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IMPROVING THE EFFICIENCY OF SOIL AND FOLIAR FERTILIZATION WITH UREA USING UREASE INHIBITORS

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SYNOPSIS

Urea is the most recommended foliar N source due to its relatively low toxicity, quick absorption, and low cost. However, in the literature reports of yield increases with foliar urea application are inconsistent. Field and growth chamber studies were conducted to study the action and benefit the urease inhibitor N-butyl thiophosphoric triamide (NBPT) with soil and foliar urea application. Both field and growth chamber studies had treatments: (T1) untreated control, (T2) full recommended N rate with urea, (T3) 75% of the recommended N rate with urea, (T4) 75% of the recommended N rate with urea plus NBPT and, (T5) 75% of the recommended N rate with urea plus NBPT and DCD, and at two temperatures 86F and 100F in the growth room study. Addition of NBPT to foliar urea inhibited urease activity, and exhibited a trend for increased leaf urea content and improved cell membrane integrity. In the field study the addition of NBPT to foliar urea resulted in an increase in seedcotton yield in 2011 but not in 2012 due to the hot dry season masking the effects.

In conclusion, the N fertilization treatment of urea with NBPT increased N uptake and dry matter production and yield of cotton compared to urea alone. High temperature also had a positive effect on N uptake but it did not influence in the performance of NBPT. In this research the application of 75% of the full N rate with urea plus NBPT resulted in lower N uptake and dry matter production compared to the full N rate with urea alone. Thus, when using urea with NBPT, a higher rate than 75% of the full recommend N should be considered. In field experiments application of urea with NBPT at 75% of the full recommend had similar lint yields compared to urea application at the full N rate. Overall, NBPT was effective in inhibiting cotton leaf urease, and in improving nitrogen use efficiency and yield in field-grown cotton.

JUSTIFICATION

Foliar N application has been used as a supplement to meet cotton N requirements (Oosterhuis, 1999). Cotton root capacity for absorbing nutrients declines when the plants are developing fruit (Maples and Baker, 1993), and therefore at this stage it is reasonable to supply N to the plants by foliar application. Foliar application of N has the advantages of low cost and rapid response of the plant, and the disadvantages of possible foliar burn, compatibility problems with other chemicals and limitations on the amount of nutrient that can be applied (Oosterhuis, 1999). Many studies have been done testing the use of foliar urea in cotton; however results in yield have been inconsistent (Maples and Barker, 1993; Oosterhuis and Bondada, 2001; Wilborn et al., 2006).

Crops are usually known to have low N use efficiency, recovering only 30 -35% of the N supplied (Constable and Rochester, 1988; Daberkow et al., 2000). Different practices have been recommended to increase crop N use efficiency and much attention has been focused on the use of urease and/or nitrification inhibitors to decrease losses of N by volatilization and leaching. Urease inhibitors (i.e. N-(n-butyl) thiophosphoric triamide - NBPT) delay hydrolyzes of urea fertilizer and thereby diminishes ammonia volatilization losses, and nitrification inhibitors (i.e. Dicyandiaminde - DCD) hinder the conversion of ammonium to nitrate lowering N loss by leaching.).

Cotton yields in the U.S.A. are negatively affected by periods of extreme high temperatures during flowering and boll development (Oosterhuis. 2002). Although cotton originates from warm temperature regions, the cotton plant is known to respond negatively to high temperatures (Oosterhuis, 2002). Optimum temperature for cotton growth is around 86^{o}F (Reddy et al., 1992); however in the US Cotton Belt, temperatures commonly reach values higher than 95^{o}F (Reddy et al., 1991). High temperature during reproductive development is the main factor causing lower and variable cotton yields in the US. Although the use of urease and nitrification inhibitors to crops are widely used, there has been limited work on the effects of these inhibitors on the cotton growth and N assimilation physiology under high temperature conditions. This research is designed to address these gaps in our knowledge and provide a better understanding of the N behavior in cotton plants under condition of heat stress.

OBJECTIVES

The main objective is to study how the use of the urease inhibitor NBPT will affect the efficiency of soil and foliar urea application. An additional objective was to determine the effect of increased temperature on the effectiveness of the urease inbibitor NPBT. With a better understanding of the physiological effects of soil and foliar urea application and the use of a urease inhibitor, we expect to improve foliar N management in crops.

MATERIAL AND METHODS

Growth room and field tests were conducted to study how the urease inhibitor NBPT will affect the efficiency of foliar urea application.

Growth Room Study:

The experiment was conducted in the Altheimer laboratory, Arkansas Agricultural Research and Extension Center in Fayetteville, AR. Cotton (*Gossypium hirsutum* L.) cultivar

ST4554 B2RF was planted in 2 liters pots filled with soil from a typical cotton growing area in Marianna, AR (Loring silt loam - fine-silty, mixed, active, thermic Oxyaquic Fragiudalfs). The pots were arranged in two large walk-in growth chambers (Model PGW36, Conviron, Winnipeg, Canada) with day/night temperatures of 30/20^{o}C, 12 hour photoperiods and a relative humidity of 70%. After 6 weeks, about one week prior to flowering, the day temperature of one growth chamber was increased in 2^{o}C increments every 2 days until the temperature reached 38^{o}C, while the temperature of the other chamber was maintained at 30^{o}C. The chambers were assumed to be identical in all variables (e.g., light and relative humidity) with differences only in day temperatures (30^{o}C and 38^{o}C).Plants were watered daily with deionized water only. The experiments were arranged in a completely randomized design with two factors and 5 replications. The factors consisted of N treatment and temperature treatment.

The N treatments consisted of: (T1) untreated control, (T2) full recommended N rate with urea, (T3) 75% of the recommended N rate with urea, (T4) 75% of the recommended N rate with urea plus NBPT and, (T5) 75% of the recommended N rate with urea plus NBPT and DCD. The full recommended N rate consisted of 125 kg ha^{-1} and correspondingly 94 kg ha^{-1} of N was used for 75% of the recommended N rate treatment. Treatments with urea plus NBPT, and urea plus NBPT and DCD, were applied using the commercial fertilizers Agrotain (Agrotain Int. LLC) and Super U (Agrotain Int. LLC), respectively. Nitrogen fertilization was split-applied at pre-plant and pinhead-square (PHS) stages. At pre-plant P_2O_5, K_2O and half of the N fertilizers was placed approximately 0.1 m below the seed. At PHS, the other half of the N rate was side-dress applied, incorporated 7 days later with ample water (12mm). All nutrient fertilization was calculated for the area of one hectare with a 0.15 m furrow slice. Flowers were collected at the first-flower stage and immediately stored in an ultra-freezer at -80^{o}C for subsequent protein and enzymes determination. At 4 weeks after FF plants were harvested for growth analysis and N uptake determination.

Field Study:

A field study, a repeat of the previous year, was conducted at the University of Arkansas Lon Mann Cotton Branch Station at Marianna, AR in a Memphis silt loam (Fine-silty, mixed, active, thermic Typic Hapludalfs) soil. The experiment was uniformly fertilized following preseason soil tests and state extension recommended rates, except for N, which was applied according to the treatments. Treatments consisted of: (T1) full recommended N soil rate with no foliar N application; (T2) 75% of recommended N soil rate with no foliar application; (T3) 75% of recommended N soil rate with two foliar urea applications (at first flower and two weeks later); (T4) 75% of recommended N soil rate with two foliar urea plus NBPT applications (at first flower and two weeks later). Each foliar urea application was calculated to supply 11.2 kg of N per hectare. The treatment with urea plus NBPT was applied using the commercial fertilizers Agrotain (Agrotain Int. LLC). The full recommended N rate consisted 125 kg N ha^{-1} and 93.7 kg N ha^{-1} was used for 75% of the recommended N rate treatment. Soil-applied N fertilization was side-dressed at planting and at the pinhead-square stage using urea. Weed, insect control and irrigation were performed according to state extension recommendations. The experiment was conducted using a plot size of 4 rows spaced 0.96 m apart by 15 m length. A randomized complete block design with 5 replications was used to conduct the experiment. Seedcotton yield was measured from the two middle rows using a mechanical harvester.

Statistical Analyses: In the growth chamber study a three factor factorial analysis was used, with the factors being treatment application, time of measurement and experiment. The objective of this analysis was to observe the interaction effect between treatment and time of measurement and the main effect of treatment. For the field study a two factor factorial analysis was used, in which the factors consisted of treatment application and year of the study. The software JMP version 8.1 (SAS Institute Cary, NC) was used to perform the statistical analyses. Mean and standard error values were calculated to assemble graphs using the Sigma Plot software version 10 (MMIV Systat Software, Inc., San Jose, CA). Analysis of Variance and LSD test (α=0.05) were used to analyze statistical significance between means. A probability less than 0.05 was considered significant.

RESULTS

<u>Field Study:</u>

In the previous year, there was a significant (P=0.0029) treatment effect (see 2011 Report) with the treatments 100% N Soil–No Foliar and 75% N Soil–Urea+NBPT Foliar exhibiting the highest yields. Significant differences were observed between the treatments 100% N Soil–No Foliar and 75% N Soil–No Foliar (P=0.0013), between 100% N Soil–No Foliar and 75% N Soil–Urea Foliar (P=0.0167), between 75% N Soil–No Foliar and 75% N Soil–Urea+NBPT Foliar (P=0.0017), and between 75% N Soil–Urea Foliar and 75% N Soil–Urea+NBPT Foliar (P=0.0221). No differences were observed between the treatments 100% N Soil–No Foliar and 75% N Soil–Urea+NBPT Foliar (P=0.8831), and between 75% N Soil–No Foliar and 75% N Soil–Urea Foliar (P=0.1901). Comparative analysis of the treatments indicated that 75% N Soil–Urea+NBPT Foliar (1997.10±108.25 kg ha^{-1}) exhibited a 20%, and 12% increase in seedcotton yield compared to the treatments 75% N Soil–No Foliar (1660.05±61.52 kg ha^{-1}) and 75% N Soil–Urea Foliar(1776.60±62.68 kg ha^{-1}), respectively. In 2010 (Fig. 4B), the treatment effect on seedcotton yield was not significant (P=0.0951). Differences were expected between the treatments 100% N Soil–No Foliar and 75% N Soil–No Foliar, but the comparison was not significant (P=0.1106).

In the current field experiment, there were no significant (P=0.05) differences between the treatments (Fig. 1). This may have been due to the extremely hot and dry season that was experienced, which may have negated the ability of the plant to efficiently use the available N.

Growth Room Study:

Statistical analysis of the data showed that there was no significant interaction effect between N treatment and temperature regime in any of the measurements collected. Significant N treatment effect was observed in the measurements of protein ($P=0.0298$) glutathione reductase ($P<0.0001$), N uptake ($P<0.0001$), and dry matter ($P<0.0001$). Temperature regime effect showed statistical significance on data of protein ($P=0.0085$), N uptake ($P<0.0001$), and dry matter ($P=0.0035$). Cotton ovary protein analysis showed a 10% increase in protein content in the high temperature (38^oC) treatment (data not shown). Protein comparison between N treatments (Table 1) showed the lowest content in the ovaries collected from unfertilized control plots and no difference between fertilized treatments. Furthermore, enzyme data (Fig. 2) indicated that flowers from the unfertilized treatment had a two-fold increase in activity of glutathione reductase compared to fertilized treatments. N measurements (Table 2) showed significantly higher N uptake in the treatment of urea at full recommended N compared to the Agrotain and Super U treatments. No difference in N uptake was observed between Agrotain and Super U treatments, however both had significantly higher uptake than urea application at 75% of the full recommended N rate. High temperature (38^oC) significantly increased N uptake (data not shown) and dry matter production (Fig. 3). N treatment effect on cotton dry matter production (Fig. 4) was similar to N uptake data, with urea full rate having the highest dry matter values, followed by Agrotain and Super U treatments. Urea application at 75% of full N rate exhibited significantly lower dry matter than Agrotain and Super U treatments.

In summary the results of this experiment indicated that high temperature increased N uptake which resulted in higher protein and dry matter production. The performance of the sources of N in this experiment was not affected by high temperature, since no significant interaction was detected. As expected N deficiency decreased cotton protein content and increased glutathione reductase activity in cotton ovaries. The addition of NBPT to urea fertilization was effective in improving N uptake of cotton plants. On the other hand, no benefit of addition of DCD was observed in any of the measurements collected.

Discussion

In last year’s growth chamber study the application of NBPT decreased membrane leakage and decreased urease activity measured 24 h after application. In addition, there was no effect on antioxidants and protein, indicating that the plants in the different nitrogen treatments were not stressed. In the current study, the addition of NBPT to foliar urea application decreased urease activity and it showed trends for increasing leaf urea content and improving cell membrane integrity. Addition of NBPT to the 75% urea treatment increased N uptake to the level of the 100% urea treatment. Similarly, the addition of NBPT to the 75% urea treatment, increased the dry matter, although not completely to the level of the 100% urea treatment. High temperature had no significant effect on the urease inhibitor.

In the previous field study seedcotton yield improvements were observed with addition of NBPT to foliar urea, but no positive effect of DCD. In the current field experiment, there were no significant (P=0.05) differences between the treatments (Fig. 1). This may have been due to the extremely hot and dry season that was experienced, which may have negated the ability of the plant to efficiently use the available N.

In conclusion, the N fertilization treatment of urea with NBPT increased N uptake and dry matter production and yield of cotton compared to urea alone. High temperature also had a positive effect on N uptake but it did not influence in the performance of NBPT. In this research the application of 75% of the full N rate with urea plus NBPT resulted in lower N uptake and dry matter production compared to the full N rate with urea alone. Thus, when using urea with NBPT, a higher rate than 75% of the full recommend N should be considered. However, in field experiments application of urea with NBPT at 75% of the full recommend had similar lint yields compared to urea application at the full N rate. An explanation for these conflicting results could be related to the fact that in this growth room study cotton plants were grown in pots capable of holding only two liter of soil.

PROJECT DURATION:

The results warrant a third year of field studies to confirm findings. This is because we had such clear positive effects of urease improving plant nitrogen use efficiency in the first year, whereas in the second year, we did not show significant differences due to the extremely hot and dry season causing a general stress on the crop which masked any significant urease effects. Also, we would like to determine the effect of soil salinity on activity and benefit of urease inhibitor.

BUDGET:

Item	**Requested ($/year)**
Personnel:	
Hourly student labor	500
Attend Fluid Fertilizer Forum	
Airfare, accommodation and meals	950
Operating Supplies:	
Biochemical analyses	500
Growth chamber rent and maintenance	1,000
Field plot maintenance	1,300
Harvest measurements	100
Physiological measurements & analyses	300
Soil analysis	100
Tissue analysis	200
Miscellaneous supplies and chemicals	50
Travel to field plots and annual meeting	1,500
Total:	6,500

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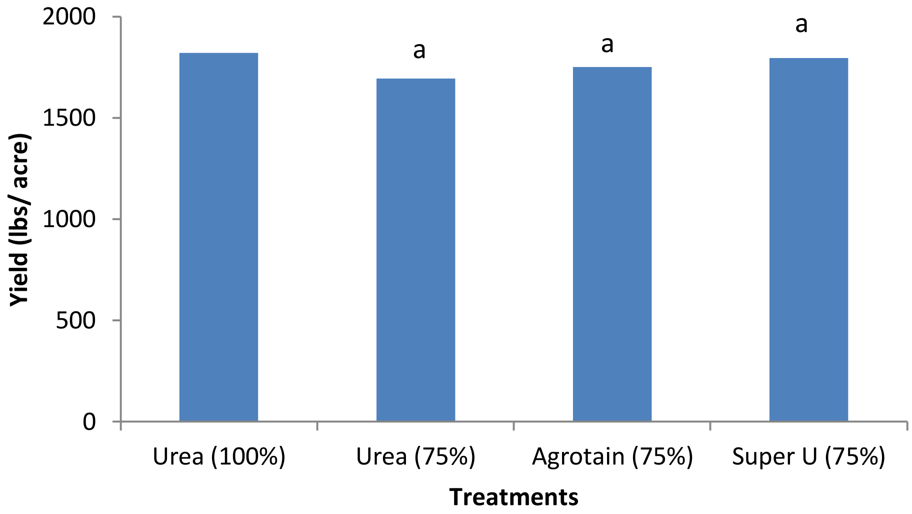

Figure 1. Effect of urea with and without NBPT and DCD on cotton fiber yield (lbs./acre). Columns with the same letter are no significantly different (P=0.05).

Table 1. Effect of temperature and urea with and without NBPT and DCD on cotton ovary protein content. Rows with the same letter are no significantly different (P=0.05).

N Treatment	**Protein mg g^{-1} FW**
Control	0.550 b
Full Urea (100%)	0.651 a
Urea 75 %	0.644 ab
Agrotain 75 %	0.729 a
Super U 75%	0.700 a

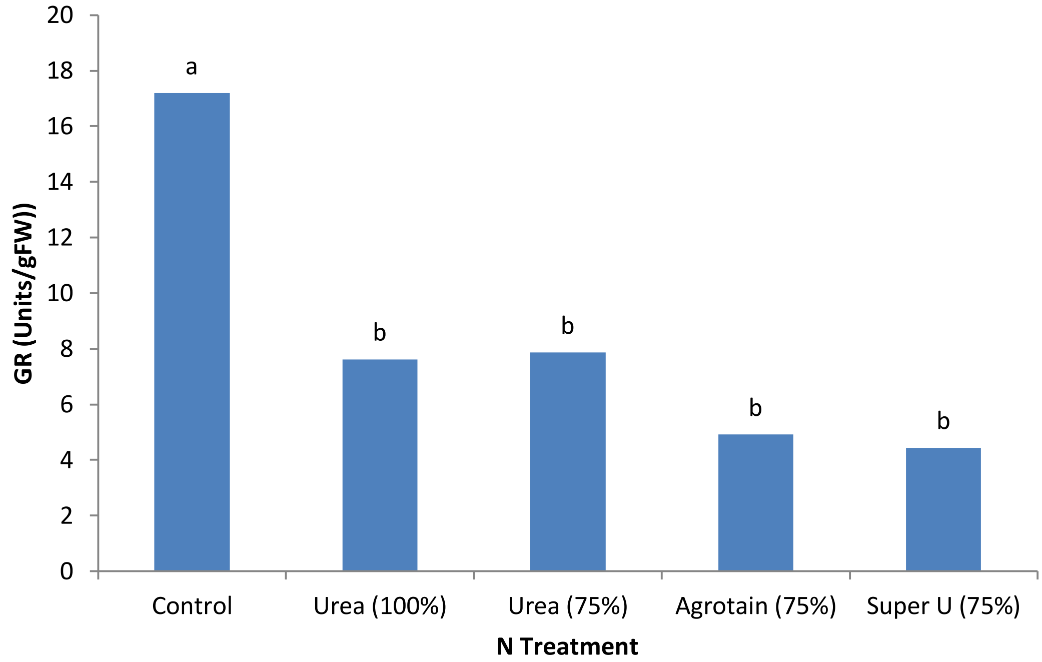

Figure 2. Effect of urea with and without NBPT and DCD on GR activity. Columns with the same letter are no significantly different (P=0.05).

Table 2. Effect of temperature and urea with and without NBPT and DCD on cotton N uptake. Rows with the same letter are no significantly different (P=0.05).

N Treatment	**N Uptake (g)**
Control	0.024 d
Full Urea (100%)	0.095 a
Urea 75 %	0.069 c
Agrotain 75 %	0.084 b
Super U 75%	0.085 b

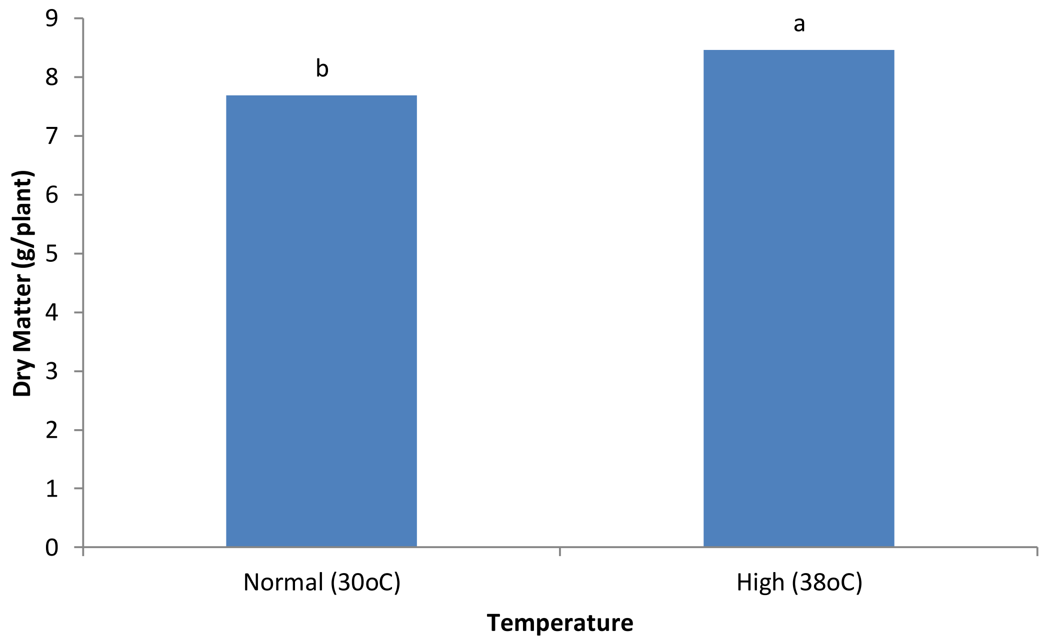

Figure 3. Effect of temperature on cotton dry matter production. Columns with the same letter are no significantly different (P=0.05).

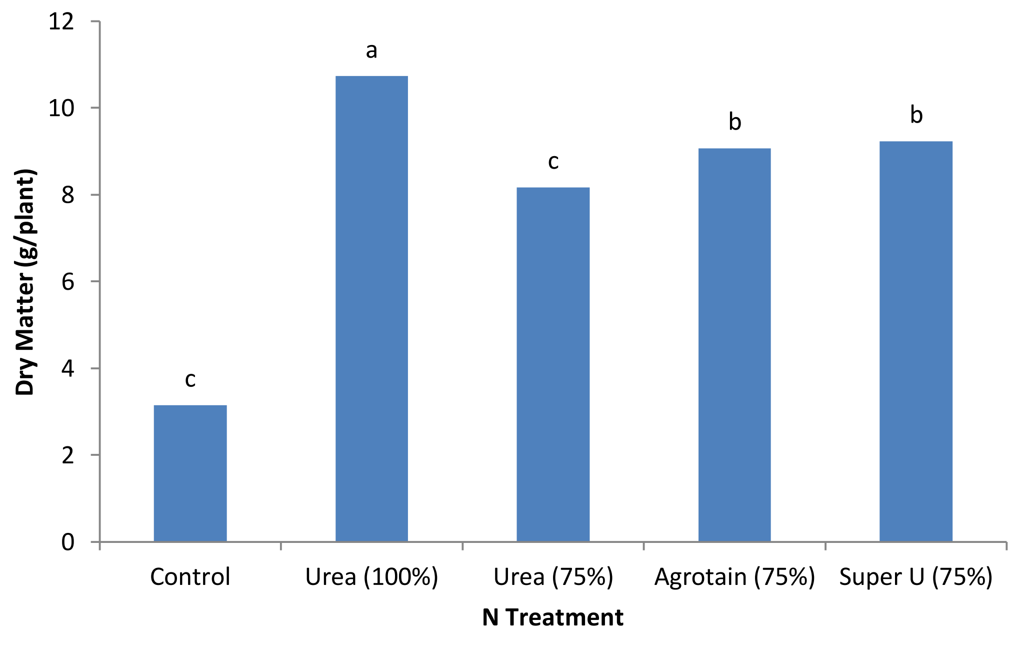

Figure 4. Effect of urea with and without NBPT and DCD on cotton dry matter production. Columns with the same letter are no significantly different (P=0.05).