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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 applications. 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. Reducing the N application by 75% of the full N rate with urea resulted in lower N uptake and dry matter production and lower yields compared to the full N rate with urea alone. However, **soil** application at 75% N of urea with NBPT increased N uptake and dry matter production and yield of cotton compared to urea alone. 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 a significant increase in seedcotton yield in 2011 but not in 2012 and 2013 due to the environmental stress during the season masking the effects. High temperature, 86F and 100F in the growth room study, had a positive effect on N uptake but it did not influence in the performance of NBPT. Thus, when using urea with NBPT, a reduced rate of the recommend N should be considered. In our 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.

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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 inhibitor 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.

Field Study:

Field studies were conducted in 2010 and 2012 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 experiments 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. Measurements included plant dry matter, nitrogen uptake, N Use Efficiency, N partitioning (stem, leaves, capsule wall, and seeds), fiber quality and seedcotton yield. Seedcotton yield was measured from the two middle rows using a mechanical harvester.

Growth Room Studies:

Two studies were conducted: a high temperature stress study and a salinity study. Experiments were 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%.

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.

High Temperature Study: 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.

Salinity Study: Three levels of salinity treatment were used for this experiment, low (0.45 dS m_1), moderate (8 dS m_1) and high (16 dS m_1). The low-salinity treatment was based on the conductivity value of the soil from Marianna, AR, and the medium- and high-salinity treatments were based on the 7.7 dS m_1 threshold previously stated in the literature. To determine the amount of salt to be added in each treatment, a preliminary calibration study was carried out by incubating different quantities of NaCl for 48 h to a known volume of soil and measuring conductivity using the saturated paste method (Rhoades et al. 1989). Sodium chloride was dissolved in distilled water and added to each pot according to the treatments when cotton seedlings exhibited unfolded cotyledons. At the pinhead-square (PHS) stage, measurements of stomatal conductance, quantum yield and leaf chlorophyll content were taken. Subsequently, growth analysis was conducted separately for each plant. One plant was oven-dried for dry matter and N uptake determination. Leaves of another plant were collected and immediately stored in an ultra-freezer (_80 °C) for subsequent protein and enzyme determination. For the different enzyme assays, a homogeneous subsample of leaves was used for each procedure. The experiment was terminated at PHS to determine the salinity and N treatment effect on cotton seedlings. Measurements included proteins, superoxide dismutase, glutathione reductase, glutathione synthetase, nitrate reductase, stomatal conductance, chlorophyll content, growth analysis and nutrient content. Details of the methods are given in the attached manuscript (KAWAKAMI E.M., OOSTERHUIS D.M. and SNIDER J.L. 2013. Nitrogen assimilation and growth of cotton in response to NaCl salinity and urea application with NBPT and DCD. *Journal of Agronomy and Crop Science*. *(in press DOI: 10.1111/jac.12002).*

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

Field Study:

Soil Applied Study:

The objective of this study was to evaluate the performance of the urease inhibitor NBPT and the nitrification inhibitor DCD with urea fertilizer on the physiology and yield of irrigated field-grown cotton. Field experiments were conducted for two years with the following treatments: untreated control, full recommended N rate with urea, 75% recommended N rate with urea, 75% recommended N rate with urea + NBPT, and 75% recommended N rate with urea + NBPT + DCD. Plant growth in the five treatments is shown in Figure 1. The growth reflects the yield differences that were recorded (Fig. 2). Yields were significantly increased by the recommended N rate compared to the control, as expected. However, the treatment Urea-75% plus NBPT improved cotton N uptake by 17% (Fig. 2) and N use efficiency by 41% (Fig. 3) when compared to the Urea-75% alone. NBPT addition to urea also positively affected leaf chlorophyll content (Fig. 4), plant growth (Fig 5) and fiber quality (data not shown). In addition, there was an indication that protein content was improved by addition of NBPT (Table 1), glutathione reductase activity was reduced indicating less plant stress (Fig. 6), and N uptake was improved (Table 2). The use of NBPT improved cotton lint yield by 14% compared to a similar urea application rate without NBPT. However, addition of DCD to urea fertilizer limited NBPT performance. The use of DCD resulted in decreased N uptake, N use efficiency, leaf chlorophyll, plant growth and yields. Under the field conditions of the current studies, it is concluded that addition of NBPT to urea, but not DCD in combination with NBPT, improved N fertilization in cotton. For more details see: 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 NBPT and DCD. *European Journal of Agronomy* 43:147-154.

Foliar Applied Study:

In the first year there was a significant (P=0.0029) treatment effect with the treatments 100% N Soil–No Foliar and 75% N Soil–Urea+NBPT Foliar exhibiting the highest yields (Fig. 7). 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. Leaf urea content was significantly increased by NBPT (Fig.8) and there was a trend for NBPT and DCD to improve membrane leakage (cell integrity) (Fig. 9). In the second year, 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 third year there were no significant (P=0.05) differences between the treatments. 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, addition of NBPT to foliar-applied urea inhibits leaf urease activity and has the potential of increasing cotton yield. However, a significant yield response only occurred in 1 of 3 years, and this was attributed to plant growth activity and environmental conditions at the time of application.

High Temperature Stress 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. Temperature had a significant effect on plant dry matter production as expected (Fig. 10). Significant N treatment effect was observed in the measurements of N uptake, protein, glutathione reductase, and dry matter (data not shown). Temperature regime effect showed statistical significance on data of N uptake (P<0.0001) (Fig. 11) and N Use Efficiency (Fig. 12), as well protein (P=0.0085) and dry matter (P=0.0035).

N measurements (Fig. 11) 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^{o}C) significantly increased N uptake and dry matter production. N treatment effect on cotton dry matter production 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. 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.

Salinity Effect of NBPT:

The effect of salinity on the growth and stress response of cotton seedlings, and the effect on N use efficiency from the use of the inhibitors of urease (NBPT) and nitrification (DCD) under salinity stress were studied in growth chambers. Three levels of salinity treatment were used for this experiment, low (0.45 dS m^{-1}), moderate (8 dS m^{-1}) and high (16 dS m^{-1}) and five N treatments: unfertilized control, 100% N rate with urea, 80% N rate with urea, 80% N rate with urea +NBPT, and 80% N rate with urea +NBPT+DCD.

The results indicated that salinity stress reduced plant growth (low leaf area and plant dry matter), decreased N assimilation (low NR, GS and protein), increased plant stress response (high GR and SOD), and decreased leaf chlorophyll, stomatal conductance, and quantum yield. Addition of NBPT to urea improved N uptake by 22% under low salinity (Fig. 13); however, this effect was not observed with increasing salinity. No benefit of addition of DCD was observed in any of the parameters collected. In conclusion, salinity stress hindered the performance of the additive NBPT and negatively affected the growth and physiology of cotton. More details of the results are available in the publication: KAWAKAMI E.M., OOSTERHUIS D.M. and SNIDER J.L. 2013. Nitrogen assimilation and growth of cotton in response to NaCl salinity and urea application with NBPT and DCD. *Journal of Agronomy and Crop Science*. *(DOI: 10.1111/jac.12002).*

CONCLUSIONS:

In conclusion, the **soil** N fertilization treatment of urea with NBPT increased N uptake and dry matter production and yield of cotton compared to urea alone. Reduction of the recommended fertilizer N rate to 75% decreased N uptake, dry matter production and yield, but addition of NBPT increased the yield similar to the 100% N fertilizer rate. Addition of NBPT to **foliar** urea inhibited urease activity, and exhibited a trend for increased leaf urea content, improved cell membrane integrity, and increased yield. The addition of NBPT to **foliar** urea resulted in a significant increase in seedcotton yield in 2011 but not in 2012 and 2013 due to the environmental stress during the season masking the effects. High temperature also had a positive effect on N uptake but it did not influence in the performance of NBPT. Salinity negatively affected the growth and physiology of cotton and reduced N uptake, but the addition of NBPT to the urea fertilizer still improved N uptake. Overall, NBPT was effective in inhibiting cotton leaf urease, and in improving nitrogen use efficiency and yield in field-grown cotton.

PUBLICATIONS FROM THE RESEARCH:

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 NBPT and DCD. *European Journal of Agronomy* 43:147-154.
http://onlinelibrary.wiley.com/doi/10.1111/jac.12002/abstract (see attached pdf)

KAWAKAMI E.M., OOSTERHUIS D.M. and SNIDER J.L. 2013. Nitrogen assimilation and growth of cotton in response to NaCl salinity and urea application with NBPT and DCD. *Journal of Agronomy and Crop Science*. *(DOI: 10.1111/jac.12002).* (see attached pdf)

OOSTERHUIIS, D.M. and KAWAKAMI, E.M. 2013. Increasing yields using urea with urease inhibitors. *Fluid Journal* 21(1):4-9. *(online)* (see attached pdf)

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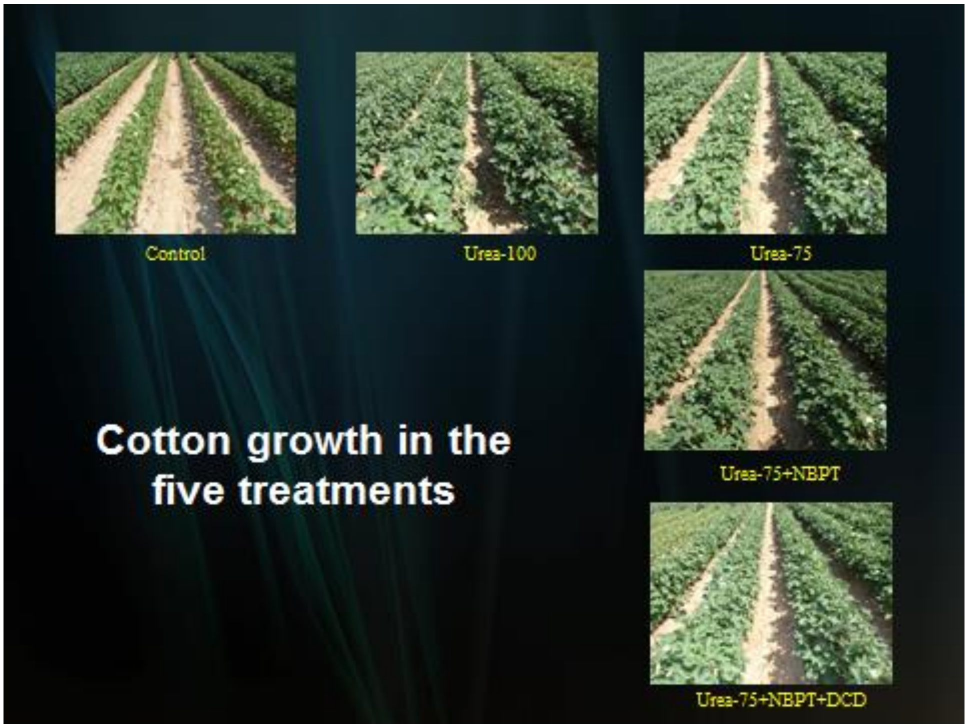

Figure 1. Photographs of field plots showing the treatment differences in growth.

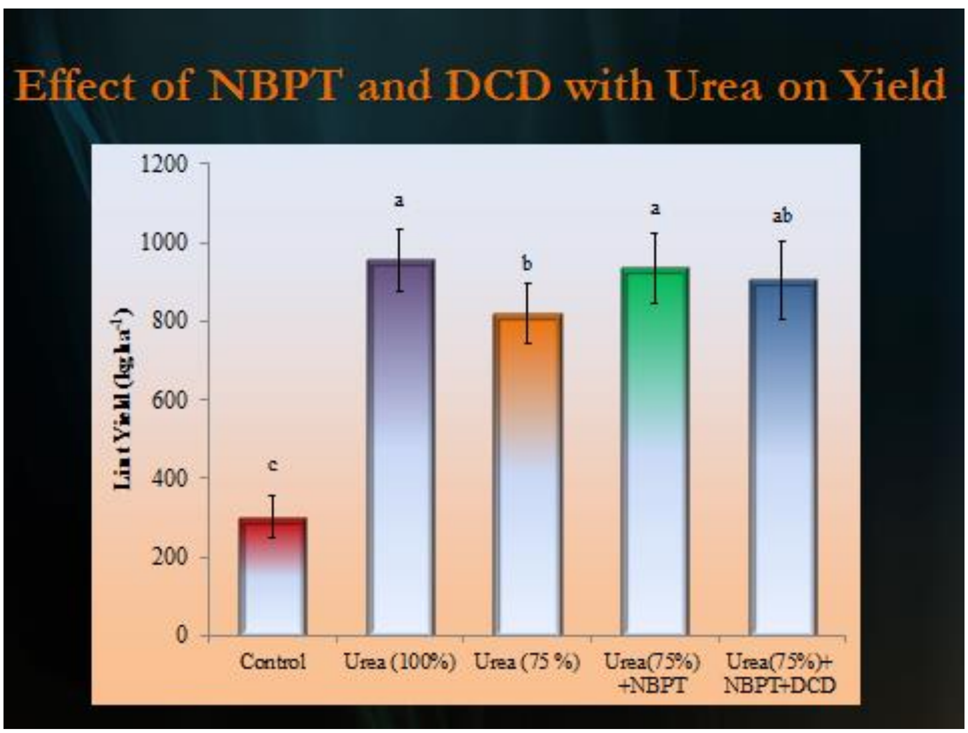

Figure 2. Effect of urea with and without NBPT and DCD on cotton lint yield. Columns with the same letter are no significantly different (P=0.05).

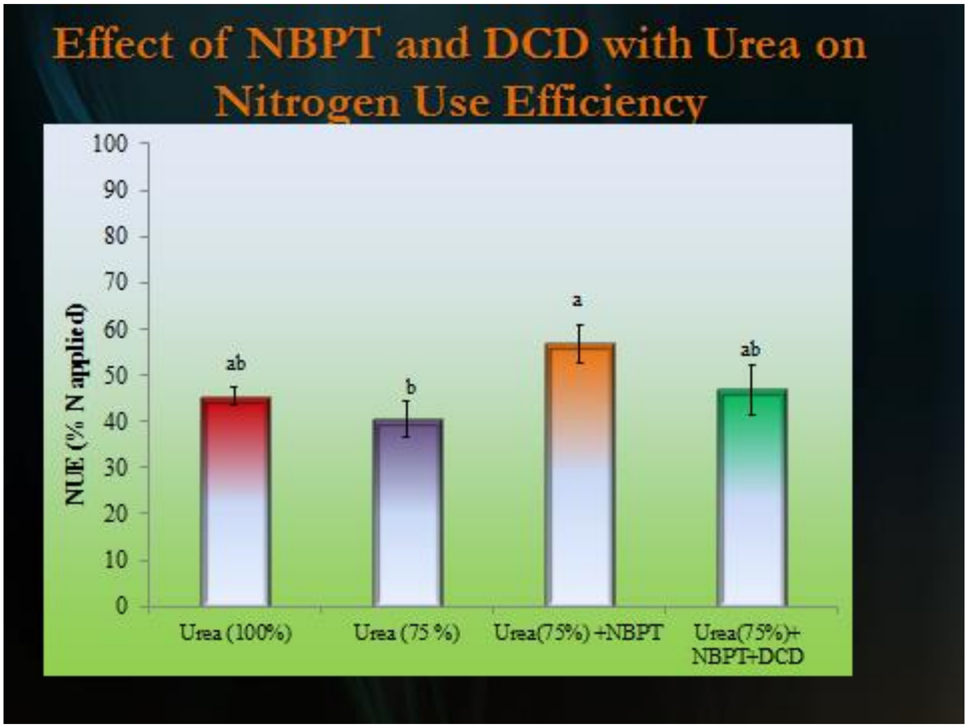

Figure 3. Effect of urea with and without NBPT and DCD on nitrogen use efficiency. Columns with the same letter are no significantly different (P=0.05).

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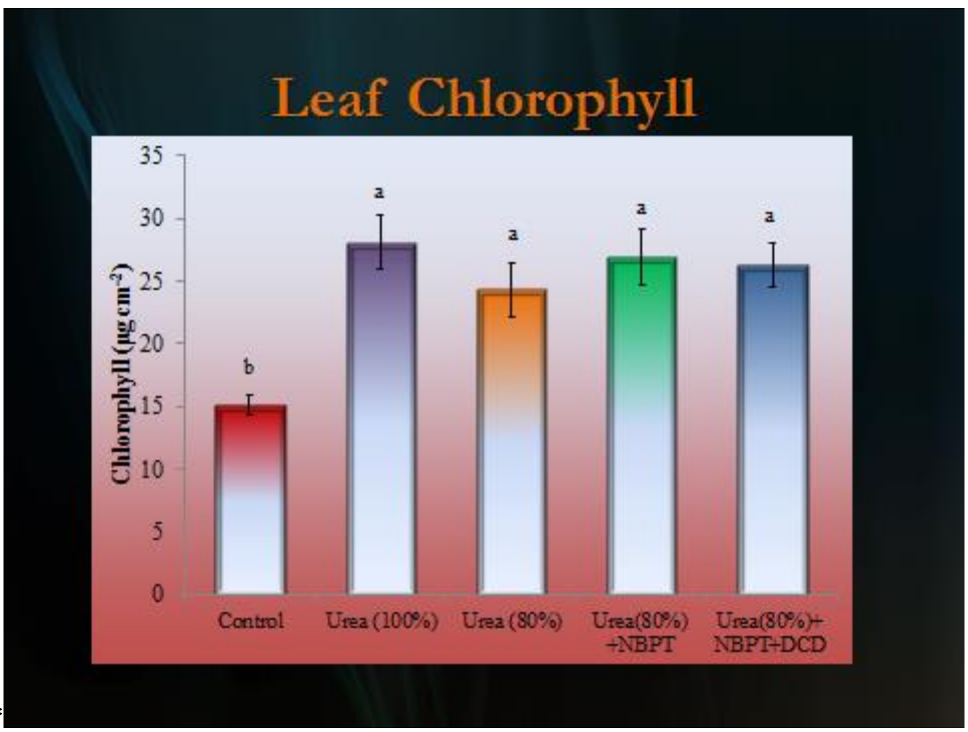

Figure 4. Effect of urea with and without NBPT and DCD on nitrogen use efficiency. Columns with the same letter are no significantly different (P=0.05).

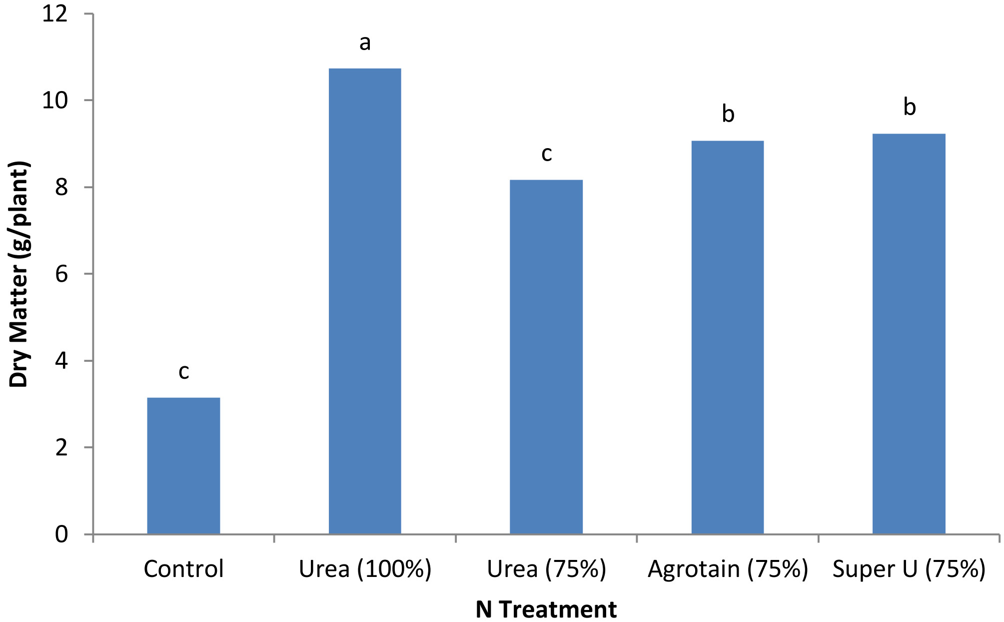

Figure 5. Effect of urea with and without NBPT and DCD on cotton dry matter production. Columns with the same letter are no significantly different (P=0.05).

Table 1. Effect of treatment (urea with and without NBPT and DCD) on cotton ovary protein content. Columns 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

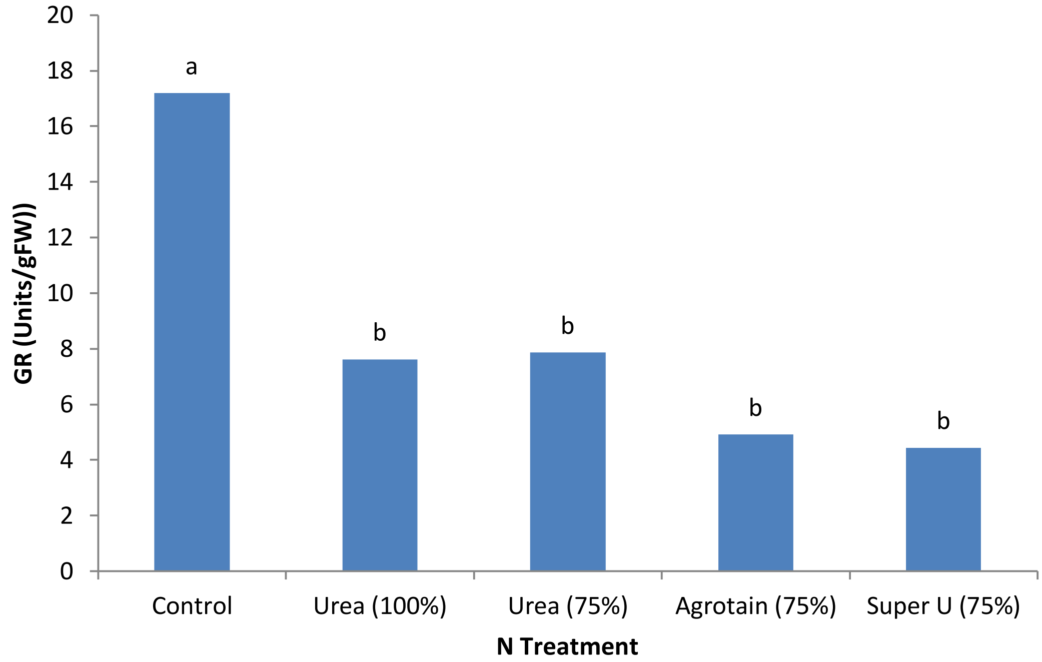

Figure 6. Effect of urea with and without NBPT and DCD on GR activity. Columns with the same letter are no significantly different (P=0.05).

Table 2. Effect of treatment (urea with and without NBPT and DCD) on cotton N uptake. Columns 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

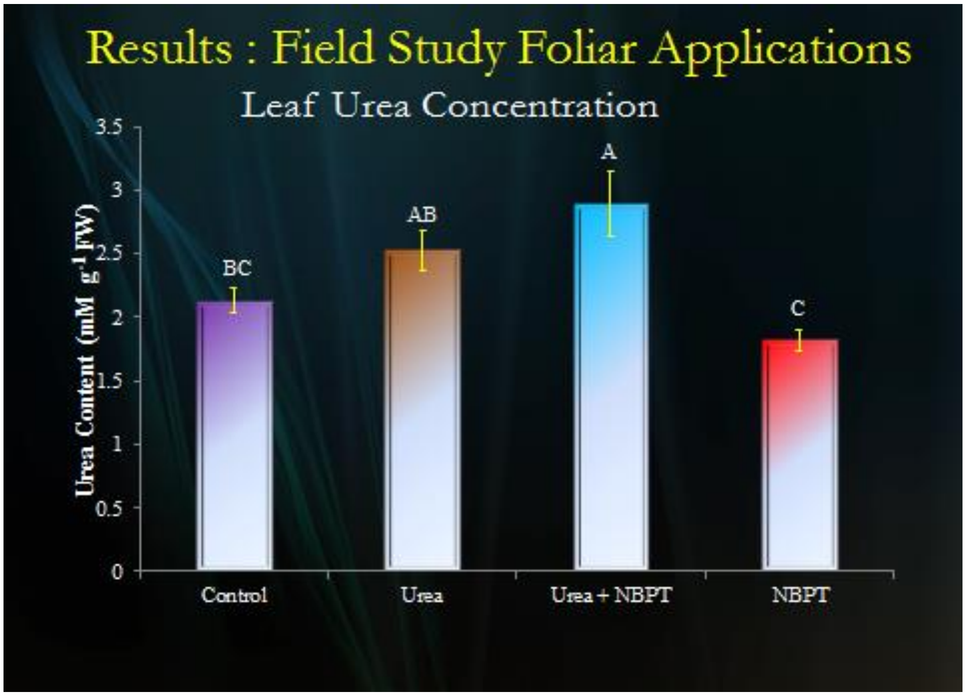

Figure 7. Effect of foliar-applied applications of urea with and without NBPT and DCD on leaf urea content. Columns with the same letter are no significantly different (P=0.05).

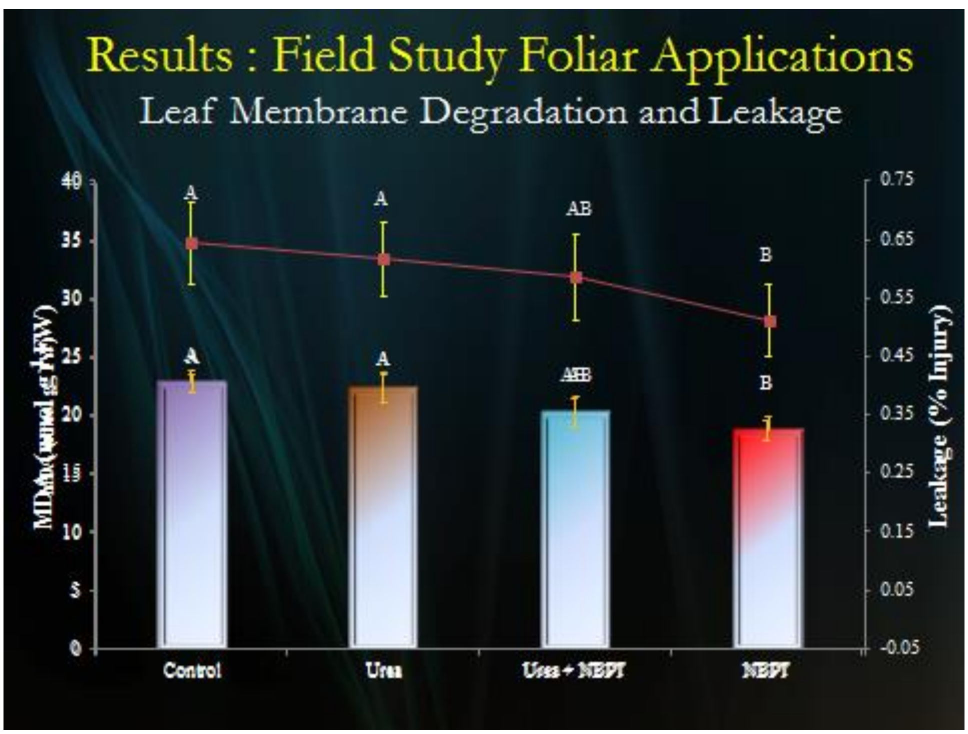

Figure 8. Effect of foliar-applied applications of urea with and without NBPT and DCD on membrane leakage (indication of cell integrity). Columns with the same letter are no significantly different (P=0.05).

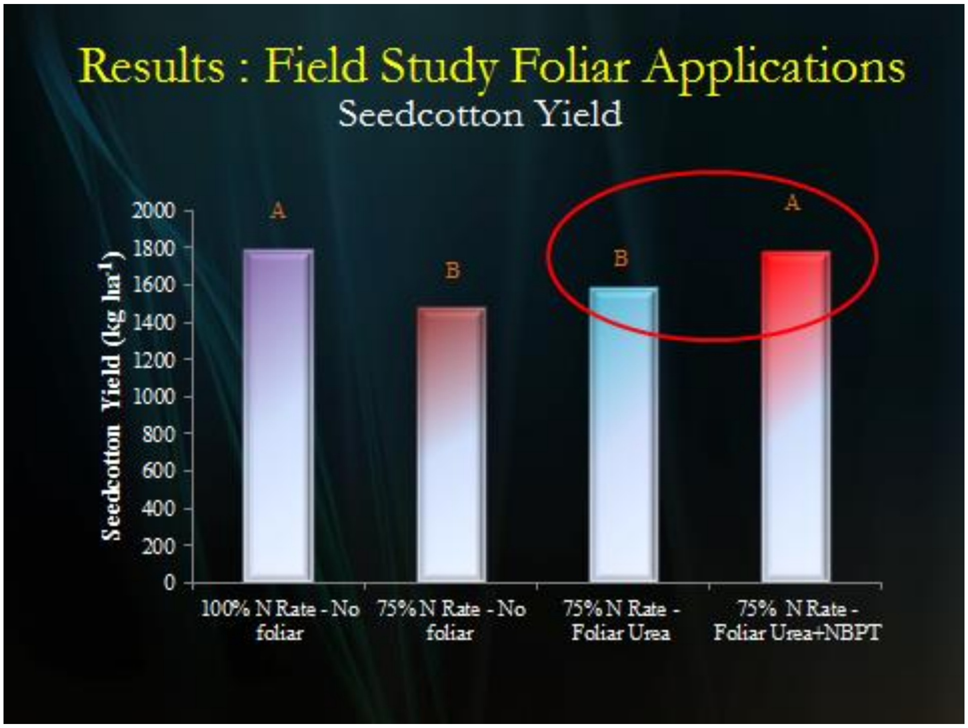

Figure 9. Effect of foliar-applied applications of urea with and without NBPT and DCD on seedcotton yield. Columns with the same letter are no significantly different (P=0.05).

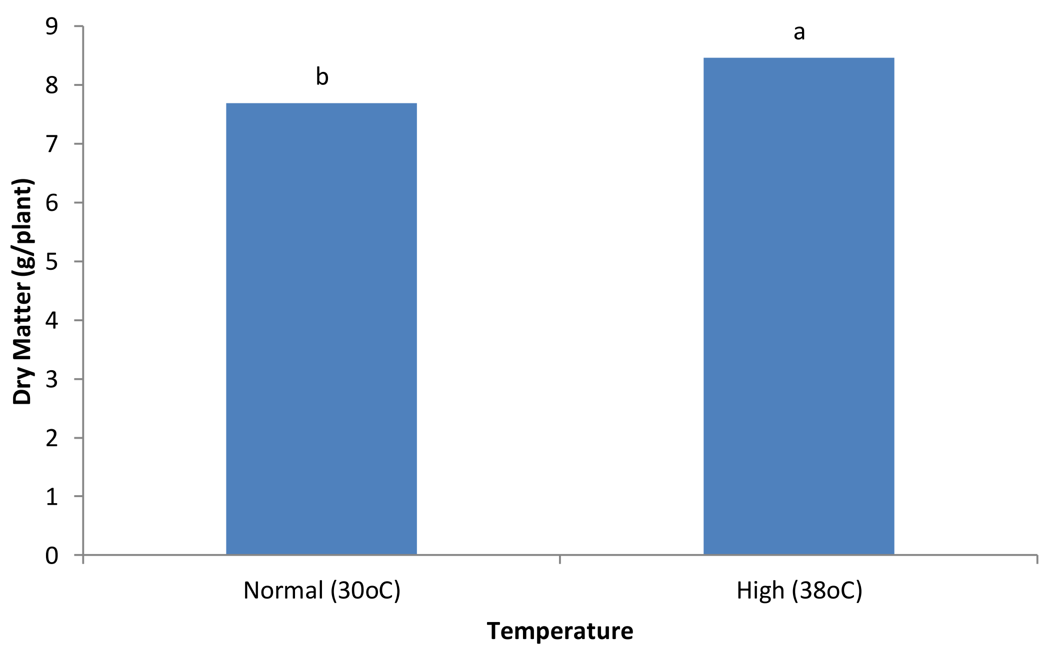

Figure 10. Effect of temperature on cotton dry matter production. Columns with the same letter are no significantly different (P=0.05).

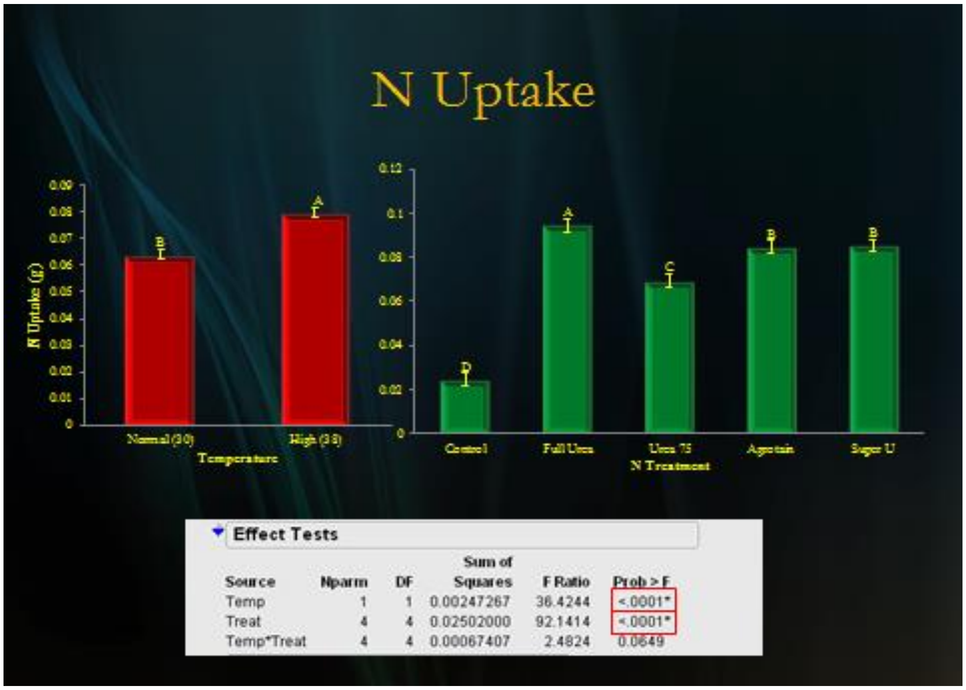

Figure 11. Effect of temperature and treatment (urea with and without NBPT and DCD) on nitrogen uptake. Columns with the same letter are no significantly different (P=0.05).

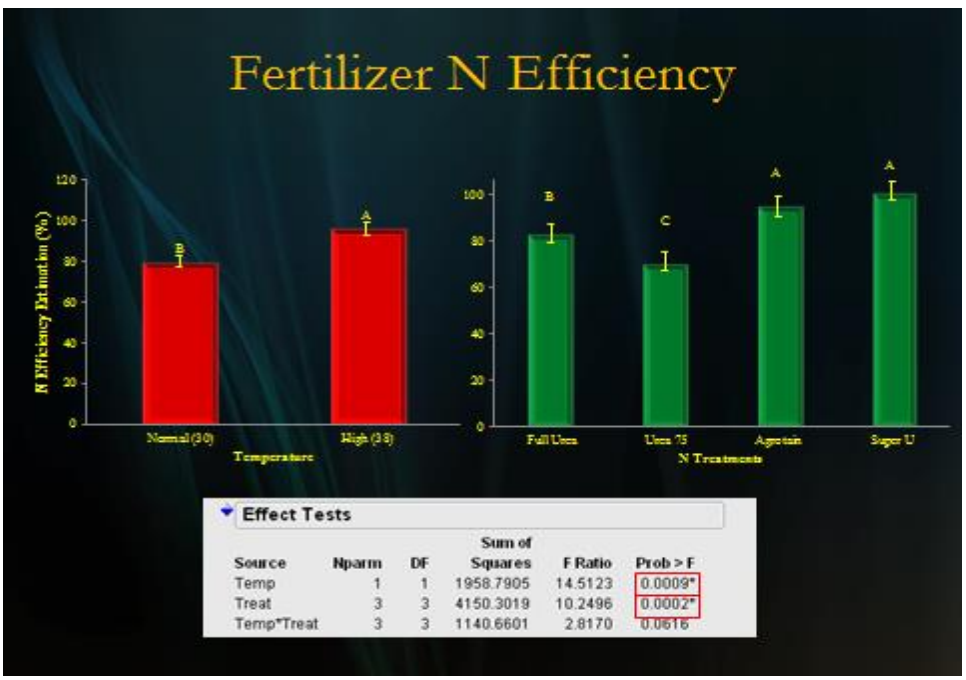

Figure 12. Effect of temperature and treatment (urea with and without NBPT and DCD) on nitrogen use efficiency. Columns with the same letter are no significantly different (P=0.05).

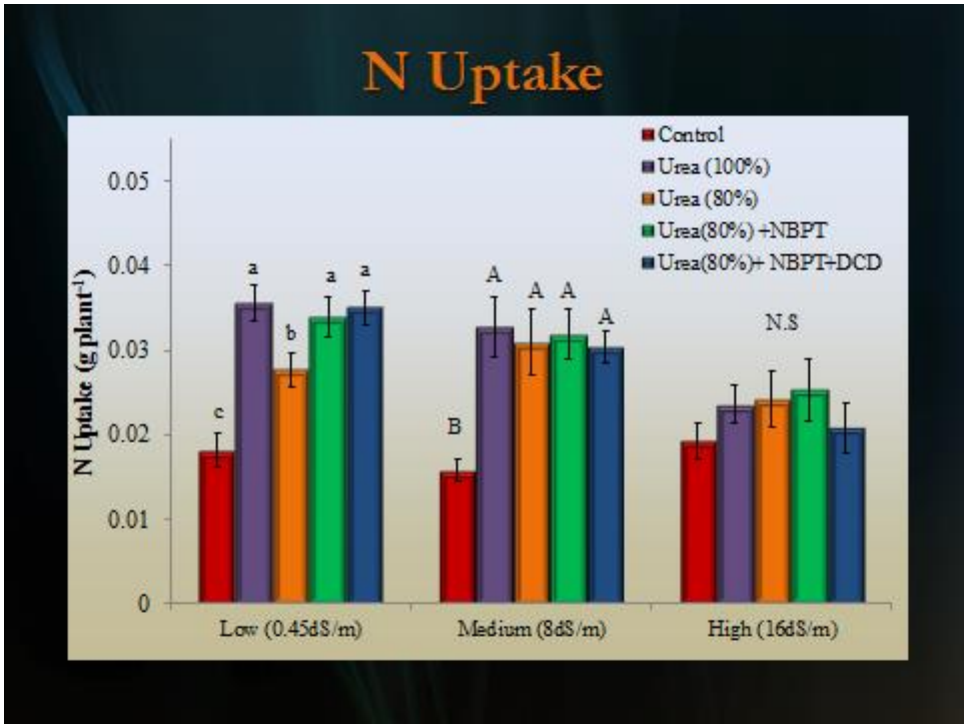

Figure 13. Effect of salinity and treatment (urea with and without NBPT and DCD) on nitrogen uptake. Columns with the same letter are no significantly different (P=0.05).