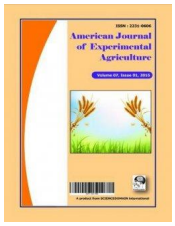

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Effects of High Night Temperatures on Cotton Leaf Gas Exchange and ATP Levels at Flowering

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Authors' contributions

This work was carried out in collaboration between both authors. Author DAL designed and executed the study, performed the statistical analysis and wrote the manuscript. Author DMO managed the corrections to the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To monitor the effects of high night temperatures on leaf photosynthesis and respiration, stomatal conductance and adenosine triphosphate (ATP) levels of cotton during its reproductive stage.
Study Design: A two-factor factorial, the two factors being temperature and time (weeks), with 40 replications in each of the temperature treatment.
Place and Duration of Study: Altheimer Laboratory, Department of Crop, Soil and Environmental Sciences, University of Arkansas, between September 2013 and June 2014.
Methodology: Growth chamber experiments were conducted using cotton (*Gossypium hisrsutum* L.) cultivar ST5288B2F with the treatments consisting of normal day/night temperatures (32/24ºC) and high night temperatures (32/30ºC) for two weeks at flowering. Measurements of leaf photosynthesis, respiration, stomatal conductance and ATP levels were conducted in the end of the first and the second week after imposition of stress.
Results: Leaf photosynthetic rates and stomatal conductance rates remained unaltered under higher night temperatures during both weeks of the experiment. In contrast, a significant increase in leaf respiration rates was observed at the end of the second week of the experiment with plants grown under conditions of high night temperatures increasing their respiration rates by 30%

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compared to those grown under normal temperatures. Conversely, leaf ATP levels were significantly decreased under conditions of elevated night temperatures.
Conclusion: It was concluded that higher than optimum temperatures during flowering had no significant effect on cotton leaf photosynthesis and stomatal conductance in contrast to leaf respiration and ATP levels that were significantly decreased.

Keywords: Cotton; photosynthesis; respiration; stomatal conductance; night temperatures; ATP levels.

1. INTRODUCTION

Heat stress constitutes a major threat to crop production worldwide and significant increases in global temperatures are anticipated by the end of the century [1]. Extensive research has been dedicated on the negative effects increased temperatures have on plant growth and development but interestingly enough, night temperatures are expected to increase faster and to a greater extent than day temperatures as a result of increased cloudiness and consequently decreases in heat radiant loss [2]. Cotton (*Gossypium hirsutum* L.) may be a perennial that originates from hot and arid areas but with its physiological optimum at 28ºC [3] heat stress becomes a constraint more often than not during the growing season. Considerable research has been conducted on the effects of higher than optimum temperatures during daytime on cotton recording significant inhibition of photosynthetic rates [4-6] and increases in respiration [7] that are accompanied by reductions in pollination and fertilization [8] leading ultimately to compromised reproductive efficiency and decreased yields [9]. Significantly less attention has been given on the subject of elevated night temperatures and their effects on cotton physiology and growth. Previous research has reported that night temperatures between 28 and 42.5ºC caused an in increase in the Q_{10} of respiration rates by 1.86 [10] while Reddy et al. [11] indicated that high night temperatures inhibit photosynthesis. However, in their experiments, day and night temperatures were raised proportionally to each other and therefore it was not possible to determine the effect of night temperature alone. Loka and Oosterhuis [12] on experiments with higher than optimum night temperatures during cotton's vegetative stage of growth observed significant increases in respiration rates. Consequently depletion in leaf carbohydrates content and significant reductions in leaf energy levels were reported [12] ultimately resulting in yield reduction [13].

The reproductive stage appears to be more heat susceptible to heat stress than the vegetative stage [14] and research in other crops has indicated that high night temperatures occurring during the reproductive stage result in significant yield reductions due to increases in male sterility and floral abscission [15,16], delays in flowering [17] and decreases in pollen viability and grain filling [18]. Variable responses have been reported on photosynthesis [19-22] while respiration have been shown to increase under conditions of high night temperature stress [18, 23,24].

As mentioned above, little or no attention has been given to the effects of increasing night temperatures during the reproductive stage on leaf gas exchange and energy levels. Since night temperatures are projected to increase faster and to a longer extent compared to day temperatures it is important that research should focus on their effects. It was hypothesized that high night temperatures will have a negative effect on cotton photosynthesis, respiration, and stomatal conductance that would result in significant reductions of energy levels. The objective of these studies was to evaluate and quantify the effect of high night temperatures on cotton photosynthesis, respiration, stomatal conductance and subsequent ATP content.

2. MATERIALS AND METHODS

Growth chamber studies were conducted and repeated at the Altheimer Laboratory, University of Arkansas. Cotton (*Gossypium hirsutum* L.) cultivar ST5288B2F was planted in 2L pots containing horticulture mix (SunGro Distribution Inc., Bellevue, WA). Pots were arranged in a growth chamber (Conviron PGW36) that was equipped with incandescent and fluorescent lamps and set for a 14 h photoperiod with a photosynthetic flux density (PPFD) of 800-850 µmol/m^2s, relative humidity of 60% and CO_2 concentration was 340-370 ppm. Half-strength Hoagland's nutrient solution was applied daily to all pots in order to maintain adequate nutrients and water. Plants were grown until first flower stage (approximately eight weeks after planting) under normal day/night temperatures of 32/24ºC (maximum during the day, minimum during the night, respectively) simulating a normal diurnal

variation. At the first flower stage, pots were divided in two groups and one group was transferred into a second growth chamber, with similar conditions of photon flux density, humidity and photoperiod as the first chamber, but with night temperatures raised to 30ºC for 6 h at the start of the dark period (18:00-24:00) and a gradual decrease to 24ºC, for an overall duration of two weeks, while the control plants remained under normal conditions (32/24ºC). Measurements of photosynthesis, respiration, stomatal conductance and ATP levels were conducted at the end of the first and second week from a total of 40 plants (10 replications for each week-group). All measurements were taken with the plants inside the growth chambers.

2.1 Photosynthesis and Respiration Measurements

A Li-Cor Model 6200 portable photosynthesis system (LiCor Inc., Lincoln, NE) was used to determine photosynthetic and respiratory rates from the fourth uppermost main-stem fully expanded leaf (n=10). Photosynthesis measurements were taken at 13:00 at the end of the first and second week. Respiratory rates were taken at 22:00 at the end of the first and second week. The results were expressed as $\mu mol/m^2s$.

2.2 Stomatal Conductance Measurements

Stomatal conductance measurements were taken at the end of each week from the fourth uppermost main-stem fully expanded leaf from 11:00 until 12:00 using a Decagon SC-1 Porometer (Decagon Inc. Pullman, WA). Three measurements of various areas of the leaf were taken and then averaged and the results were expressed as mmol /m²s.

2.3 ATP Content Measurements

ATP content was measured according to Loka and Oosterhuis [12]. Six leaf disks per subtending leaf were excised using a cork borer (d=1cm) at the end of the dark period. The leaf disks were placed immediately in tubes containing 10 ml TRIS solution and the tubes were put in a boiling waterbath for 10 min, for the extraction of ATP. The resulting aliquot was then stored in 1.5 ml microcentrifuge tubes at -80ºC. The substrate-enzyme complex of luciferin-luciferase (ATP bioluminescent assay kit, Sigma Chemical Company, St. Louis, MO) that converts the chemical energy associated with ATP into light was used and the light produced (proportional to the ATP content of the sample) was determined with a 20/20n Luminometer (Turner Biosystems Inc. Sunnyvale, CA) and the help of a standard curve. The concentrations of the standards were 0, 0.00001, 0.0001, 0.001, 0.01, 0.04 µg ATP/ml.

2.4 Statistical Analysis

The experimental design was a two-factor factorial, the two factors being temperature and time (weeks), with 40 replications in each of the temperature treatment. The trends of the two growth chamber studies were similar and the data were pooled. No significant interaction was observed between the two factors across all measurements and the effects of temperature were analyzed for each week using a Student's *t*-test. Means were considered significantly different at $P \leq 0.05$.

3. RESULTS

3.1 Photosynthesis and Respiration

Photosynthetic rates were not significantly affected by high night temperatures (Fig. 1). No significant interaction was observed between the main factors (time and temperature, $P = 0.89$ for photosynthesis and $P = 0.45$ for respiration) and the effect of temperature was analyzed using Student's *t*-test for each week. Plants grown under high night temperatures increased their photosynthetic rates by 5% and 6.5% the first and the second week respectively, after initiation of treatment compared to the control. In contrast to photosynthesis, respiration rates significantly increased at the end of the second week of high night temperatures with heat-stressed plants having a 30% higher respiration rates compared to the control (Fig. 2).

3.2 Stomatal Conductance

Stomatal conductance measurements indicated that high night temperatures had no significant effect on the function of stomates. Again, no significant interaction was observed between the main factors (time and temperature, $P = 0.49$) and the effect of temperature was analyzed using Student's *t*-test for each week. A non-statistically significant increase of 6% and 2.5% was recorded the first and the second week respectively (Fig. 3), compared to the control.

3.3 ATP Levels

Contrary to both photosynthesis and stomatal conductance functions, leaf ATP levels were considerably affected by high night temperatures (Fig. 4). No significant interaction was observed between the main factors (time and temperature, P = 0.35) and the effect of temperature was analyzed using Student's t-test for each week. Heat-stressed plants had 14% and 18% lower ATP levels at the end of the first and the second week respectively, after initiation of stress compared to control.

4. DISCUSSION

Temperature plays a major role regulating plant growth and development and ultimately yield since it determines the function of several physiological and metabolic processes. Photosynthesis, a function closely related with crop productivity is considered to be directly affected by temperature, being one of the most heat-sensitive processes in plants. Leaf photosynthetic rates are known to decrease under conditions of high day temperature stress

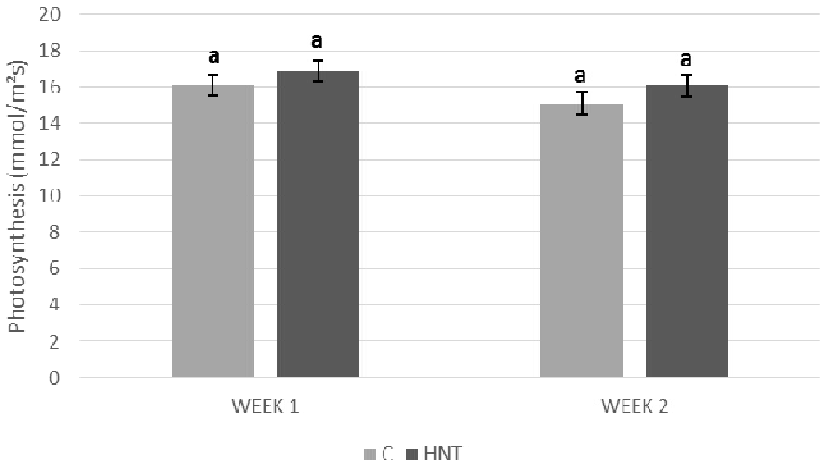

Fig. 1. Effect of high night temperatures on leaf photosynthesis rates one and two weeks after the night temperatures were raised

Pairs of columns of columns within each time interval with the same letter are not significantly different (P ≤ 0.05). C = 24ºC night temperature, HNT = 30ºC night temperature. Error bars indicate ±1 standard error

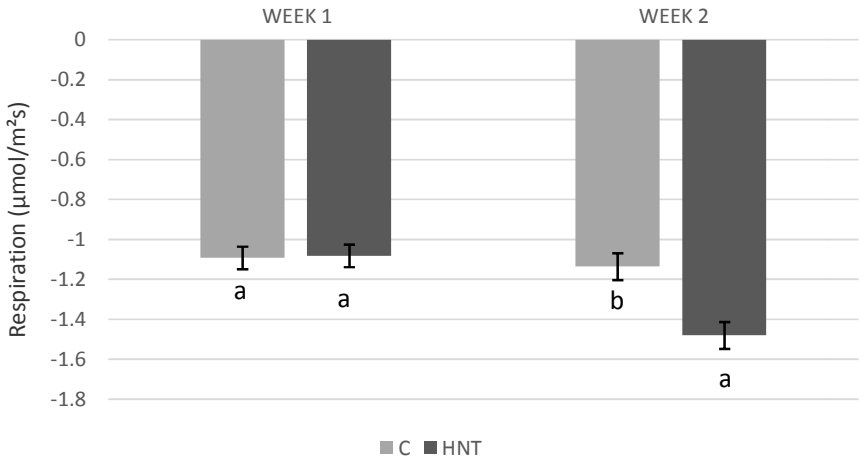

Fig. 2. Effect of high night temperatures on leaf respiration rates one and two weeks after the night temperatures were raised

Pairs of columns of columns within each time interval with the same letter are not significantly different (P ≤ 0.05). C = 24ºC night temperature, HNT = 30ºC night temperature. Error bars indicate ±1 standard error

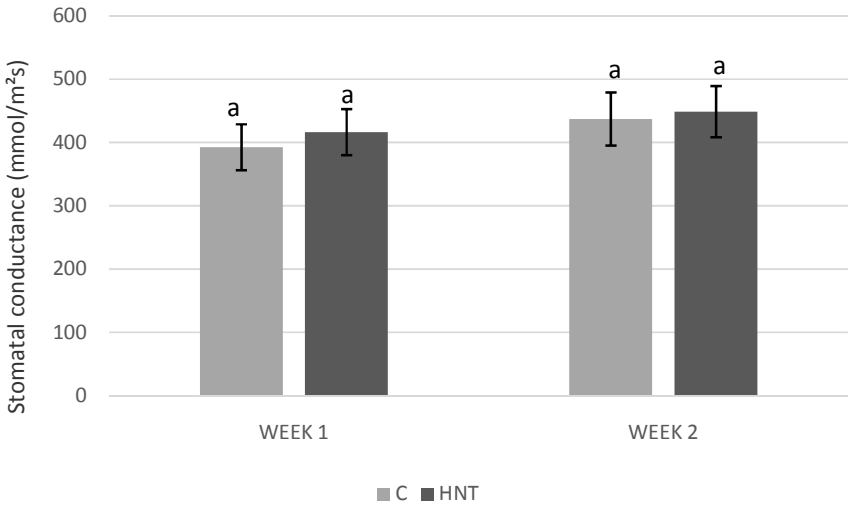

Fig. 3. Effect of high night temperatures on leaf stomatal conductance rates one and two weeks after the night temperatures were raised

Pairs of columns of columns within each time interval with the same letter are not significantly different (P ≤ 0.05). C = 24ºC night temperature, HNT = 30ºC night temperature. Error bars indicate ±1 standard error

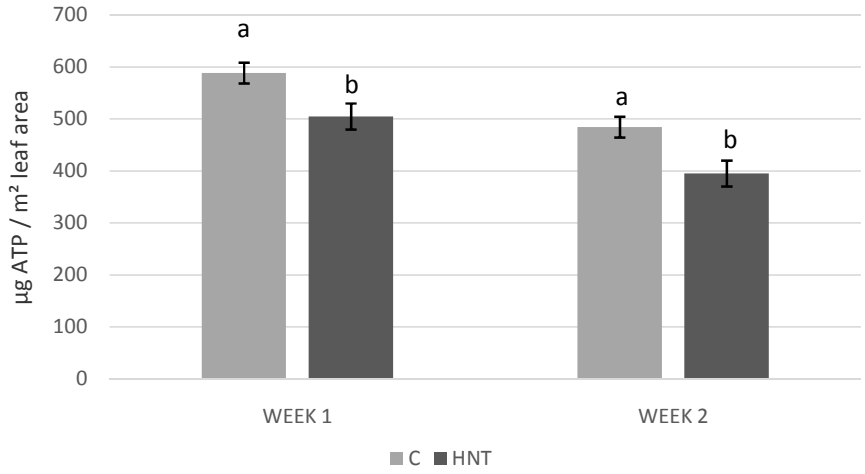

Fig. 4. Effect of high night temperatures on leaf ATP content one and two weeks after the night temperatures were raised

Pairs of columns of columns within each time interval with the same letter are not significantly different (P ≤ 0.05). C = 24ºC night temperature, HNT = 30ºC night temperature. Error bars indicate ±1 standard error

[4,6,25]; however, controversial reports exist on the photosynthetic response under high night temperature stress. Kanno et al. [22] and Turnbull et al. [19] with experiments on rice (*Oryza* sativa) and cottonwood (*Populus deltoids*) respectively, indicated that leaf photosynthetic rates were increased under higher than optimum night temperatures, while the opposite was reported by Prasad et al. [26] and Djanaguiraman et al. [24] on wheat (*Triticum aestivum*) and soybean (*Glycine max*) respectively. In our study leaf photosynthetic rates remained unaffected from the high night temperature regime during the first and the second week of our experiment which is in accordance with Mohammed and Tarpley [18] and Prasad et al. [26], where no change in the leaf photosynthetic rates of rice and sorghum (*Sorghum bicolor*) respectively, was observed. In studies with cotton, Reddy et al. [11] reported that photosynthesis decreased after heat stress;

however, in their experiment day and night temperatures were increased.

Carbon dioxide supply for photosynthesis is dependent on stomatal function and in our study higher than optimum night temperatures had no significant effect on leaf stomatal function. Contrary to our results, Prasad et al. [26] and Djanaguiraman et al. [24] reported significant decreases in leaf stomatal conductance rates under conditions of high night temperature stress which were accordingly to their photosynthetic rates. They suggested that this could be due to indirect effects of high night temperatures on leaf senescence.

Respiration, is another key metabolic process controlling crop growth. Dark respiration (in contrast to photorespiration and photosynthesis) occurs during day and night with the energy being produced divided between growth and maintenance components [27]. Similarly to photosynthesis, respiration is greatly controlled by temperature with Salvucci and Crafts-Brandner [10] reporting high night temperatures between 28 and 42.5ºC causing an increase in the Q_{10} of respiration rates by 1.86 in cotton plants. As our results have shown, high night temperatures of 30C increased leaf respiration rates during the second week of our experiments but not the first. Loka and Oosterhuis [12] also reported significant increases in respiration rates under high night temperature conditions during cotton's vegetative stage indicating that reproductive and vegetative stage are equally sensitive to high night temperatures. Research in other crops such as sorghum [28], soybean [24,29] and rice [18] also noticed that higher than optimum night temperatures resulted in increasing rates of respiration.

The significantly lower leaf ATP levels observed in plants grown at higher than optimal night temperatures compared to the levels of those grown at normal temperatures are attributed, we assume, to the increased respiration rates that resulted in significant decreases in leaf ATP levels. Similar to our results, Djanaguiraman et al. [24] reported significant decreases in leaf ATP levels of soybean plants grown under high night temperatures conditions and they speculated that this was due to increases in thylakoid membrane's permeability which resulted in leakage of protons. In support of our assumption, Loka and Oosterhuis [12] reported substantially lower levels of leaf ATP in cotton under increased night temperatures during cotton's vegetative stage. Furthermore, Lawrence and Holaday [30] observed lower ATP values in expanding cotton leaves for plants grown and 30/28C compared to those for plants grown at 30/19ºC leading us to assume that leaf ATP levels are susceptible to increased night temperatures at all growth stages of cotton.

5. CONCLUSION

In conclusion, contrary to our expectations and hypothesis, high night temperatures had no significant effect on leaf photosynthesis and stomatal conductance with plants grown at higher temperatures having almost similar photosynthetic and stomatal conductance rates with those grown under normal conditions. However, leaf respiration rates were significantly increased under high night temperatures which consequently resulted in considerable decreases in leaf ATP pools leading us to conclude that both functions, respiration and ATP production during cotton's reproductive stage are as sensitive, to higher than optimum night temperatures, as during its vegetative stage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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