IMPROVING THE EFFICIENCY OF 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. The objectives of this research were to study foliar urea assimilation in cotton and to test the effect of the urease inhibitor N-butyl thiophosphoric triamide (NBPT) with foliar urea application. The study consisted of a growth chamber experiment with the treatments: (1) control; (2) foliar urea; (3) foliar urea+NBPT; and (4) foliar NBPT, and a field experiment with the treatments: (A) full recommended N soil rate with no foliar N application; (B) 75% of recommended N soil rate with no foliar application; (C) 75% of recommended N soil rate with two foliar Urea applications; (D) 75% of recommended N soil rate with two foliar Urea+NBPT applications. Each foliar urea application was calculated to supply 11.2 kg of N per hectare. In the growth room study the addition of NBPT to foliar urea inhibited urease activity. In addition, NBPT 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 conclusion, 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 studied 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).

Once in the plant urea is converted to ammonia, by the enzyme urease, and ammonia is incorporated to glutamate, by the enzyme glutamine synthetase (Sirko and Brodzik, 2000). In the literature it is still not clear whether leaf burn resulted from foliar urea application is caused by

toxic accumulation of urea or ammonia. In soybean, foliar urea leaf burn is mainly associated with urea accumulation (Bremmer 1995; Krogmeier et al., 1989). However; to our knowledge in the literature there is no research done in cotton. Use of urease inhibitor with foliar urea application could be an effective method to help elucidate the fate of urea in cotton leaves. A well known urease inhibitor is N-(n-butyl) thiophosphoric triamide (NBPT) applied in the soil with urea, NBPT has been proved to have high efficiency in inhibiting urease at low concentration in a wide variety of soils (Vittori et al., 1996; Rawluk et al., 2001).

Preliminary data indicated that addition of NBPT to foliar urea application increased cotton yield, with values significantly higher than urea alone. Furthermore, seedcotton yield of NBPT + foliar urea treated plots that received only 75% of the full recommended N rate was statistically equivalent to the plots that had 100 % of the N rate. Thus, the use of urease inhibitor with foliar urea fertilization could have the potential of enhance N assimilation in plant leaves, which could help improve foliar N management in crops.

OBJECTIVES

The main objective is to study foliar urea assimilation in cotton plants and how the use of the urease inhibitor NBPT will affect the efficiency of foliar urea application. An additional objective is to understand if cotton leaves treated with urea, suffers from toxicity of urea or ammonia. With a better understanding of the physiological effects of 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 determine if use of the urease inhibitor NBPT will affect the efficiency of foliar urea application.

Growth Room Study:

Cotton (*Gossypium hirsutum* L.) cultivar ST4554B2RF was planted in 1.5-liter pots filled with soil from a representative cotton growing area in Marianna, AR (Memphis silt loam - fine-silty, mixed, active, thermic Typic Hapludalfs). The pots was arranged in a large walk-in growth chamber (Model PGW36, Conviron, Winnipeg, Canada) with day/night temperatures of 30/20^{o}C, relative humidity of 70% and 14 hour photoperiods at 500 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR). The P_2O_5 and K_2O fertilization rates were 45 and 73 kg ha^{-1} calculated using a soil volume of 1 ha and 0.15 m furrow slice. No soil N fertilization was applied in this experiment and pots were watered daily only with double deionized water. The treatments consisted of: (T1) untreated control with no foliar urea application; (T2) foliar urea application; (T3) foliar urea applications with NBPT (T4) foliar NBPT without urea. 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 fertilizer Agrotain (Agrotain Int. LLC) and the foliar NBPT rate was calculated based on reports that Agrotain contains 0.84% of NBPT. Treatments were applied at 8:00 AM, 4 weeks after planting. Spraying was carried with a CO_2 backpack sprayer regulated to deliver 93.22 l ha^{-1}. Photosynthesis and chlorophyll fluorescence were measured 2 and 24 hours after application. Leaf discs for membrane leakage were collected 2 and 24 hours after application, and immediately after, leaves were sample for subsequent biochemical measurements. Leaves were kept in a -80^{o}C freezer for protein, glutathione reductase, glutamine synthetase, urea and urease determination. The experiment was repeated twice in 2010 and a complete randomized design with 5 replications was used to conduct the experiment.

Measurements included: Leaf photosynthesis was recorded using a Licor 6200 photosynthesis portable system; Chlorophyll fluorescence was done using a Modulated Fluorometer OS1-FL; Membrane leakage was measured as a percent injury method; Malondialdehyde (MDA) extraction procedure followed the method of Goel & Sheoran (2003); Glutathione Reductase (GR) Activity was measured using the method of Gomez et al. (2004); Leaf protein content was measure using the method of Bradford (1976); Glutamine Synthetase (GS) with a modified leaf extraction method of Yajun et al. (2008); Urea measured using a modified method of hot water extraction of Lang and Kaiser (1994); and Urease measured using the method of Gerendas and Sattelmacher (1997.

Field Study:

A field study 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

Growth Room Study:

There was a significant main treatment effect was observed for membrane leakage (*P=0.*0031) and MDA (*P=0.0270*). There was a significant decrease in membrane leakage and MDA for the NBPT treatment. For example compared with the control, the NBPT treatment had a decrease of 20% (*P=0.0051*) in membrane leakage and 18% (*P=0.0070*) in MDA content. The treatment Foliar Urea+NBPT (58.59±7.41 % injury) had only a numerical decrease (*P=0.0827*) in membrane leakage (Fig. 1A) compared to the Foliar Urea treatment (61.65±6.38 % injury). Similarly, data of MDA (Fig. 1B) also indicated only a numerical (*P=0.1761*) decrease in the values of the Foliar Urea+NBPT (20.38±1.17 mmol g^{-1} FW) compared to the Foliar Urea (22.44±1.24 mmol g^{-1} FW) treatment.

Glutathione reductase data (Fig. 1C) did not have any significant interaction or treatment effect (*P=0.1191)*. The Foliar Urea+NBPT treatment had a numerical increase in GR values compared to the rest of the treatments; however due to the high variability in the measurements the data were not significantly different.

Data of urease activity (Fig. 2) had a significant (*P=0.0349*) interaction effect between the parameters treatment and time of measurement. The analysis indicated that no significant treatment effect (*P=0.7913*) was observed in the measurements made a 2 h after foliar application (Fig. 2A). However measurements collected at 24 h after foliar application (Fig. 2B) showed a significant (*P=0.0114)* treatment effect, in which the foliar urea treatment exhibited significantly higher urease activity values than the rest of the treatments. In comparison to the Foliar Urea+NBPT (0.007±0.0001 units g^{-1} FW) treatment, the Foliar urea (0.011 ±0.0001 units g^{-1} FW) treatment had a 42% increase in urease activity (*P=0.02335*) when measurement were made 24 h after foliar application. Furthermore, the Foliar Urea+NBPT treatment did not exhibit increased urease activity; its values were not significantly different than the control treatment (*P=0.4909).*

Leaf urea content (Fig. 3) measurement also indicated a significant (*P=0.0382*) interaction effect between the parameters treatment and time of measurement. In the measurement made 2h after foliar application (Fig. 3A) a significant treatment effect was observed (*P=0.0200*); however, the only statistical differences observed were when the Foliar NBPT treatment was compared with the treatments Foliar Urea (*P=0.*0129) and Foliar Urea+NBPT (*P=0.*0034). At the measurement made at 24h after foliar application (Fig. 3B), also a significant treatment effect was observed (*P<0.0001*). Compared to the Control treatment a significant increase in leaf urea content was observed in the treatments Foliar Urea (*P=0.*0013) and Foliar Urea+NBPT (*P=0.*0006). In this case, the treatments Foliar Urea (3.15±0.18 mM g^{-1}FW) and Foliar Urea+NBPT (3.57±0.44 mM g^{-1}FW) had respectively, a 48% and 68% increase in leaf urea content compared to the Control treatment (2.12±0.11 mM g^{-1}FW). Significant differences were also observed when the Foliar NBPT treatments was compared with the treatments Foliar Urea (*P=0.*0003) and with Foliar Urea+NBPT (*P=0.*0002). On the otherhand, comparative analysis of the Foliar Urea with Foliar Urea+NBPT (*P=0.*4780) and of the Control with Foliar Urea (*P=0.*5887) were not significant.

The data of GS (Table 1) and leaf protein (Table 1) content did not have any significant interaction or treatment effect. The treatment effect P values for GS and protein were respectively 0.4354 and 0.1193. Similarly the measurement of photosynthesis (Table 2 and chlorophyll fluorescence (Table 2) had no statistical effect of interaction or treatment. In this case the treatment effects P-values for photosynthesis and chlorophyll fluorescence were 0.1961 and 0.8531, respectively.

Field Study:

A significant (*P=0.*0012) interaction effect between treatment and year of the experiment was observed in the data of seedcotton yield. There was a significant (*P=0.0029*) treatment effect (Fig. 4A) 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 measurement of leaf burn (Fig. 5A) collected in the 2010 experiment, a significant treatment effect was observed (*P<0.0001*). However the comparative analysis only indicated that higher values of leaf burn occurred in the plots that received foliar urea application. No significant differences were observed between the treatments 75% N Soil–Urea Foliar and 75% N Soil–Urea+NBPT Foliar (*P=0.2639*).

Measurement of leaf N (Fig. 5B) and petiole nitrate (Fig. 5C) content indicated no significant interaction or treatment effect. The P-values for the treatment effect were respectively 0.4197 and 0.2955 for leaf N and petiole nitrate data.

Discussion

The summary of the growth chamber study was that: application of only NBPT decreased membrane leakage and MDA; addition of NBPT-to-foliar-urea decreased urease activity measured at 24 h after application; and had no effect in the measurements of GS, GR, protein, photosynthesis, and chlorophyll fluorescence. In the field study, addition of NBPT to foliar urea resulted in a yield increase. Furthermore, addition of NBPT to foliar urea application had no significant effect on leaf burn, leaf N, and petiole nitrate content.

In the literature, foliar urea application with the urease inhibitor phenylphosphorodiamde (PPD) has been reported to have a negative effect on soybean leaves (Krogmeier et al., 1989). The authors of this study hypothesized that soybean leaf–tip injury caused by foliar urea application was attributed to ammonia formation from urea hydrolysis; however they reported that the leaf necrosis was attributed to toxicity of urea rather than of ammonia. On the otherhand Rawluk et. al. (1999) did not observe any negative effect from NBPT with foliar applied urea in wheat. In our study the negative effect of adding the urease inhibitor to foliar urea was not evident. We observed that addition of NBPT to foliar urea was effective in inhibiting leaf urease activity measured at 24 h after application. The mode of action of NBPT is carried by a binding and deactivation of the urease receptor site for urea (Mobiley, 1989; Manuza et al., 1999). The efficacy of NBPT in inhibiting urease in the soil is well documented (Watson et al., 1994; Antisari et al., 1996; Rawluk et al., 2001); however to our knowledge there is no report of NBPT effect on leaf urease activity. Since the addition of NBPT to foliar urea decreased urease activity it was expected that NBPT would result in increased leaf urea content. However, urea measurement collected at 24h after treatment application showed no significant differences between the treatments Foliar Urea and Foliar Urea+NBPT. There was a numerical increase in leaf urea content with addition of NBPT, thus it is possible that a statistical difference could be detected if the measurements were done after the 24h period. The data of urease and urea in cotton indicated that the total hydrolization and assimialtion of the foliar applied urea is not completed in the period of 24 h. The data of membrane leakage and MDA had identical results, indicating that application of Foliar NBPT improved the cell membrane integrity of cotton leaves. The treatment Foliar Urea+NBPT showed statistically equal values compared to the Foliar NBTP treatment; however its values were not significantly different than the Foliar Urea

treatment. The process involved in the role of NBPT on cell membrane integrity is not clear; however since NBPT binds to Ni urease receptor sites, it is possible that NBPT has a Ni chelating effect in the plant. Ros et al. (1992) reported that Ni affected the cell plasma membrane properties and ATPase activity of rice plants. Furthermore, in the review of Seregin and Kozhevnikova (2006), there are reports of Ni causing oxidative stress in a variety of plants, thus NBPT in the plant could be resulting in a protective mechanism against Ni. In this experiment, no evidence of a negative effect of urea and/or NBPT was observed in the measurements of GR, GS, protein, photosynthesis and chlorophyll fluorescence. However it is possible that an effect of NBPT could occur in a measurement collected after the 24 h sampling, since a significant NBPT effect was observed in urease and membrane integrity data. Additional research is needed to address this hypothesis.

The yield data of the field experiment showed a significant interaction effect between treatment and year of the experiment. This indicated that the values of seedcotton yield responded differently to foliar treatment applications depending on the year of the experiment. We observed a significant seedcotton yield increment with addition of NBPT to foliar urea. Addition of NBPT increased yield compared to application of foliar urea alone and it resulted in equivalent seedcotton yield to the 100% N Soil application treatment. However data of leaf burn, leaf N, and petiole nitrate content did not show any significant effect of addition of NBPT to foliar urea application. The significant influence on NBPT on cotton yield could result from the NBPT effect on the inhibition of urease and improvements of cell membrane integrity indicated in the growth chamber study.

In conclusion in the growth chamber study the use of NBPT to foliar urea application decreased urease activity and it showed trends for increasing leaf urea content and improving cell membrane integrity. In the field study seedcotton yield improvements were observed with addition of NBPT to foliar urea.

Table 1: Effect of foliar treatments on glutamine synthetase and leaf protein content (Growth Room Study).

Foliar Treatment	Glutamine Synthetase (mM glutamyl hydroxamate $g^{-1}FW\ hr^{-1}$)	Leaf Protein mg g^{-1} FW
Control	0.070 ± 0.005	11.48 ± 0.21
Urea	0.064 ± 0.003	11.81 ± 0.18
Urea+NBPT	0.066 ± 0.004	11.37 ± 0.19
NBPT	0.063 ± 0.002	11.33 ± 0.21
P-Value	0.4354	0.1193

Table 2: Effect of foliar treatments on leaf photosynthesis and chlorophyll fluorescence (Growth Room Study).

Foliar Treatment	Leaf Photosynthesis µmol m^{-2} s^{-1}	Chlorophyll Fluorescence Yield (Fv/Fm)
Control	12.46 ± 0.60	708.06 ± 14.98
Urea	13.00 ± 0.47	703.98 ± 9.17
Urea+NBPT	13.36 ± 0.50	698.98 ± 6.64
NBPT	13.58 ± 0.34	702.65 ± 7.00
P-Value	0.1961	0.8531

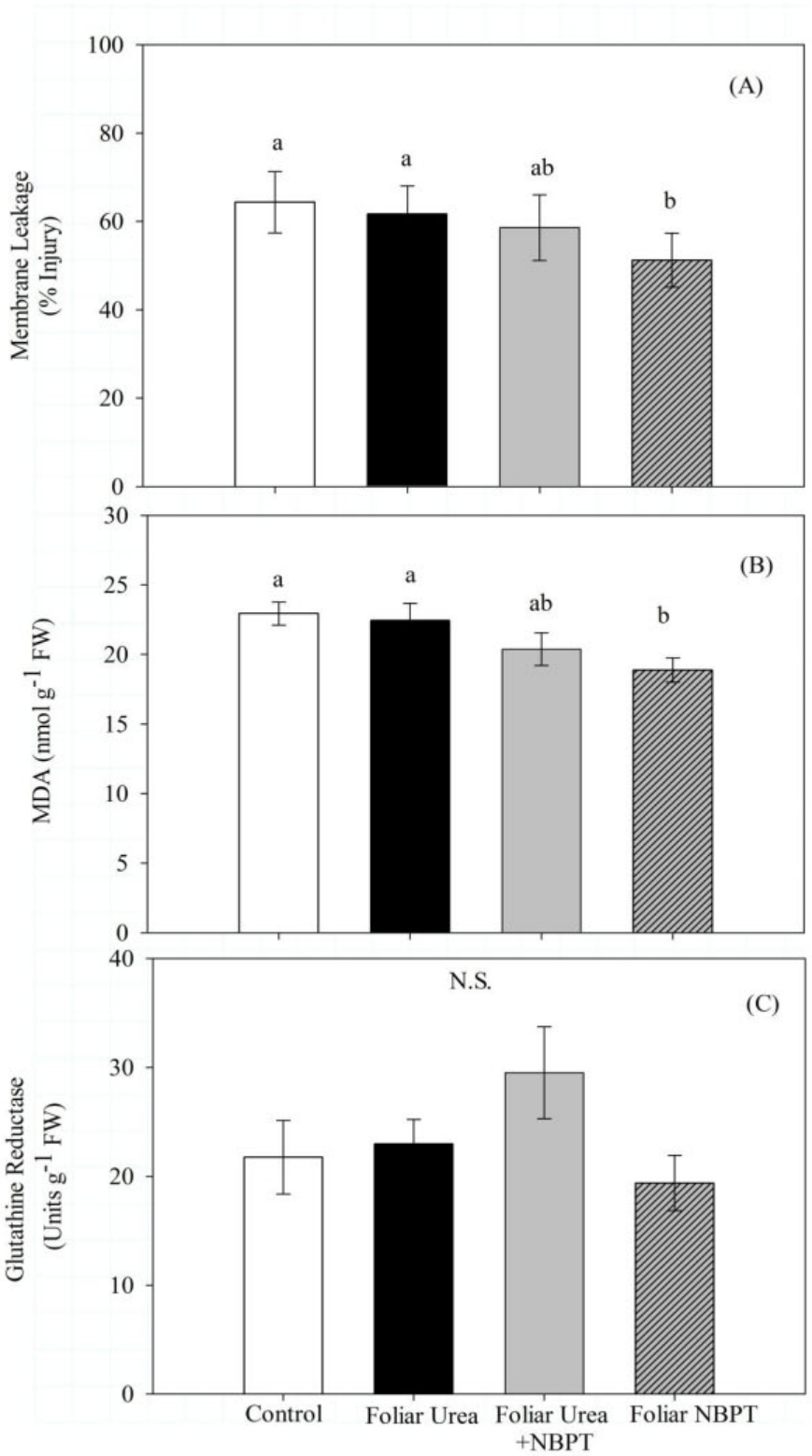

Figure 1: Effect of foliar treatments on membrane leakage (A), MDA (B), and glutathione reductase (C) in cotton grown in growth room conditions. N.S. = not significant ($P \leq 0.05$).

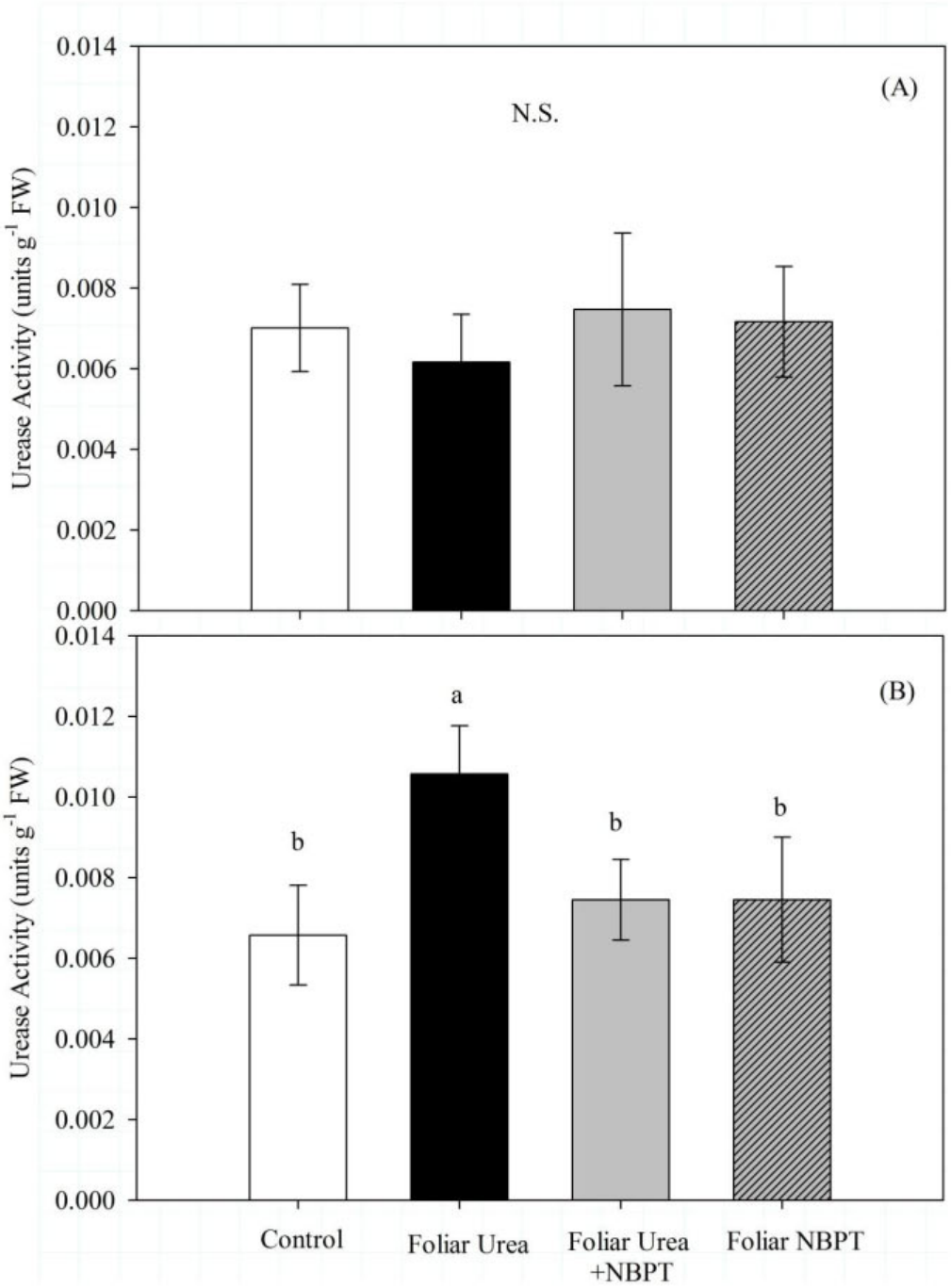

Figure 2: Effect of foliar treatments on leaf urease activity measured at 2h (A) and 24 h (B) after application in cotton grown in growth room conditions. N.S. = not significant ($P \leq 0.05$).

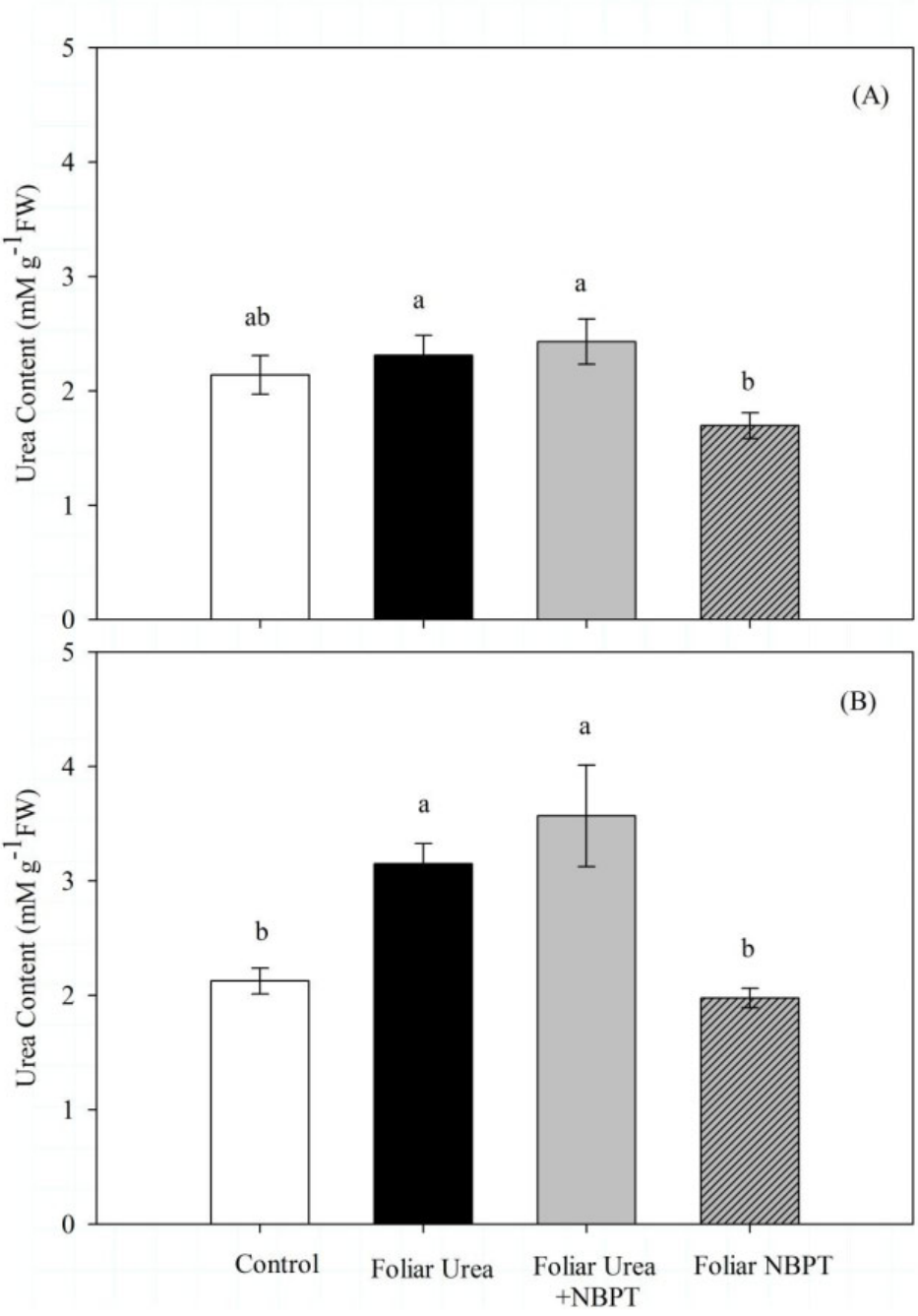

Figure 3: Effect of foliar treatments on leaf urea content measured at 2h (A) and 24 h (B) after application in cotton grown in growth room conditions.

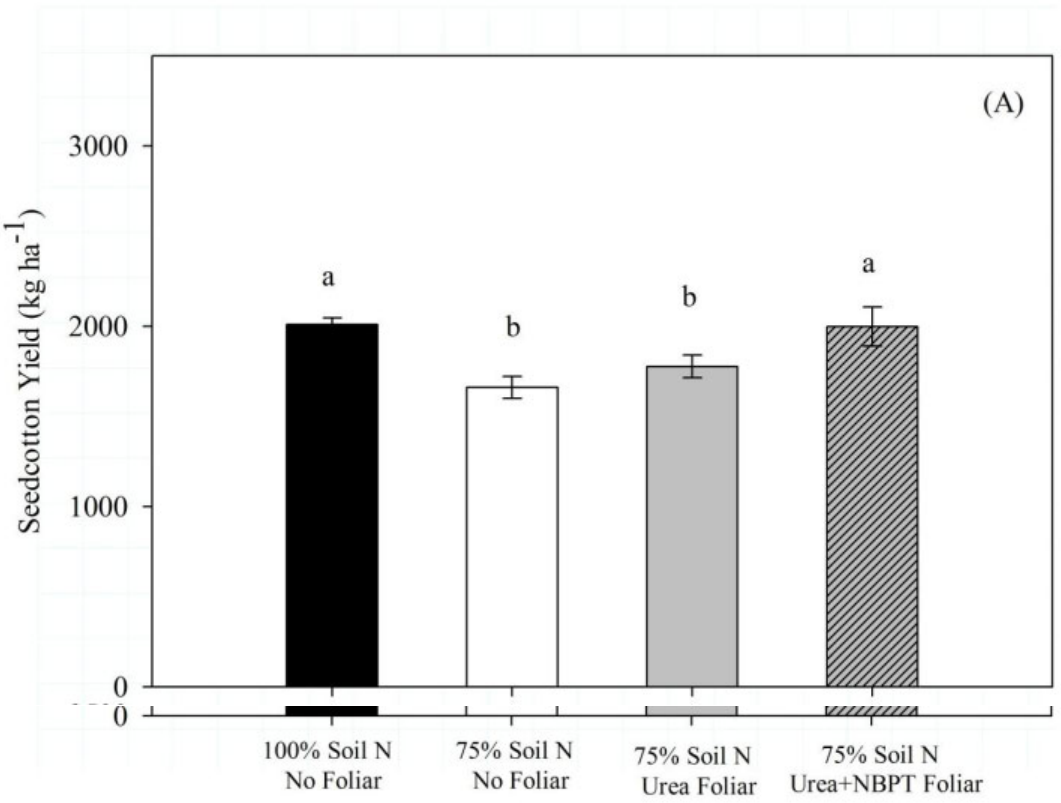

Figure 4: Effect of foliar treatments on seedcotton yield of field grown . N.S. = not significant ($P \leq 0.05$).

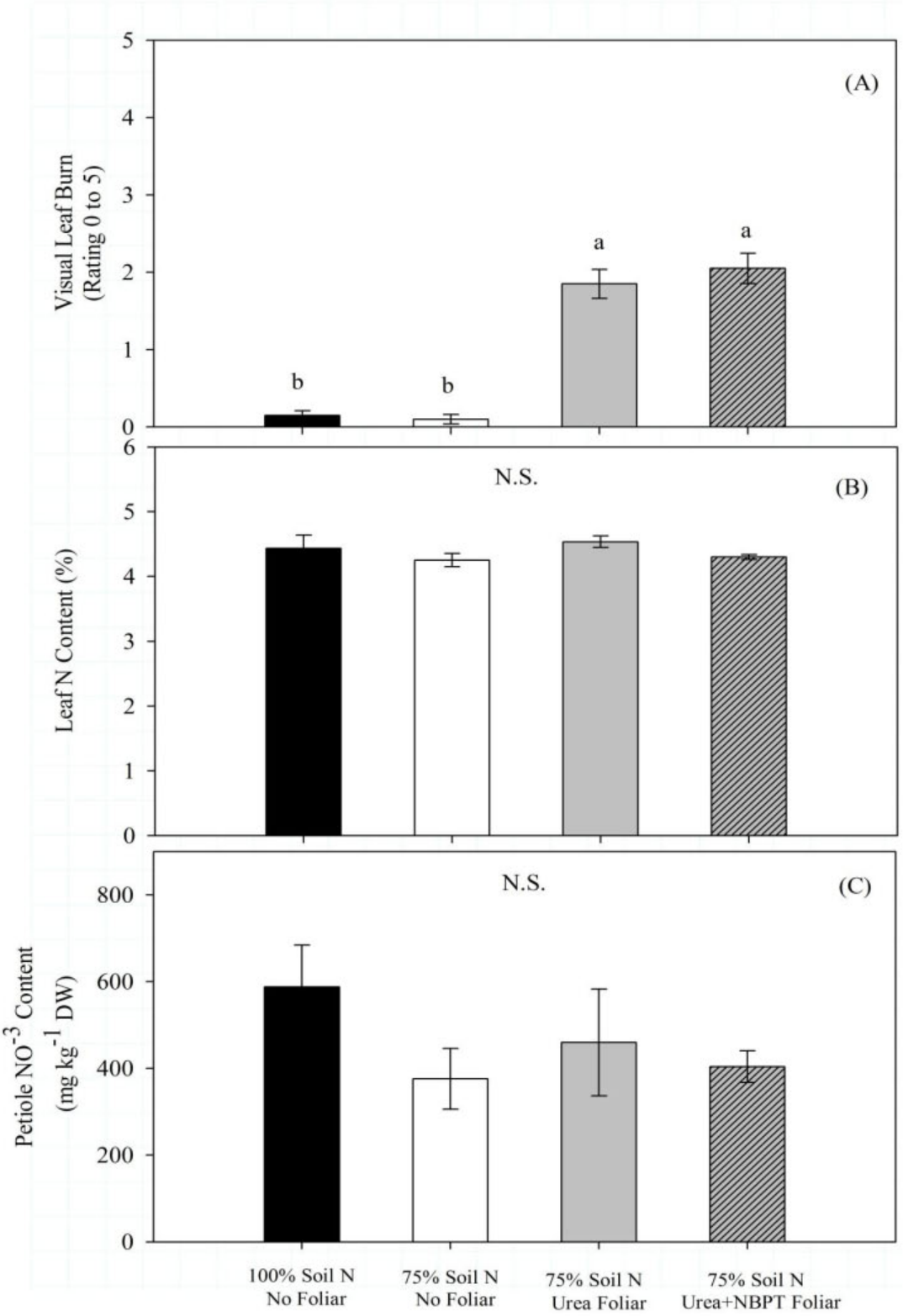

Figure 5: Effect of foliar treatments on leaf burn (A), leaf N (B), and petiole nitrate (C) of a field grown cotton (2010). N.S. = not significant ($P \leq 0.05$).

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