THE BIOCHEMICAL EFFECTS OF HEAT STRESS ON FLOWERING COTTON

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Abstract

Cotton yields in the Mississippi Delta are below optimum and suffer from significant year-to-year variability. Research has identified the correlation of high temperature stress and reduced cotton yields, however little work identifies potential acclimation effects cotton may have to stressful environmental factors such as temperature. A field study was conducted in 2011 in Fayetteville, AR to determine possible adaptive effects of cotton. Growth chamber and field experiments were undertaken in an effort to determine adaptive effects cotton may possess to tolerate high temperature stress. Field measurements from the white flower and the subtending leaf were taken during first flower when temperatures were below 32°C, above 38°C, and again below 32°C. This was followed up with a growth chamber experiment at flowering that indicated potential adaptive effects for membrane leakages comparing a week of heat stress to another cycle of stress one week following. Protein concentrations for the leaf and ovary indicated significance when comparing week 1 to week 3 for the ovaries but not for leaf tissues. The antioxidant glutathione reductase showed significance between weeks 1 and 3 for the ovaries but not for the leaf tissues. The antioxidant peroxidase also displayed significance for the ovaries but not for leaf tissues. According to our results, there appears to be an adaptive response to cotton during heat stress that can be identified across multiple different areas of the cotton plant.

Introduction

Invariably, a cotton crop in Arkansas shows great yield potential mid-way through the season, but often fails to develop their maximum yield. This variability has been associated with genetics, environmental stress and management practices. It is assumed that breeders have an overall good genetic pool to draw from in commercial varieties, as well as good management practices based upon many years of research. Hence, the wide fluctuations of the environment and in particular the mid-season temperature stresses affect reproductive success and ultimately yield. Research indicates that the timing of the stress is among the most critical for yield returns (Snider et al., 2009), with lower returns as temperatures climb beyond 32°C (Burke et al., 1988). However, high temperature stress can exhibit itself if air temperatures exceed 30°C (McMichael and Burke, 1994). Attempts to mitigate any stress by improved managing practices using adjuvants or better watering practices have significantly improved yields, but attempts to maintain a consistent yield response has been elusive. This study is unique in that it investigates the biochemical (energy production, oxidative stress, etc.) and physiological (membrane leakage and protein production) basis for adaptive responses to high temperature over time. Although much research has been done to investigate response of different stresses such as drought stress (Oosterhuis and Wullschleger 1987; Massacci et al., 2008), salt stress (Thapa et al., 2011), and low temperature stress (Guy, 1990) little work has been done to investigate potential adaptation responses of high temperature stress in cotton after exposing it to cycles of heat stress.

Materials and Methods

Cotton (*Gossypium hirsutum* L.) cultivar ST5288 B2RF was grown in a large walk-in growth chamber (Altheimer Laboratory, Arkansas Agricultural Research and Extension Center in Fayetteville, AR) 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. 80 plants were grown in nutrient deficient potting mix in 2 L pots and watered daily (as needed) using ½ strength Hoagland's solution.

Plants were completely randomized within 2 chambers, 40 plants per chamber, set to identical temperatures, humidity, light intensity, and photoperiods once squaring was established. Once flowering began in the chambers, temperatures were increased in 1 chamber (the stress chamber) to 40/24^{o}C for approximately 7 days. Measurements were taken of membrane leakage (ML) daily at approximately 1200-1400 hrs. White flowers and their subtending leaves were taken daily from each chamber for antioxidant, protein, and carbohydrate analysis and immediately stored at -80^{o}C. After seven days, temperatures were reduced in the high temperature chamber back to pre-stress

levels for 7 days. No measurements were taken at this time. Following this, the temperatures were then increased in the stress chamber for another 7 days and measurements were taken again during this time using the same methodology of the first week of collection. White flower and its subtending leaves were subsequently analyzed for protein, glutathione reductase (GR), and peroxidase (POX) analysis. Ten samples from each collection were oven dried and submitted for analysis of carbohydrates.

For field tests, cotton cultivar ST5288B2RF was grown at the University of Arkansas Agricultural Research and Extension Center in Fayetteville. Plots of four rows of 50 feet with 2 row borders and 4 replications were planted. 3 planting dates were established 2 weeks apart to optimize the chance of high temperature stress to occur during flowering. Weed and insect control was in accordance to state recommendations. Watering was managed on an as needed basis to establish good stands. Mepiquat chloride was used as needed to control vegetative growth.

Measurements were taken of ML and fluorescence daily at approximately 1200-1400 hrs. ML and fluorescence measurements, the white flowers, and their subtending leaves were taken when temperatures were below 32^{o}C. This was repeated again when temperatures rose above 38^{o}C. And again the same measurements and collections were made following heat stress when temperatures had cooled to below 32^{o}C. The white flower and its subtending leaves were subsequently analyzed for protein, GR, and POX analysis. Samples from each collection were oven dried and submitted for analysis of carbohydrates.

Results

Field 2011

Cotton leaves were observed to contain greater ML during week 2 with weeks 1 and 3 showing similar levels of leakage (Figure 1). Protein concentrations in the leaf were not significantly different during the treatment indicating that heat stress does not have a significant impact on it; however protein concentrations rose significantly during heat stress and were substantially higher in week 3 (Figure 2). POX concentrations of the leaves decreased in the leaf during week 2, but increased to levels significantly higher in week 3 than in the week 1. POX levels in the ovaries were not significantly different in weeks 1 and 2, but were significantly greater in week 3 (Figure 3). GR concentrations rose significantly in the leaves during weeks 1, 2, and 3. However, GR levels in the ovary were not significantly different during the course of the experiment (Figure 4).

Growth Chamber 2012

Cotton leaves exhibited a diminished increase in membrane permeability in week 3 compared to week 1. In both weeks, the membrane stabilized within 3 days (Figure 5). Leaf protein displayed significant differences between weeks 1 and 3, however, the increased protein response in week 3 is not adequately explained (Figure 6). Ovary protein had significant concentration differences when compared to the control if week 1 and weeks 3. However, there appears to be a rapid increase in protein concentration following day four in week 3 that is not adequately explained (Figure 7). GR concentrations in the leaf were not significantly different from each other, but had an increase on the second day of heat stress, rather than the first (Figure 8). GR concentrations in the ovary were significantly different between weeks 1 and 3 with day 2 of week 3 having a steep drop when compared to week 1 (Figure 9). Leaf POX concentrations did not show any significance between weeks 1 and 3, however peroxidase levels did trend upwards from week 1 (Figure 10). Ovary POX levels were significantly different between weeks 1 and 3 (Figure 11).

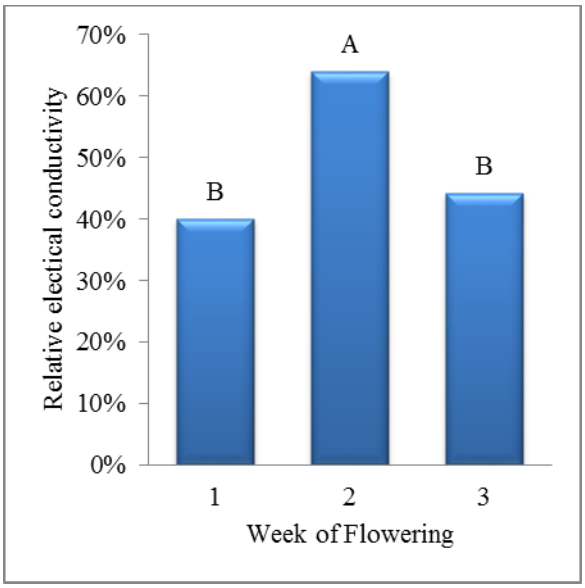

Figure 1. High temperature stress field comparisons between weeks 1, 2, and 3 for leaf ML. High significance was found between the weeks (P=0.05).

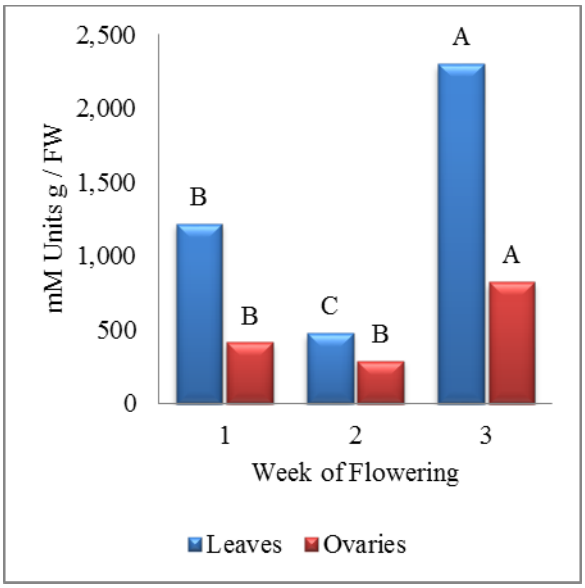

Figure 3. High temperature stress field comparisons between weeks 1, 2, and 3 for POX concentrations for both leaf and ovary tissues (P=0.05).

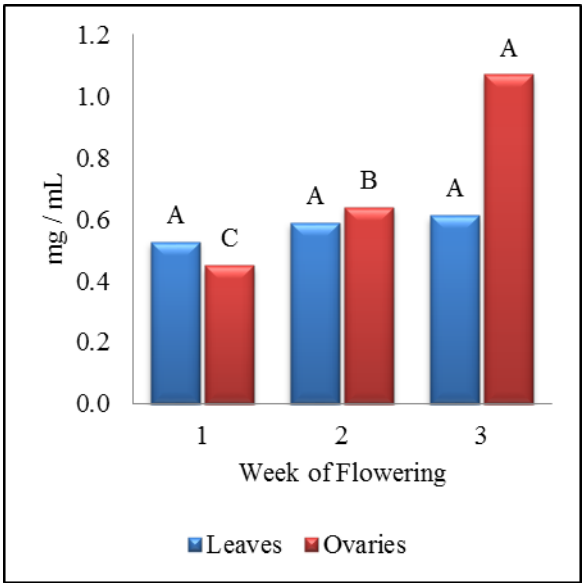

Figure 2. High temperature stress field comparisons between weeks 1, 2, and 3 for protein concentrations for both leaf and ovary tissues (P=0.05).

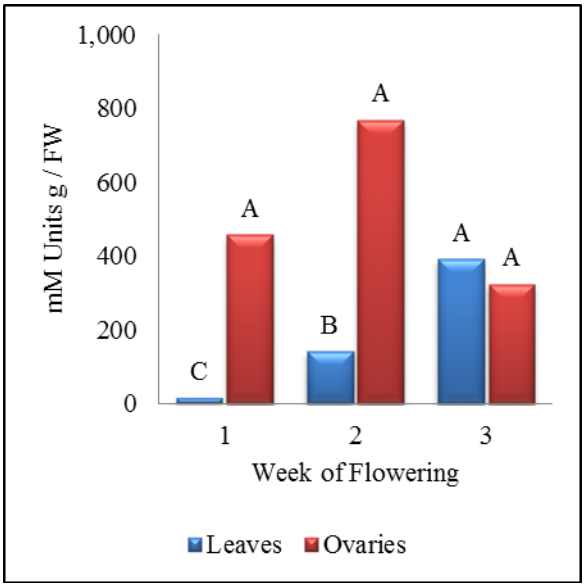

Figure 4. High temperature stress field comparisons between weeks 1, 2, and 3 for GR concentrations for both leaf and ovary tissues (P=0.05).

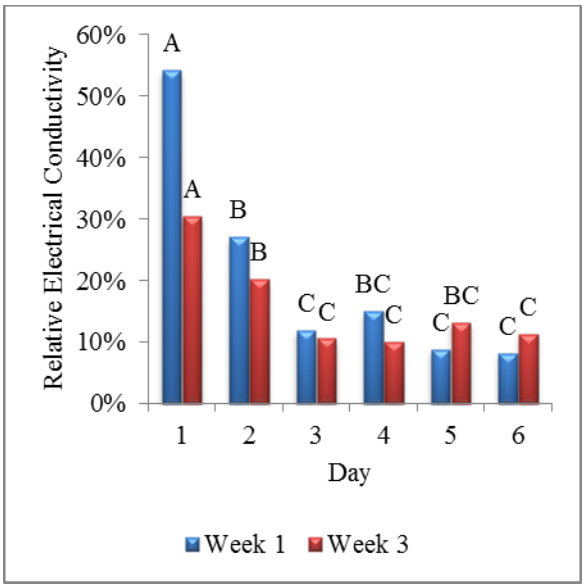

Figure 5. Daily growth chamber high temperature stress comparisons for leaf ML. Significant differences between weeks 1 and 3 were found (P=0.05).

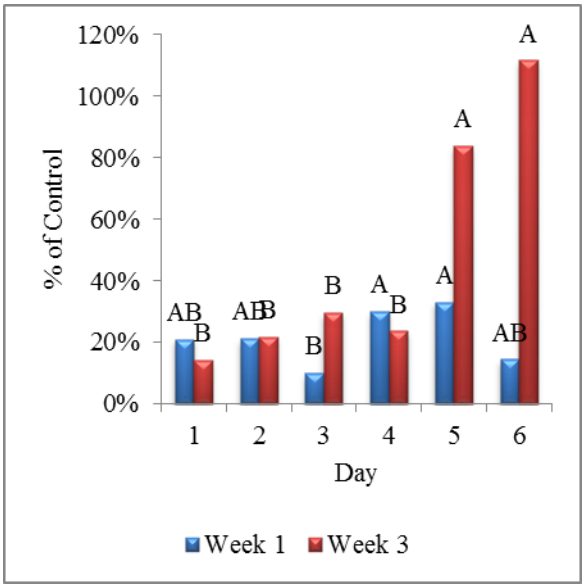

Figure 7. Daily growth chamber high temperature stress comparisons for ovary protein concentrations. Significant differences were indicated between weeks 1 and 3 (P=0.05).

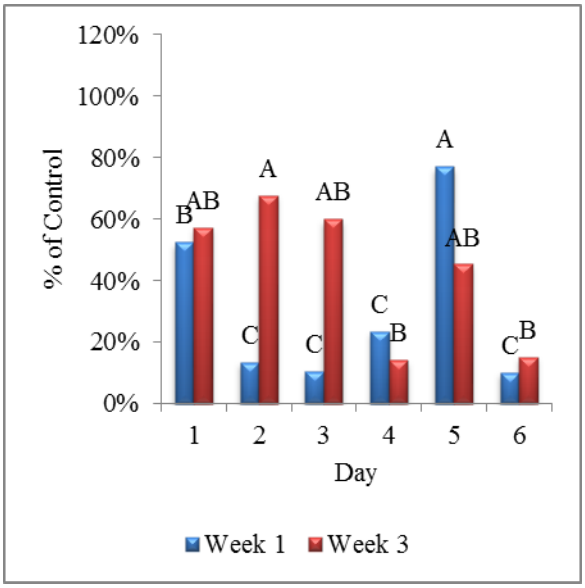

Figure 6. Daily growth chamber high temperature stress comparisons for leaf protein concentrations. Significant differences were indicated between weeks 1 and 3 (P=0.05).

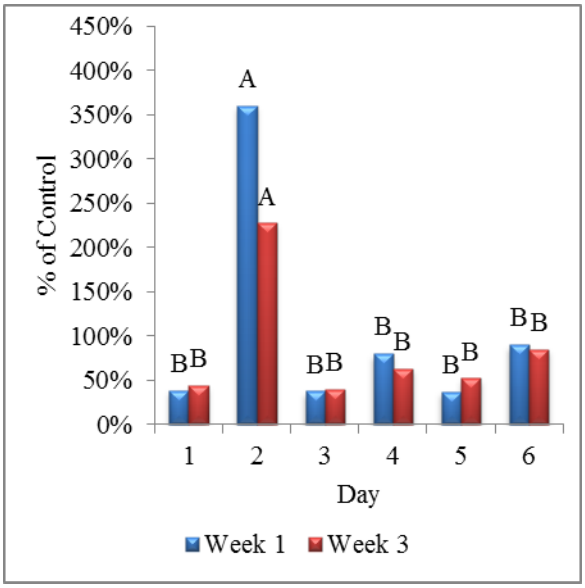

Figure 8. Daily growth chamber high temperature stress comparisons for leaf GR. No significant differences were indicated between weeks 1 and 3 (P=0.05).

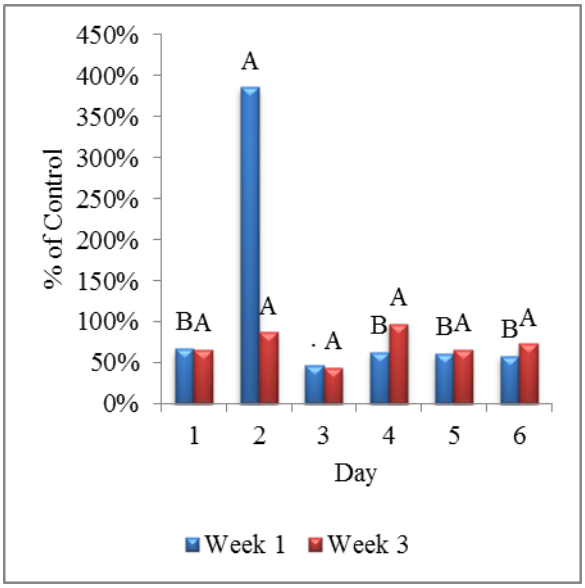

Figure 9. Daily growth chamber high temperature stress comparisons for ovary GR. Significant differences were indicated between weeks 1 and 3 (P=0.05).

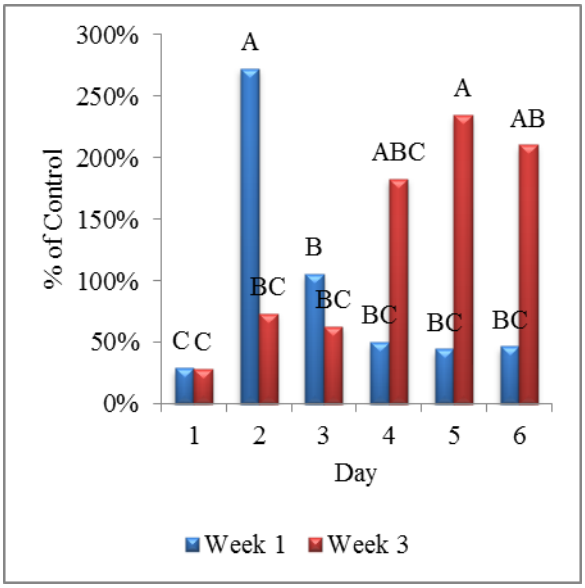

Figure 11. Daily growth chamber comparison of POX in the ovary when compared to a control. Significant differences were found between weeks 1 and 3 (P=0.05).

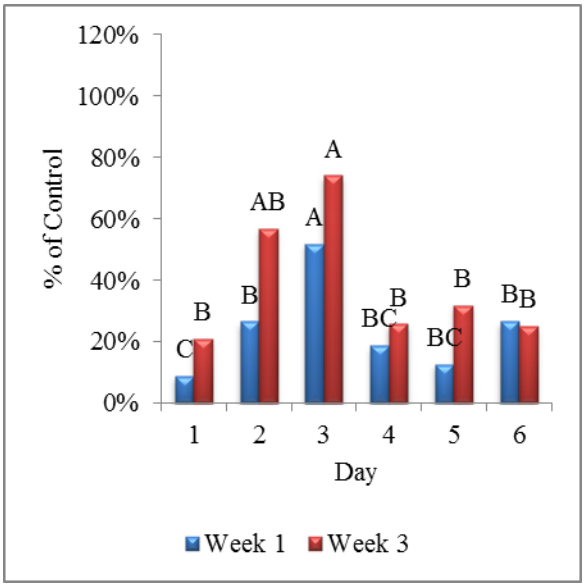

Figure 10. Daily growth chamber comparison of POX in the leaf when compared to a control. No significant differences were found between weeks 1 and 3 (P=0.05).

Summary

High temperature stress is a significant restriction to optimal yield potential for cotton grown in the Mississippi Delta region; however, the large year-to-year variability poses a significant challenge to growers seeking greater stability in their crop. Our experiment indicated that cotton does possess adaptive mechanisms that allow it to tolerate bouts of stress that can be carried forward in time to buffer the repeated effects of stress. ML quickly adapted within 3 days and after the next bout of stress, the leakage was diminished quite significantly indicating that the plant's ability to maintain homogeneity within the cell is greatly increased, and thus stress levels would be impacted. This was true for several of the other responses in the leaf, such as GR, POX, and protein showed distinct differences between weeks 1 and 3. The GR was reduced in day 2 of week 3 substantially over week 1, indicating a decreased need in up-regulation of the antioxidant. Levels of POX were higher initially in week 3 over week 1, possibly suggesting a greater response to potential oxidation sources.

Ovary responses were much more dynamic that the leaf. Protein levels in the field were much higher after the stress level and this increase can also be seen in the growth chamber experiment towards the end of week 3. GR levels at the field saw an increase in levels over time; the chamber observed levels were much lower in week 3 than week 1. This could be the result of extra factors in outside conditions that could not have been simulated accurately in the chamber. POX levels in the chamber saw a substantial rise at the end of week 3 in the chamber, which matched the results from the field when comparing to week 1, suggesting an adaptive response.

Future research is necessary to conclude whether a timing of stress may be an important protective mechanism for temperature variations that occur throughout the growing season. Additionally, further research should look into the possibilities that this adaptive response may provide another alternative to chemical adjuvants to smooth year-to-year variability by instigating a mild stress prior to flowering to perhaps decrease the high flower abscission rates that can occur in high temperatures. This research may prove valuable to breeders and growers alike who may be seeking cultivars that can be quick to recover from stress and able to carry that protective response forward rather than have the stress be a repeatable detriment to the plant.

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