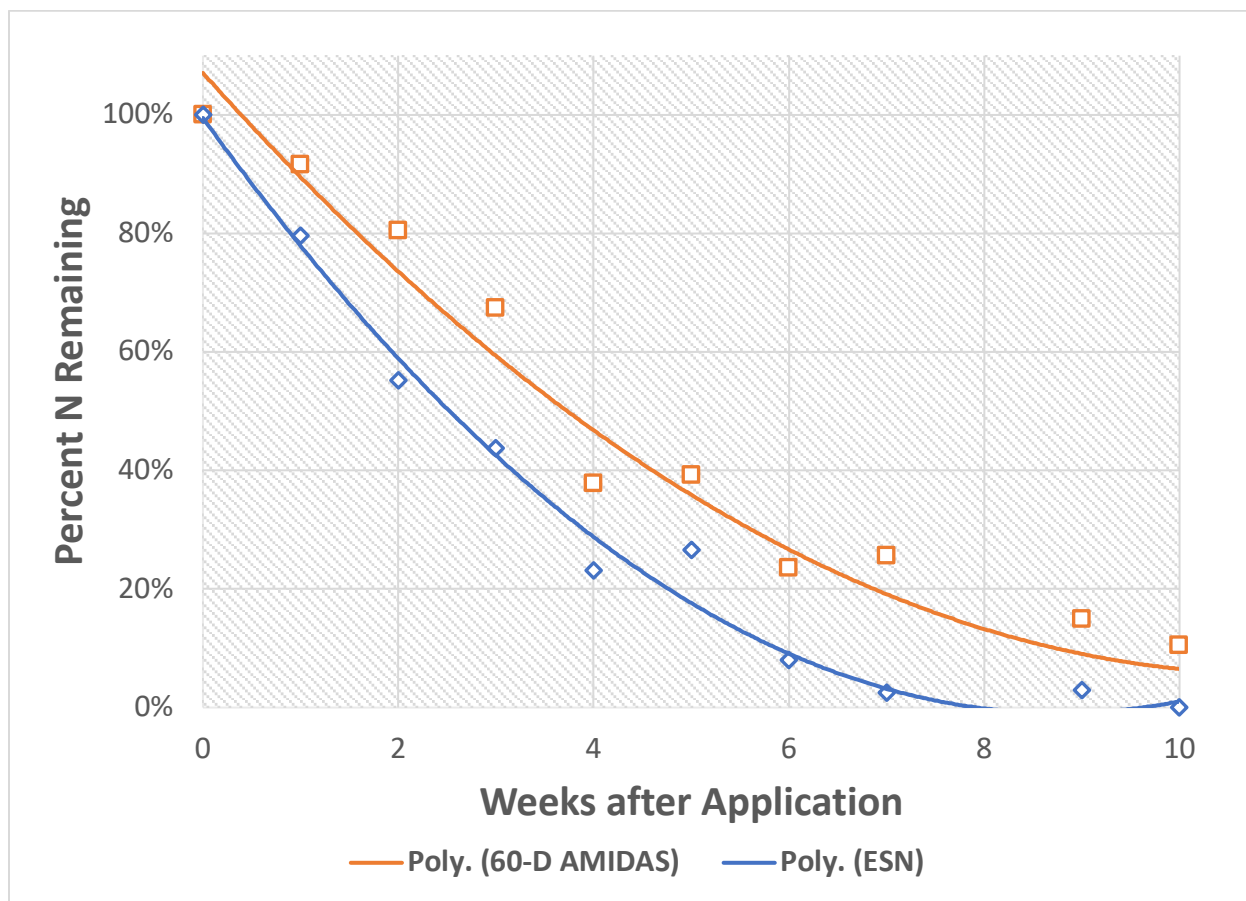


Figure A1. Differences between fertilizer treatments of polymer coating release across a 10-week period. Nitrogen Release was measured for Poly (60-Day AMIDAS®) and Poly (ESN).