5-2016

Heat Stress Effects on Cotton During Reproductive Development and Subsequent Acclimation Responses

Toby Ryan Fitzsimons
University of Arkansas, Fayetteville

Heat Stress Effects on Cotton During Reproductive Development and Subsequent Acclimation Responses

A Dissertation Submitted in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy in Crop, Soil, and Environmental Sciences

by

Toby R. FitzSimons
University of Central Oklahoma
Bachelor of Science in Biology, 2006
University of Central Oklahoma
Master of Science in Biology, 2010

May 2016
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

_____
Dr. Derrick M. Oosterhuis
Dissertation Director

_____
Dr. Fred M. Bourland
Committee Member

_____
Dr. Curt R. Rom
Committee Member

_____
Dr. Richard J. Norman
Committee Member

_____
Dr. Bobbie L. McMichael
Committee Member

Abstract

High temperature stress is among the most difficult to control abiotic factors affecting crop yields in the Southern United States due to its wide regional influence. Cotton (*Gossypium hirsutum* L.) though a tropical plant in origin, it is sensitive to the effects of high temperature. This is of particular concern when the warmest temperatures coincide with the most sensitive developmental stage of flowering. Thus, the capacity to improve cotton's ability to tolerate heat stress has been a significant focus for many decades. Therefore, this research was composed of several different components all designed to investigate heat stress effects. Using a combination of environmental growth chamber, field, and exploratory data modeling studies it appears that high temperatures affect cotton fruit production ubiquitously. This conclusion, based upon the results of several novel experiments of identifying heat stress effects were summarized under the following objectives:

1) Identify historic regional effects of high temperature for both irrigated and non-irrigated fields in the Mississippi Delta

2) Determine the impact heat has on the carbohydrate status of the flower and subtending leaf the days surrounding anthesis

3) Characterize acclimation potential to repeated periods of heat stress that emphasizes that the timing of analytical collections is an important underreported factor in plant development

4) Investigate well-irrigated cotton and its response to heat stress to ascertain if irrigation could provide protection from increased temperatures

Acknowledgements

The author would like to extend his deepest gratitude towards all who provided support and guidance for this massive undertaking. I would like to first thank Dr. Derrick M. Oosterhuis, my graduate professor, who was willing to take a chance on a student whom had near zero agricultural experience. His guidance and criticism has kept me in line as I explored new techniques and methods to extract the correct results. He has been a teacher, a moderator, and more importantly a friend who has continually pushed me to do more.

I appreciate the efforts of my committee members, Dr. Fred M. Bourland, Dr. Bobbie L. McMichael, Dr. Richard J. Norman, and Dr. Curt R. Rom. They have been a quiet support team who have on occasion been a valuable resource in helping me complete this degree.

I greatly appreciate the work of Johnathan R. Clark. He has been invaluable as a lab assistant, but most importantly as a friend. This project would not have been possible without his assistance in the field and countless hours helping with the environmental chamber experiments.

Additionally, all the former graduate students who have come through the program with me, who taught me so much about foreign culture. Each in some way challenged me to become a better person now than when I started. The experience gleaned from assisting each of your experiments has had a positive impact on this manuscript; it is as much yours as it is mine.

Finally, I would like to thank my family who have consistently pushed me to succeed even under the most challenging times during my career here. It has been a journey, and I am thankful that you came along for the ride.

Table of Contents

List of Figures

List of Tables

Original Articles

1) Chapter III – Published

   A. FitzSimons, T.R., and D. Oosterhuis. 2016. Historical sensitivity to temperature and precipitation for irrigated and non-irrigated cotton yields in the Mississippi Delta. Am. J. Agric. Sci. 3: 11–20.

CHAPTER I

Introduction

Heat stress for a plant is any temperature above which damage to the plant will impair either its growth or development. It is common for plants to experience significant temperature fluctuations over the course of a single day, let alone the entire growing season. In general, any transient temperature exceeding the thermal capacity of a plant is considered heat stress (Wahid et al., 2007). Therefore, the ability of a plant to phenotypically express heat tolerance, or the ability to produce economic yield under heat stress, is considered a desirable trait (Singh et al., 2007). For cotton, (*Gossypium hirsutum* L.) maximal temperatures for several growth characteristics are established. For instance, the greatest rate of leaf area development occurs at 26 ^{o}C (Reddy et al., 1997) while the overall growth optimal growth curve lies between 20 – 30 ^{o}C (Reddy et al., 1991). However, this poses an interesting situation for cotton growers in the southern United States, as it is common to exceed these optimal temperatures in the summer. Furthermore, these temperatures most often occur during anthesis, which is the most sensitive of development stages to environmental stress (Reddy et al., 1992; Snider et al., 2009). Heat stress significantly limit productivity due to disruption of the fruiting period (Wullschleger and Oosterhuis, 1990) which leads to subsequent yield losses (Stewart, 1986).

Currently, producers have only a few practical methods to help lessen the damage incurred by heat stress. First, frequent irrigation is a common technique used to depress canopy temperatures by transpiration (Burke and Upchurch, 1989). Second, planting crops as early in the season as possible despite the possibility of cooler temperatures stunting vegetative growth (Wrather et al., 2008). Third, the use of earlier maturing cultivars that set a larger number of

bolls, but at the expense of possibly greater flower abscission if heat stress occurs (Reddy et al., 1992). Finally, planting of heat tolerant cultivars that are capable of maintaining a greater flower set during elevated temperatures, albeit at the possible expense of lower yields, reduced fiber properties, and lessened insect resistance (Bauer, 1994).

Due to the unique environmental conditions of a shallow water table, deep alluvial soils, an annual rainfall exceeding 1.5 meters per year, and access to the Mississippi River Valley Alluvial Aquifer (Bengtson et al., 1995; Snipes and Nichols, 2005), many producers choose to use irrigation. This irrigation serves two primary purposes of increasing yield potential (Kebede et al., 2014) and depressing canopy temperature relative to the ambient air (Burke and Upchurch, 1989). However, a significant portion of research utilizes a well-watered regime as a control parameter to compare other stresses (Blum and Ebercon, 1981; Burke, 2007; Snider et al., 2011). Yet, even irrigated cotton could potentially suffer effects of high temperature stress despite the cooling capability of the plant. It is plausible that the controls may have their heat related responses overshadowed by larger variations in the experimental factors.

In Arkansas, there has been a historical trend of increasing yields due to improved cultivar and field management practices (Malo et al., 2000). Typically, irrigation and nutrient inputs have improved overall yields, but there remains significant year-to-year yield variation attributed to heat stress (Oosterhuis, 1999). Anthesis remains the most sensitive period to temperature stress (Reddy et al., 1992). In Arkansas, anthesis occurs during the warmest months of the year in July and early August. Research is limited investigating the regional influence of temperature on yield. In the Mississippi River Delta, flowering occurs during the warmest period of the year in July and early August with temperatures sometimes exceeding 40 ^{o}C, well above cotton's thermal maximum. Additionally, there is increasing evidence that warmer night

temperatures may also negatively impact the plant and subsequent yields (Loka and Oosterhuis, 2016). Though irrigated fields are the majority in Arkansas, a number of non-irrigated fields are still capable of producing an acceptable crop yield. For these reasons, historical investigations of regional effects upon both irrigated and non-irrigated fields to identify if heat limitations are as inherent in current cultivars as in the past.

Likewise, acclimation effects appear overlooked in studies examining heat stress responses. There is significant research identifying the application of heat stress and subsequent negative results on physiological factors such as reproductive success (Snider et al., 2009), antioxidant responses (Bibi et al., 2010), and subsequent yield (Azhar et al., 2009), acclimation does not appear to be represented in the results of many researchers. Acclimation is an important physiological trait of plants that can alter a plant's response to their environment. Acclimation to heat stress induces significant alterations in genome responses (Larkindale and Vierling, 2008), antioxidant control of reactive oxygen species (Xu et al., 2006), and the production of more heat tolerant proteins (Law et al., 2001; Liu and Huang, 2008). Thus, if analysis relies upon examining the first introduction of heat stress, the effects of such a response would be severe, as plants had not had exposure to such environmental conditions. If the timing of the heat stress is delayed until further in the growing season, then plants would be expected to maintain greater fruit loads as bolls are generally not shed after 14 days (Mauney, 1986).

Upon first exposure to an environmental stress like high temperature cotton plants will shed reproductive units due to changes within the carbohydrate balance (Guinn, 1982). Yet, the changes of carbohydrate pools within the flower and subtending leaf due to high temperatures has had limited investigations. Evidence indicates that high levels of sucrose within the ovary may lead to spontaneous abortion of developing bolls due to developmental changes of the fruit

(Aloni et al., 1997). As the flower acquires a significant portion of its carbon supply from the subtending leaf (Ashley, 1972), the effects heat stress has upon the carbohydrate pools within the tissues should be rapidly identified within the developing flower. Nonetheless, research for cotton ovaries typically assess carbohydrate changes to the flower several days following anthesis (Zhao and Oosterhuis, 2002; Marcelis et al., 2004). This leaves a gap in the research of carbohydrate changes during anthesis as carbohydrate pools adjustments may be a factor in increased reproductive abortion rates.

Heat stress remains the single most uncontrollable regional influencer of cotton yield today. As such, research on multiple levels in this dissertation examined various parameters of heat stress in an effort to better characterize its negative effects. The objectives of this dissertation were to:

1. Utilize historical public yield and regional environmental factors across the Mississippi River Delta to identify when heat stress effects were most sever to yield for both irrigated and non-irrigated cotton.
2. Identify carbohydrate changes within the flower and its subtending leaf during anthesis that may offer insight to the sensitivity of reproductive structures to heat stress.
3. Assess if acclimation is an important missing component of investigative research that may bias results dependent upon the timing of data collection.
4. Determine if well-watered cotton is also susceptible to the effects of high temperature stress, despite the transpirational cooling of the canopy.

References

Aloni, B., L. Karni, Z. Zaidman, and A.A. Schaffer. 1997. The relationship between sucrose supply, sucrose-cleaving enzymes and flower abortion in pepper. Ann. Bot. 79: 601–605.

Ashley, D. 1972. 14C-labelled photosynthate translocation and utilization in cotton plants. Crop Sci. 12: 69–74.

Azhar, F., Z. Ali, M. Akhtar, A. Khan, and R. Trethowan. 2009. Genetic variability of heat tolerance, and its effect on yield and fibre quality traits in upland cotton (*Gossypium hirsutum* L.). Plant Breed. 128: 356–362.

Bauer, P.J. 1994. Cotton crop production. C.J. Arntzen and E.M. Ritter, Eds. Encycl. Agric. Sci.: 485–493.

Bengtson, R.L., C.E. Carter, J.L. Fouss, L.M. Southwick, and G.H. Willis. 1995. Agricultural drainage and water quality in Mississippi Delta. J. Irrig. Drain. Eng. 121: 292–295.

Bibi, A.C., D.M. Oosterhuis, and E.D. Gonias. 2008. Photosynthesis, quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes. J. Cotton Sci. 12: 150–159.

Bibi, A.C., D.M. Oosterhuis, E.D. Gonias, and J.M. Stewart. 2010. Comparison of responses of a ruderal G*ossypium hirsutum* L. with commercial cotton genotypes to high temperature stress. Am. J. Plant Sci. Biotechnol: 87-92

Blum, A., and A. Ebercon. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21: 43–47.

Burke, J.J. 2007. Evaluation of source leaf responses to water-deficit stresses in cotton using a novel stress bioassay. Plant Physiol. 143: 108 –121.

Burke, J.J., and D.R. Upchurch. 1989. Leaf temperature and transpirational control in cotton. Environ. Exp. Bot. 29: 487–492.

Guinn, G. 1982. Causes of square and boll shedding. USDA Tech. Bull. 1672: 1–21.

Kebede, H., D. Fisher, R. Sui, and K. Reddy. 2014. Irrigation methods and scheduling in the delta region of Mississippi: Current status and strategies to improve irrigation efficiency. Am. J. Plant Sci. 5: 2917–2928.

Larkindale, J., and E. Vierling. 2008. Core genome responses involved in acclimation to high temperature. Plant Physiol. 146: 748–761.

Law, D.R., S.J. Crafts-Brandner, and M.E. Salvucci. 2001. Heat stress induces the synthesis of a new form of ribulose-1,5-bisphosphate carboxylase/oxygenase activase in cotton leaves.

Planta 214: 117–125.

Liu, X., and B. Huang. 2008. Photosynthetic acclimation to high temperatures associated with heat tolerance in creeping bentgrass. J. Plant Physiol. 165: 1947–1953.

Loka, D.A., and D.M. Oosterhuis. 2016. Increased night temperatures during cotton's early reproductive stage affect leaf physiology and flower bud carbohydrate content decreasing flower bud retention. J. Agron. Crop Sci.: 1-12.

Malo, J.P., L.D. Parsch, and N.P. Tugwell. 2000. Arkansas cotton: Stagnant yield and increased risk? Arkansas Agric. Exp. Stn. Spec. Rep. 198: 275–278.

Marcelis, L.F.M., E. Heuvelink, L.R.B. Hofman-Eijer, J. Den Bakker, and L.B. Xue. 2004. Flower and fruit abortion in sweet pepper in relation to source and sink strength. J. Exp. Bot. 55: 2261–2268.

Mauney, J.R. 1986. Vegetative growth and development of fruiting sites. Cott. Found. 1: 11–28.

Oosterhuis, D. 1999. Yield response to environmental extremes in cotton. pp. 30–38. *In* Proceedings of the 1990 Cotton Research Meeting.

Reddy, K., H. Hodges, and J. McKinion. 1997. Modeling temperature effects on cotton internode and leaf growth. Crop Sci. 37: 503–509.

Reddy, K.R., V.R. Reddy, and H.F. Hodges. 1992. Temperature effects on early season cotton growth and development. Agron. J. 84: 229–237.

Singh, R.P., P.V.V. Prasad, K. Sunita, S.N.N. Giri, K.R. Reddy, and Donald L. Sparks. 2007. Influence of high temperature and breeding for heat tolerance in cotton: A review. Adv. Agron. 93: 313–385.

Snider, J.L., D.M. Oosterhuis, D.A. Loka, and E.M. Kawakami. 2011. High temperature limits in vivo pollen tube growth rates by altering diurnal carbohydrate balance in field-grown *Gossypium hirsutum* pistils. J. Plant Physiol. 168: 1168–1175.

Snider, J.L., D.M. Oosterhuis, B.W. Skulman, and E.M. Kawakami. 2009. Heat stress-induced limitations to reproductive success in *Gossypium hirsutum*. Physiol. Plant. 137: 125–138.

Snipes, C.E., and S.P. Nichols. 2005. Current agricultural practices of the Mississippi Delta. Bulletin 1143. Mississippi State

Stewart, J. 1986. Integrated events in the flower and fruit. p. 261–300. *In* Mauney, J. and Stewart, J.M. (eds.), Cotton Physiology. The Cotton Foundation, Memphis, TN.

Wahid, A., S. Gelani, M. Ashraf, and M. Foolad. 2007. Heat tolerance in plants: An overview. Environ. Exp. Bot. 61: 199–223.

Wrather, J.A., B.J. Phipps, W.E. Stevens, A.S. Phillips, and E.D. Vories. 2008. Cotton planting date and plant population effects on yield and fiber quality in the Mississippi Delta. J. Cott. Sci. 7: 1–7.

Wullschleger, S.D., and D.M. Oosterhuis. 1990. Photosynthetic and respiratory activity of fruiting forms within the cotton canopy. Plant Physiol. 94: 463–469.

Xu, S., J. Li, X. Zhang, H. Wei, and L. Cui. 2006. Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. Environ. Exp. Bot. 56: 274–285.

Zhao, D., and D.M. Oosterhuis. 2002. Cotton carbon exchange, nonstructural carbohydrates, and boron distribution in tissues during development of boron deficiency. Field Crop. Res. 78: 75–87.

CHAPTER II

Literature Review

Cotton (*Gossypium hirsutum* L.) has a rich and diversified history in the world of agriculture. Few other crops have the multiplicity of uses ranging from a natural fiber for clothing and textiles, a food source, a fuel source, and a commercial feed for livestock. Likewise, there are few crops grown in as many environmentally varied parts of the world. With ranges extending from the irrigated fields of the United States to the arid drylands of Pakistan and Australia. Cotton has had a rich agricultural narrative and continues to spawn further insight for genomic researchers, plant growth and development scientists, and maintains a role as a model organism for both biotic and abiotic stresses.

The genus *Gossypium* belongs to the *Malvaceae* family, sharing similarity with other agricultural crops such as okra (*Abelmoschus esculentus* L. Moench) (Lamont, 1999) and cacao (*Theobroma cacao* L.) (Bayer and Kubitzki, 2003), and other important fiber crops such as kenaf (*Hibiscus cannabinus* L.) (Dempsey, 1975). More than 50 species in *Gossypium* currently are identified, but only four are domesticated for their fiber production, two from Africa and the Asia continents and two from the Americas (Fryxell, 1979). Species from the African and Asian continents include *G. arboretum* and *G. herbaceum*, while from the Americas the species *G. hirsutum* and *G. barbadense* originated. *Gossypium hirsutum* has become the dominant crop species in the world, with more than 95% of the world's crop (Zhang et al., 2014). In American production, the ratio remains consistent with 99% of cotton production belonging to upland cotton, *G. hirsutum*, and the remaining 1% to Pima cotton, *G. barbadense* (Hu et al., 2013).

Major concerns exist concerning the thermal boundaries inherent to cotton and its production climate. Particularly, that cotton has an optimum temperature range at a modest 20 to 30 ºC (Reddy et al., 1991), with significant injuries to yield occurring beyond 35 ºC (Bibi et al., 2004). This despite cotton being of tropical origin, its upper temperature limit is limited by its optimal enzyme kinetics at 33 ºC, and beyond leading to yield decreases and growth restrictions (Burke et al., 1988). This is troubling as most cotton growing regions within the United States often exceed this thermal upper limit in the summer. Most concerning is that the warmest summer temperatures align with the most thermally sensitive period of cotton development, anthesis (Reddy et al., 1992). Hence, the development of more heat resistant cotton has been a primary focus for breeders (Iqbal et al., 2005), geneticists (Azhar et al., 2009; Khan et al., 2011), and physiologists examining heat stress related effects with chemical ameliorants (Bibi et al., 2006; Kawakami et al., 2013). Development of greater heat tolerance in cotton is limited due to the difficulties integrating more heat tolerant genes from other *Gossypium* species into modern cotton lineages (Iqbal et al., 2005; Khan et al., 2011). High temperature remains the single greatest limiter of cotton production due to its broad regional effects, management difficulties, and alignment with sensitive reproductive development.

Millennia of cotton's domestication has not enhanced cotton's ability to tolerate warmer temperatures compared to wildtype cotton cultivars (Bibi et al., 2004; Brown and Oosterhuis, 2010). Investigations of cotton in the early 20th century by W. Lawrence Balls reported that the growth of Egyptian cultivars was reduced when temperatures exceeded the "thermotoxic" limit of 35 ºC (Balls, 1912). More recently, Bibi et al. (2004) examined twelve commercial cultivars for thermal tolerance up to 42 ºC. As was similarly indicated in Balls' study in 1912, as temperatures exceeded 35 ºC current commercial cultivars exhibited decreases in cellular

membrane stability, a proxy for heat related injury. Yet, the wildtype cotton of var. *Palmeri* reportedly had no significant cell membrane injury until temperatures exceeded 41 ^{o}C. Thus, current cultivars grown today still exhibit the same thermal boundaries that obsolete cultivars during Balls' time once expressed.

Mahan et al. (1995), submitted a review discussing management strategies that could assist in mitigating heat stress effects of cotton. Their proposals to minimize heat stress effects included that breeders should focus on optimal canopy architectures to provide shade for developing flowers, deeper rooting systems, and higher thermal boundaries to reduce yield decreases or stagnation. Better canopy structure could better assist transferring heat energies from the plant to the environment with greater efficiency. Ehleringer and Mooney (1978) indicated that the leaves of the desert shrub of *E. farinosa*, in an environment that frequently exceeds 40 ^{o}C, were significantly cooler and possessed a higher rate of photosynthesis with leaf pubescence than without. This led to the development of "okra" lobed-leaf cotton cultivars. These cultivars have been researched extensively for greater light penetrations (Peng et al., 1991) and reduced boundary layers, encouraging better heat transfer to the environment (Singh et al., 2007).

This hard thermal ceiling, however, is not completely devoid of more heat tolerant cultivars within the current germplasm. For instance, a significant impediment in heat tolerance is pollen. Pollen germination on the stigma occurs in the morning between 0700 h and 1100 h within 30 minutes of pollen to stigma contact (Pundir, 1972). The development of pollen tubes within the style is very temperature sensitive (Snider et al., 2009, 2011a). Burke et al. (2004) identified that pollen grains removed from shaded anthers germinated at a much higher percentage compared to grains exposed to direct sunlight during flowering. He determined that

the optimal temperature for germination should not exceed 28 ºC, which sun exposed flowers often surpassed. Rodriguez-Garay and Barrow (1988) investigated *G. barbadense* pollen grains first exposed to 35 ºC for germination studies. They crossed the heat-treated pollen grains with *G. hirsutum* cultivars to transfer improved thermotolerance to the *G. hirsutum* hybrids. Their results indicated that greater heat tolerance was present in the subsequent offspring, as well as F_2 generations, and backcross progeny. Yet, heat tolerant cultivars often come at the expense of unwanted traits such as reduced yields, diminished fiber properties, and decreased insect resistance which precludes their widespread adoption (Bauer, 1994).

Thermotolerance is of large concern given that modern cultivars appear to have similar limitations to high temperature stress effects as long obsolete cultivars (Balls, 1912; Bibi et al., 2008; Brown and Oosterhuis, 2010). It has been suggested that breeders reevaluate their selections based upon success under duress rather than of greatest yield potentials (Constable et al., 2001). Utilizing yield as a sole indicator for a cultivar's merit disregards the influence of generational developmental time and yearly environmental stress impacts (Constable et al., 2001). Hall and Hartwell Jr. (1993) hypothesized that cultivars displaying heat tolerance during reproductive development should have the following characteristics: a high harvest index, high photosynthetic capacity per unit leaf area, small leaves, and low leaf area per unit ground area. Breeding work has been successful in many of these areas, such as decreases in leaf size and increased stomatal conductance in *G. barbadense* (Lu et al., 1994). Bibi et al. (2008) utilized the nondestructive method of photosynthetic fluorescence, and the minimally destructive cellular membrane integrity to determine differences in several current and ancestral cultivars. They determined that both techniques were able to identify differences within the different cultivars under increasing heat stress. Recently, Cottee et al. (2010) utilized a series experiments to

determine which biochemical stress markers to identify stress could be most reliable. Their research focused on three biochemical stress proxies of fluorescence, cellular membrane stability, and enzymatic viability, and indicated that both breeders and researchers should utilize these proxies as rapid cultivar identifying techniques.

The developing boll is highly susceptible to high temperatures within the first 14 days after anthesis with homeostatic disruptions leading to the developing boll's abortion (Stewart, 1986). A recent article from Pettigrew (2008) noted that cotton exposed to a modest increase of 0.5 to 0.8°C throughout the growing season negatively affected yields by as much as 10%. Zhao et al. (2005) reported that heat-stressed cotton ovaries the day following anthesis experienced increased ovule sucrose concentrations while glucose and fructose concentrations declined. Similarly, carbon partitioning within maize (*Zea mays* L.*)* kernels is also disrupted by heat stress with carbon being directed towards sucrose and hexoses as opposed to starch (Cheikh and Jones, 1995). Heat-stressed starch granules in immature rice (*Oryza sativa* L.) grains were smaller and starch reserves were decreased (Zakaria et al., 2002). Increased ambient temperatures in cotton reduced the soluble carbohydrates concentrations within the ovary (Snider et al., 2011b). Most likely due to respiration rates within the tissues increasing at a linear rate with increased temperatures (Perry et al., 1983).

Further, as temperatures increase beyond the optimum the plasma membrane both of the cell and of the thylakoid begin to become more porous (Gerner et al., 1980; ur Rahman et al., 2004). As temperatures increase, the plasma membrane undergoes lipid peroxidation which permits ions to pass from organelles unchecked, disrupting cellular stability and efficiency (Schrader et al., 2004; Sharkey, 2005). This permeability activates a series of calcium dependent protein kinases that spur cellular production of increased antioxidants (Wahid et al., 2007). These

antioxidants are necessary to react to the increasing concentration of reactive oxygen species (ROS) present within the cell as temperatures increase (Gür et al., 2010). Normally, small amounts of ROS function as signaling markers that precipitate beneficial downstream events such as plant growth and development (Schroeder et al., 2001; Foreman et al., 2003; Arasimowicz and Floryszak-Wieczorek, 2007). As ROS levels increase within the cell, they initiate enzymatic pathways that serve as protective stress responses (Dat et al., 1998; Foyer and Noctor, 2005). If stress conditions exceed that of the protective measures, ROS finally initiate programmed cellular death (Gechev et al., 2006). Therefore, it is important that cells maintain sufficient levels of antioxidants during stressful situations to avoid premature apoptosis.

Temperatures beyond the optimal thermal limits of the species will hinder the photosynthetic capability (Bibi et al., 2008). This is the result of photosynthesis transitioning from non-cyclic photophosphorylation pathways to a cyclic phosphorylation pathway. Increasing heat forces the photosystem II (PSII) complex to decouple from photosystem I (PSI) to preserve to more thermosensitive PSII from excessive quantum energy injury (Bukhov et al., 2001; Bukhov and Carpentier, 2004). This decoupling preserves PSII from heat related damage while allowing the Calvin cycle to continue providing intermediates during photorespiration and ensuing starch breakdown (Weise et al., 2006). In *G. barbadense*, heat stress related increases in cyclic phosphorylation was sufficient to compensate for the permeability of the thylakoid membrane maintaining adenosine triphosphate (ATP) outputs (Schrader et al., 2004). Yet, cyclic phosphorylation is an inherently inefficient compensation mechanism as it creates ATP but does not allow for quantum energy to be transferred to nicotinamide adenine dinucleotide phosphate (NADP), which is a necessary energy carrying molecule for carbon fixation (Chain and Arnon, 1977).

PSII has been categorized as being the most heat-sensitive component of the photosynthetic apparatus (Berry et al., 1980). Recent work though, indicate that PSII is more robust than previously thought. For example, it was determined by Gombos et al. (1994) that lasting negative effects on the PSII apparatus rarely occur until temperatures exceed 45 ^{o}C. Additionally, the negative effects of high temperature on PSII can be mitigated by increasing atmospheric CO_2 concentrations. Cotton grown under increased CO_2 and higher temperatures had greater leaf area, increased photosynthetic capacity, and a higher boll retention when compared to normal CO_2 and higher temperatures (Radin et al., 1987; Mauney et al., 1994).

Work performed by Reddy et al. (1998) indicated that cotton was especially buffered to higher temperatures as CO_2 levels increased photosynthetic rates during photosynthesis. Cotton maintained significantly higher rates of photosynthesis at 36 ^{o}C with no reduction in rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) activase activity at CO_2 concentrations of 450 ppm, compared to plants at the same temperature but lower CO_2 concentrations. This preservation of activity was due to a directional push towards carbon fixation by rubisco. Research by Crafts-Brandner and Salvucci (2000) suggested that rubisco was not the limiting photosynthetic factor affecting photosynthetic efficiencies during high temperature stress. Isolated rubisco from cotton leaves failed to decrease activity until temperatures exceeded 50 ^{o}C, far outside normal growing conditions. In contrast, the rate for its isolated activase was limited at 42 ^{o}C. Thus, the upper maximum temperature limit for photosynthetic capacity was instead limited by rubisco activase and not rubisco itself.

Heat stress can have negative impacts upon sucrose related enzyme activities modifying the soluble sugar homeostasis within the cell. Work within tomatoes (*Solanum lycopersicum* L.) identified that heat shocked fruit embryos significantly decreased sucrose synthase activities,

which catalyzes the reversible reaction of sucrose to fructose and glucose with NDP (nucleoside diphosphate) (Wang et al., 1993). This reduction in sucrose synthase activity led to an increase of sucrose concentration within the developing pericarp. Excess sucrose can reduce the gradient between source and sink, decreasing phloem flow to the developing ovary (Walker et al., 1978). Increased sucrose concentrations within the developing boll has been known to cause ovary swelling, fruit deformities, and increased rates of abscission in cotton (Darnell, 2013). Additionally, increased temperatures also affect respiration rates within the cotton ovule. The optimal temperature for cellulose synthesis is approximately 28 ^{o}C with higher temperatures reducing cellulose deposition, substantially increase respiration rates, and decreasing fiber elongation (Roberts et al., 1992).

PSI is unable to utilize all of photonic energies that strike the leaf. Excess energies must be dissipated into different pathways or risk cellular damage (Müller et al., 2001; Zhang et al., 2011). Heat stress also increases the amount of ROS present within the cell (Allakhverdiev et al., 2008), which must be reduced to avoid photoinhibition of PSII. Two of the dominant pathways assisting in quenching excess photonic energies include the regulation of photosynthetic reactions through the redox mediation of the plastoquinone pool, and the xanthophyll regulatory pathway. The xanthophyll cycle is capable of mitigating significant quantities of excess light energies into other heat dissipating pathways. In the xanthophyll cycle, the conversion of violaxanthin to zeaxanthin is responsible for the photo-protection of photosynthesis by dissipating excess light energy from chlorophyll into heat energy to protect PSII (Müller et al., 2001). Zeaxanthin is also capable of stabilizing disrupted thylakoid membranes by alleviating lipid peroxidation effects (Havaux et al., 2007). As described by Mubarakshina and Ivanov (2010), plastoquinone, which is a quinone molecule directly involved in the electron transport

chain, performs a dual role in reactive oxygen species regulation. First, it is able to reduce oxygen to a superoxide by plastosemiquinone. Second, it can transform superoxide into hydrogen peroxide by plastohydroquinone. The hydrogen peroxide produced from this reaction can subsequently be utilized as a signaling molecule for gene expression (Gechev et al., 2002), or as a protectant from both photoinhibition and photo-oxidation (Karpinski et al., 1999). Though, if ROS within the cell and chloroplast become too great then photoinhibition will occur, leading to increased cellular damage (Allakhverdiev et al., 2008). Cellular damages that exceed the capacity of the antioxidant mechanisms, then production of antioxidants decrease significantly and senescence is initiated within the tissues (Liu and Huang, 2000). This correlates with the susceptibility of the developing boll, which is most sensitive to stress within the first 14 days after anthesis and possibly leading to higher rates of reproductive unit abortion (Gipson, 1986; Stewart, 1986)

Every summer season, heat stress is a looming obstacle for producers to tackle. Traditionally, producers have used irrigation as the primary method to alleviate heat stress within the canopy. Cotton leaf temperature reductions by more than 10 ^{o}C can be achieved under hot low humidity conditions with ample irrigation (Burke and Upchurch, 1989). However, the conditions of these results were from the drier climates of west Texas where vapor pressure differentials can be quite significant. Higher relative humidities limit the effectiveness of transpiration due to a reduced vapor pressure difference between atmospheric and internal stomatal humidity. Moreover, transpiration rates decrease significantly during the middle of the day when root impedance becomes a significant restriction to replacing water in the leaf tissues lost to the air (Skidmore and Stone, 1964). Mott and Parkhurst (1991) reviewed the responses of multiple species and determined that using relative humidities as the basis for stomatal

conductance measurements may be problematic, as plants do not react to relative humidities, but rather to changes in transpiration rates. Thus, if the transpiration rate becomes too great for the leaf to accommodate stomata will begin narrow to limit water loss. Stomatal closures to reduce transpiration rates also reduce photosynthesis rates. A broader examination of 16 species performed by Monteith (1995) supported the theory that stomata respond not to humidity levels, but respiration rates. In agreement with Mott and Parkhurst (1991), Moneith also identified that stomata from all investigated species had patchy stomata closure in response to increased respiration rates to limit water loss.

Even under irrigated conditions, plants will adjust stomatal opening to reduce excessive transpirational water loss. A physiological basis for this phenomenon may be due to limitations of water uptake. Taylor and Keppler (1975) investigated the effects of water uptake in cotton due to the effects of soil water potential, soil hydraulic capacity, water uptake, and water density. They determined that cotton has a high resistance to water flow from the root epidermis into the root xylem. Higher root resistances reduce the quantity of water in the leaf tissues if transpiration rates are sufficiently high to exceed the rates of water incorporation.

Likewise, too much irrigation can reach conditions akin to waterlogging, where air spaces between soil granules are substituted with water. This leads to several soil dynamic changes, including decreases in soil oxygen, increases in ethylene concentration, and increases in soil CO_2 levels (Wiengweera and Greenway, 2004). Cotton in particular is relatively sensitive to decreases in soil O_2 concentrations with growth inhibition occurring after mildly hypoxic conditions (< 10% O_2) (Meyer et al., 1987). Root exposures greater than 3 hours to anoxic conditions results in complete death of the terminal apices of roots (Huck, 1970). This can limit water inflow into

developing above ground tissues forcing stomatal narrowing, limiting CO2 gas exchange, reducing photosynthesis, and increasing leaf temperature.

Thus, irrigation may prove more of a bane to yield if the recourse for mitigating heat stress is frequent watering during the warmest of the summer months. This is a serious concern in Arkansas, because it is the most heavily irrigated agricultural area in the surrounding region (Maupin and Barber, 2005). This is despite the Mississippi River Delta receiving on average more than 1.5 meters of rainfall per year (Snipes and Nichols, 2005). In addition, the insistence on using irrigation has resulted in the depletion of subsurface waters of the Mississippi River Valley Alluvial Aquifer due to excessive withdrawals, of which more than 96% of the extracted water being utilized for agricultural purposes (Sullivan and Delp, 2007). Since 1980, yields from irrigated fields in Arkansas have only been about 22% higher than dryland cotton. Yet, non-irrigated cotton yields have been increasing in parallel with irrigated crops, maintaining the same difference in yield. (FitzSimons and Oosterhuis, 2016).

Thus, research indicates that non-irrigated production is not necessarily constrained due to a lack of irrigation in the cotton growing regions of the Mississippi River Valley. If a crop maintains more fruit early in the season, then the plant will maintain a shorter stature as more carbohydrates are partitioned into fruit development rather than vegetative growth (Whitaker et al., 2008). Ritchie et al. (2009) investigated different irrigation strategies in west Texas and observed that boll distribution patterns in non-irrigated cotton were generally lower within the canopy. They attributed this decrease in cotton production to water stress, as the plants were smaller in stature and contained less vegetative growth. Ritchie and team also determined that the surface drip method and non-irrigated cotton trials both contained fewer harvestable bolls

higher in the canopy. Suggesting that the irrigation quantity, timing, or combination or the two factors may be more determinate of successful crop yield.

If irrigation could potentially cause problems for root and plant development, then a more modified irrigation strategy may be required. Mitchell et al. (1984) presented an irrigation theory for fruit trees that emphasized regulated deficit irrigations (RDI) that emphasized irrigation only certain times. The principles of RDI is to strategically time irrigations to distinct crop developmental stages in an effort to minimize excess vegetative growth while maintaining yield (Girona et al., 1993). RDI's purpose is to utilize water resources more effectively so that during critical reproductive development stages the plant does not have drought stress complications affecting yield. Although this technique originally was developed for tree orchards where deep rooting structures are common, other perennial species grown as annuals such as peanuts (*Arachis hypogaea* L.) have maintained yields while minimizing water usage (Abou Kheira, 2009; Rowland et al., 2012) . Rowland et al. (2012) investigated the effects of RDI in peanuts for the semi-arid west Texas plains. Their results indicated that reducing irrigation during early vegetative growth maintained viable economic yields by the end of the season. Additionally, plants accelerated their reproductive development cycle by more than a month in some cases. Further, plants under a the RDI strategy maintained similar NDVI (Normalized Difference Vegetation Index) values as the well-watered treatments, indicating a maintenance of photosynthetic capacity.

By utilizing an RDI irrigation strategy, it is possible to reduce the amount of water necessary while maintaining yields. There is evidence that exposure to mild drought stress during vegetative growth may produce an acclimation effect to better adjust to future stress events through changes in gene expression, modifications to the plant morphology, and photosynthetic

adjustments to the carbon balance (Flexas et al., 2006). The effects of acclimation can better ready a plant for future stresses that the plant may incur later in the season. Acclimation can maintain reproductive success rates under stressful conditions (Kozlowski and Pallardy, 2002), maintain increased levels of antioxidants to mitigate ROS (Yamasaki et al., 2002), and subsequent maintenance of photosynthetic capabilities under heat stress (Law and Crafts-Brandner, 1999; Sethar et al., 2002). Still, acclimation has not been a well-studied avenue of research in cotton under conditions of heat stress, though it has been well researched for water stress (Yordanov et al., 2003; Massacci et al., 2008). Moderate periods of heat could provide significant protection against future heat stresses (Vierling, 1991), posing an anthesis to current irrigation philosophy and acclimation potential for cotton.

References

Abou Kheira, A.A. 2009. Macromanagement of deficit-irrigated peanut with sprinkler irrigation. Agric. Water Manag. 96: 1409–1420.

Allakhverdiev, S.I., V.D. Kreslavski, V. V Klimov, D. a Los, R. Carpentier, and P. Mohanty. 2008. Heat stress: an overview of molecular responses in photosynthesis. Photosynth. Res. 98: 541–550.

Arasimowicz, M., and J. Floryszak-Wieczorek. 2007. Nitric oxide as a bioactive signalling molecule in plant stress responses. Plant Sci. 172: 876–887.

Azhar, F., Z. Ali, M. Akhtar, A. Khan, and R. Trethowan. 2009. Genetic variability of heat tolerance, and its effect on yield and fibre quality traits in upland cotton (*Gossypium hirsutum* L.). Plant Breed. 128: 356–362.

Balls, W.L. 1912. The cotton plant in egypt: studies in physiology and genetics. 1st ed. MacMillan and Co., London, England.

Bauer, P.J. 1994. Cotton crop production. C.J. Arntzen and E.M. Ritter, Eds. Encycl. Agric. Sci.: 485–493.

Bayer, C., and K. Kubitzki. 2003. Malvaceae. p. 225–311. *In* Flowering Plants · Dicotyledons. Springer Berlin Heidelberg, Berlin, Heidelberg.

Berry, J., O. Björkman, and O. Bjorkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol. 31: 491–543.

Bibi, A.C., D.M. Oosterhuis, and E.D. Gonias. 2008. Photosynthesis, quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes. J. Cotton Sci. 12: 150–159.

Bibi, A.C., D.M. Oosterhuis, E.D. Gonias, and F.M. Bourland. 2004. Screening a diverse set of cotton cultivars for high temperature tolerance. pp. 39–43. *In* Oosterhuis, D.M. (ed.), Proc 2004 Cotton Research Meeting and Summaries of Research in Progress. AAES Research Series, Fayetteville, AR.

Bibi, A.C., D.M. Oosterhuis, E.D. Gonias, and J.D. Mattice. 2006. Exogenous application of putrescine on cotton ovaries under two temperature regimes. Summ. Arkansas Cott. Res., AAES, Research Series: 54–57.

Brown, R., and D. Oosterhuis. 2010. High daytime temperature stress effects on the physiology of modern versus obsolete cotton cultivars. Am. J. Plant Sci. Biotechnol. 4: 93–96.

Bukhov, N., and R. Carpentier. 2004. Alternative photosystem I-driven electron transport routes: Mechanisms and functions. Photosynth. Res. 82: 17–33.

Bukhov, N.G., G. Samson, and R. Carpentier. 2001. Nonphotosynthetic reduction of the intersystem electron transport chain of chloroplasts following heat stress. The pool size of stromal reductants. Photochem. Photobiol. 74: 438–443.

Burke, J.J., J.R. Mahan, and J.L. Hatfield. 1988. Crop-specific thermal kinetic windows in relation to wheat and cotton biomass production. Agron. J. 80: 553–556.

Burke, J.J., and D.R. Upchurch. 1989. Leaf temperature and transpirational control in cotton. Environ. Exp. Bot. 29: 487–492.

Burke, J.J., J. Velten, and M.J. Oliver. 2004. In vitro analysis of cotton pollen germination. Agron. J. 96: 359–368.

Chain, R.K., and D.I. Arnon. 1977. Quantum efficiency of photosynthetic energy conversion. Proc. Natl. Acad. Sci. U. S. A. 74: 3377–3381.

Cheikh, N., and R.J. Jones. 1995. Heat stress effects on sink activity of developing maize kernels grown in vitro. Physiol. Plant. 95: 59–66.

Constable, G.A., P.E. Reid, and N.J. Thomson. 2001. Approaches utilized in breeding and development of cotton cultivars in australia. p. 1–16. *In* Jenkins, J.N., Saha, S. (eds.), Genetic improvement of cotton: Emerging technologies. Science Publishers, Enfield, NH.

Cottee, N.S., D.K.Y. Tan, M.P. Bange, J.T. Cothren, and L.C. Campbell. 2010. Multi-level determination of heat tolerance in cotton (*Gossypium hirsutum* L.) under field conditions. Crop Sci. 50: 2553-2564.

Crafts-Brandner, S.J., and M.E. Salvucci. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. Proc. Natl. Acad. Sci. U. S. A. 97: 13430–13435.

Darnell, R. 2013. Night temperature and source-sink effects on growth, leaf carbon exchange rate, and carbohydrate accumulation in bell pepper ovaries. J. Am. Soc. Hortic. Sci. 138: 331–337.

Dat, J.F., H. Lopez-Delgado, C.H. Foyer, and I.M. Scott. 1998. Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol. 116: 1351–1357.

Dempsey, J.M. 1975. Fiber crops. Universtiy Press of Florida, Gainesville, Florida.

Ehleringer, J.R., and H.A. Mooney. 1978. Leaf hairs: effects on physiological activity and adaptive value to a desert shrub. Oecologia 37: 183–200.

FitzSimons, T.R., and D. Oosterhuis. 2016. Historical sensitivity to temperature and precipitation

for irrigated and non-irrigated cotton yields in the Mississippi Delta. Am. J. Agric. Sci. 3: 11–20.

Flexas, J., J. Bota, J. Galmés, H. Medrano, and M. Ribas-Carbó. 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. Physiol. Plant. 127: 343–352.

Foreman, J., V. Demidchik, J.H.F. Bothwell, P. Mylona, H. Miedema, M.A. Torres, P. Linstead, S. Costa, C. Brownlee, J.D.G. Jones, J.M. Davies, and L. Dolan. 2003. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422: 442–446.

Foyer, C.H., and G. Noctor. 2005. Oxidant and antioxidant signalling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. Plant, Cell Environ. 28: 1056–1071.

Fryxell, P.A. 1979. The natural history of the cotton tribe. Texas A&M University Press, College Station, Texas.

Gechev, T.S., F. Van Breusegem, J.M. Stone, I. Denev, and C. Laloi. 2006. Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. BioEssays 28: 1091–1101.

Gechev, T., I. Gadjev, F. Van Breusegem, D. Inzé, S. Dukiandjiev, V. Toneva, and I. Minkov. 2002. Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. Cell. Mol. Life Sci. 59: 708–714.

Gerner, E.W., A.E. Cress, D.G. Stickney, D.K. Holmes, and P.S. Culver. 1980. Factors redulating membrane permeability alter thermal resistance. Ann. N. Y. Acad. Sci. 335: 215–233.

Gipson, J.R. 1986. Temperature effects on growth, development, and fiber properties. p. 47–56. *In* Mauney, J.R. and McD. Stewart, J. (eds.), Cotton Physiology. The Cotton Foundation.

Girona, J., M. Mata, D. A. Goldhamer, R.S. Johnson, and T.M. DeJong. 1993. Patterns of soil and tree water status and leaf functioning during regulated deficit irrigation scheduling in peach. J. Am. Soc. Hortic. Sci. 118: 580–586.

Gombos, Z., H. Wada, E. Hideg, and N. Murata. 1994. The unsaturation of membrane lipids stabilizes photosynthesis against heat stress. Plant Physiol. 104: 563 –567.

Gür, A., U. Demirel, M. Özden, A. Kahraman, O. Opur, and O. Çopur. 2010. Diurnal gradual heat stress affects antioxidant enzymes, proline accumulation and some physiological components in cotton (*Gossypium hirsutum* L.). African J. Biotechnol. 9: 1008–1015.

Hall, A.E., and A.L. Hartwell, Jr. 1993. Designing cultivars for the climatic conditions of the next century. p. 291–297. *In* Boxton, D.R., Shibles, R., Forsberg, R.A., Blad, B.L., Asay,

K.H., Paulsen, G.M., Wilson, R.F. (eds.), International Crop Science I. Crop Science Society of America, Madison, WI.

Havaux, M., L. Dall'Osto, and R. Bassi. 2007. Zeaxanthin has enhanced antioxidant capacity with respect to all other xanthophylls in A*rabidopsis* leaves and functions independent of binding to PSII antennae. Plant Physiol. 145: 1506–1520.

Hu, G., J. Koh, M.J. Yoo, K. Grupp, S. Chen, and J.F. Wendel. 2013. Proteomic profiling of developing cotton fibers from wild and domesticated *Gossypium barbadense*. New Phytol. 200: 570–582.

Huck, M.G. 1970. Variation in taproot elongation rate as influenced by composition ofthe soil air. Agron. J. 62: 815–818.

Iqbal, M.J., M.Z. Iqbal, R.S.A. Khan, and K. Hayat. 2005. Comparison of obsolete and modern varieties in view to stagnancy in yield of cotton (*G. hirsutum* L.). Asian J. Plant Sci. 4: 374–378.

Karpinski, S., H. Reynolds, B. Karpinska, G. Wingsle, G. Creissen, and P. Mullineaux. 1999. Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. Science 284: 654–657.

Kawakami, E.M., D.M. Oosterhuis, J.L. Snider, and T.R. FitzSimons. 2013. High temperature and the ethylene antagonist 1-methylcyclopropene alter ethylene evolution patterns , antioxidant responses , and boll growth in *Gossypium hirsutum*. Am. J. Plant Sci.: 1400–1408.

Khan, A.I., I.A. Khan, F.S. Awan, H.A. Sadaqat, and S. Bahadur. 2011. Estimation of genetic distance based on RAPDs between 11 cotton accessions varying in heat tolerance. Genet. Mol. Res. 10: 96–101.

Kozlowski, T.T., and S.G. Pallardy. 2002. Acclimation and adaptive responses of woody plants to environmental stresses. Bot. Rev. 68: 270–334.

Lamont, W.J. 1999. Okra - a versatile vegetable crop. Horttechnology 9: 179–184.

Law, R., and S. Crafts-Brandner. 1999. Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant Physiol. 120: 173–182.

Liu, X., and B. Huang. 2000. Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. Crop Sci. 40: 503–510.

Lu, Z., J.W. Radin, E.L. Turcotte, R. Percy, and E. Zeiger. 1994. High yields in advanced lines of Pima cotton are associated with higher stomatal conductance, reduced leaf area and lower leaf temperature. Physiol. Plant. 92: 266–272.

Mahan, J.R., B.L. McMichael, and D.F. Wanjura. 1995. Methods for reducing the adverse effects of temperature stress on plants: a review. Environ. Exp. Bot. 35: 251–258.

Massacci, A., S.M. Nabiev, L. Pietrosanti, S.K. Nematov, T.N. Chernikova, K. Thor, and J. Leipner. 2008. Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. Plant Physiol. Biochem. 46: 189–95.

Mauney, J., B. Kimball, P. Pinterjr, R. Lamorte, K. Lewin, J. Nagy, and G. Hendrey. 1994. Growth and yield of cotton in response to a free-air carbon dioxide enrichment (FACE) environment. Agric. For. Meteorol. 70: 49–67.

Maupin, M.A., and N.L. Barber. 2005. Estimated withdrawals from principal aquifers in the United States, 2000. Reston, VA.

Meyer, W.S., D.C. Reicosky, H.D. Barrs, and R.C.G. Smith. 1987. Physiological responses of cotton to a single waterlogging at high and low N-levels. Plant Soil 102: 161–170.

Mitchell, P.D., P.H. Jerie, and D.J. Chalmers. 1984. The effects of regulated water deficits on pear tree growth, flowering, fruit growth, and yield. J. Am. Soc. Hortic. Sci. 109: 604–606.

Monteith, J.L. 1995. A reinterpretation of stomatal responses to humidity. Plant. Cell Environ.: 357–364.

Mott, K. a., and D.F. Parkhurst. 1991. Stomatal responses to humidity in air and helox. Plant. Cell Environ. 14: 509–515.

Mubarakshina, M.M., and B.N. Ivanov. 2010. The production and scavenging of reactive oxygen species in the plastoquinone pool of chloroplast thylakoid membranes. Physiol. Plant. 140: 103–110.

Müller, P., X.P. Li, and K.K. Niyogi. 2001. Non-photochemical quenching. a response to excess light energy. Plant Physiol. 125: 1558–1566.

Peng, S., D.R. Krieg, and F.S. Girma. 1991. Leaf photosynthetic rate is correlated with biomass and grain production in grain sorghum lines. Photosynth. Res. 28: 1–7.

Perry, S.W., D.R. Krieg, and R.B. Hutmacher. 1983. Photosynthetic rate control in cotton. Plant Physiol. 73: 662–665.

Pettigrew, W.T. 2008. The effect of higher temperatures on cotton lint yield production and fiber quality. Crop Sci. 48: 278–285.

Pundir, N.S. 1972. Experimental embryology of *Gossypium arboreum* L. and *G. hirsutum* L. and their reciprocal crosses. Bot. Gaz. 133: 7–26.

Radin, J.W., B. a. Kimball, D.L. Hendrix, and J.R. Mauney. 1987. Photosynthesis of cotton plants exposed to elevated levels of carbon dioxide in the field. Photosynth. Res. 12: 191–203.

Reddy, V.R., K.R. Reddy, and D.N. Baker. 1991. Temperature effect on growth and development of cotton during the fruiting period. Agron. J. 83: 211–217.

Reddy, K.R., V.R. Reddy, and H.F. Hodges. 1992. Temperature effects on early season cotton growth and development. Agron. J. 84: 229–237.

Reddy, A.R., K.R. Reddy, and H.F. Hodges. 1998. Interactive effects of elevated carbon dioxide and growth temperature on photosynthesis in cotton leaves. Plant Growth Regul. 26: 33–40.

Ritchie, G.L., J.R. Whitaker, C.W. Bednarz, and J.E. Hook. 2009. Subsurface drip and overhead irrigation: a comparison of plant boll distribution in upland cotton. Agron. J. 101: 1336–1344.

Roberts, E.M., N.R. Rao, J.Y. Huang, N.L. Trolinder, and C.H. Haigler. 1992. Effects of cycling temperatures on fiber metabolism in cultured cotton ovules. Plant Physiol. 100: 979–986.

Rodriguez-Garay, B., and J.R. Barrow. 1988. Pollen selection for heat tolerance in cotton. Crop Sci. 28: 857–859.

Rowland, D.L., W.H. Faircloth, P. Payton, D.T. Tissue, J.A. Ferrell, R.B. Sorensen, and C.L. Butts. 2012. Primed acclimation of cultivated peanut (*Arachis hypogaea* L.) through the use of deficit irrigation timed to crop developmental periods. Agric. Water Manag. 113: 85–95.

Schrader, S.M., R.R. Wise, W.F. Wacholtz, D.R. Ort, and T.D. Sharkey. 2004. Thylakoid membrane responses to moderately high leaf temperature in pima cotton. Plant, Cell Environ. 27: 725–735.

Schroeder, J., G. Allen, V. Hugouvieux, J. Kwak, and D. Waner. 2001. Guard cell signal transduction. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52: 627–658.

Sethar, M.A., V.M. Pahoja, and Q. Chachar. 2002. Heat acclimation potential of chlorophyll fluorescence of cotton cultivars. Pakistan J. Bot. 34: 275–282.

Sharkey, T.D. 2005. Effects of moderate heat stress on photosynthesis: Importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. Plant, Cell Environ. 28: 269–277.

Singh, R.P., P.V.V. Prasad, K. Sunita, S.N.N. Giri, K.R. Reddy, and Donald L. Sparks. 2007. Influence of high temperature and breeding for heat tolerance in cotton: A review. Adv. Agron. 93: 313–385.

Skidmore, E.L., and J.F. Stone. 1964. Physiological role in regulating transpiration rate of the cotton plant. J. Am. Soc. Agron. 56: 405–410.

Snider, J.L., D.M. Oosterhuis, and E.M. Kawakami. 2011a. Diurnal pollen tube growth rate is slowed by high temperature in field-grown *Gossypium hirsutum* pistils. J. Plant Physiol. 168: 441–448.

Snider, J.L., D.M. Oosterhuis, D.A. Loka, and E.M. Kawakami. 2011b. High temperature limits in vivo pollen tube growth rates by altering diurnal carbohydrate balance in field-grown *Gossypium hirsutum* pistils. J. Plant Physiol. 168: 1168–1175.

Snider, J.L., D.M. Oosterhuis, B.W. Skulman, and E.M. Kawakami. 2009. Heat stress-induced limitations to reproductive success in G*ossypium hirsutum*. Physiol. Plant. 137: 125–138.

Snipes, C.E., and S.P. Nichols. 2005. Current agricultural practices of the Mississippi Delta. Bulletin 1143. Mississippi State.

Stewart, J. 1986. Integrated events in the flower and fruit. p. 261–300. *In* Mauney, J., Stewart, J.M. (eds.), Cotton Physiology. The Cotton Foundation, Memphis, TN.

Sullivan, M.E., and W.M. Delp. 2007. How a systems approach to irrigation promotes sustainable water use. p. 145–159. *In* NBAC Report 24: Water Sustainability in Agriculture.

Taylor, H.M., and B. Keppler. 1975. Water uptake by cotton root systems: an examination of assumptions in the single root model. Soil Sci. 120: 57–67.

ur Rahman, H., S.A. Malik, and M. Saleem. 2004. Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane thermostability. Field Crop. Res. 85: 149–158.

Vierling, E. 1991. The roles of heat shock proteins in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 579–620.

Wahid, A., S. Gelani, M. Ashraf, and M. Foolad. 2007. Heat tolerance in plants: An overview. Environ. Exp. Bot. 61: 199–223.

Walker, A.J., L.C. Ho, and D.A. Baker. 1978. Carbon translocation in the tomato: Pathways of carbon metabolism in the fruit. Ann. Bot. 42: 901–909.

Wang, F., A. Sanz, M.L. Brenner, and A. Smith. 1993. Sucrose synthase, starch accumulation, and tomato fruit sink strength. Plant Physiol. 101: 321–327.

Weise, S.E., S.M. Schrader, K.R. Kleinbeck, and T.D. Sharkey. 2006. Carbon balance and circadian regulation of hydrolytic and phosphorolytic breakdown of transitory starch. Plant Physiol. 141: 879–886.

Whitaker, J.R., G.L. Ritchie, C.W. Bednarz, and C.I. Mills. 2008. Cotton subsurface drip and overhead irrigation efficiency, maturity, yield, and quality. Agron. J. 100: 1763–1768.

Wiengweera, A., and H. Greenway. 2004. Performance of seminal and nodal roots of wheat in stagnant solution: K+ and P uptake and effects of increasing O_2 partial pressures around the shoot on nodal root elongation. J. Exp. Bot. 55: 2121–2129.

Yamasaki, T., T. Yamakawa, Y. Yamane, H. Koike, and K. Satoh. 2002. Temperature acclimation of photosynthesis and related changes in photosystem II electron transport in winter wheat. Plant Physiol. 128: 1087–1097.

Yordanov, I., V. Velikova, and T. Tsonev. 2003. Plant responses to drought and stress tolerance. Bulg. J. Plant Physiol.: 187–206.

Zakaria, S., T. Matsuda, S. Tajima, and Y. Nitta. 2002. Effect of high temperature at ripening stage on the reserve accumulation in seed in some rice cultivars. Plant Prod. Sci. 5: 160–168.

Zhang, R., D.M. Kramer, J.A. Cruz, K.R. Struck, and T.D. Sharkey. 2011. The effects of moderately high temperature on zeaxanthin accumulation and decay. Photosynth. Res. 108: 171–181.

Zhang, J., R.G. Percy, and J.C. McCarty. 2014. Introgression genetics and breeding between upland and Pima cotton: A review. Euphytica 198: 1–12.

Zhao, D., K.R. Reddy, V.G. Kakani, S. Koti, and W. Gao. 2005. Physiological causes of cotton fruit abscission under conditions of high temperature and enhanced ultraviolet-b radiation. Physiol. Plant. 124: 189–199.

CHAPTER III

Historical Sensitivity to Temperature and Precipitation for Irrigated and Non-Irrigated Cotton Yields in the Mississippi Delta

Abstract

An investigation of cotton yield between irrigated and non-irrigated fields in Arkansas from 1980 to the present was initiated to determine if large regional effects in yield could be attributed to heat or precipitation stress. Temperature and precipitation data from weather stations centrally located in three agricultural districts of cotton growing regions in eastern Arkansas were analyzed with cotton yields obtained from the National Agricultural Statistics Service (NASS) for both irrigated and non-irrigated fields within the state. Yield relationships were quantified between the months of June, July, and August for maximum and minimal temperature influences upon yield in relation to precipitation, as well as decadal trends of historical yield. Analyses determined that Arkansas irrigated fields were most influenced by warmer July temperatures decreasing 47.01 kg/ ha / °C with increasing maximal and decreasing 51.61 kg / ha / °C as minimum temperatures increased. Non-irrigated yields decreased 56.92 kg / ha / °C and 71.94 kg / ha / °C as July maximum and minimum temperatures increase. Historically, irrigated cotton yields have averaged near 25% greater than non-irrigated fields since the early 1980's. Effects of precipitation were limited compared to the influence of temperature. Only non-irrigated fields had significant yield increases for the month of August, increasing by 19.76 kg / ha / cm of additional rainfall. Results indicate that irrigated and non-irrigated yields historically parallel, therefore we suggest that overall yield gains are the result of better yielding cultivars and management practices. The results also suggest that modern cotton cultivars appear to be just as intolerant to increasing temperatures and mild drought stress as cultivars planted in the past.

Introduction

Cotton (*Gossypium hirsutum* L.) producers often establish long-term goals for their fields by determining if irrigation is a profitable expenditure given its installation and startup costs. One of the principal factors taken into consideration is whether the climatic conditions of their region necessitate irrigation. Temperature and precipitation are the two largest climatic factors influencing these decisions. In Arkansas, the Mississippi River Delta region is composed of a shallow water table, deep alluvial soils, flat topography, and large annual rainfall that can exceed 1.5 m a year (Bengtson et al., 1995; Snipes and Nichols, 2005). This contributes to the Mississippi River Valley as one of the most productive agricultural regions in the United States. Despite this large precipitation resource, Arkansas is the most heavily irrigated state in the surrounding region (Maupin and Barber, 2005). A majority of the irrigation is groundwater derived from the Mississippi River Valley Alluvial Aquifer which is in danger of depletion due to increasing withdrawals. Currently, only 43% of the aquifer withdrawal demand is sustainable, with more than 96% of water extracted used for agriculture (Sullivan and Delp, 2007).

Many irrigation strategies rely upon daily maximum temperatures as an influencing factor for their applications (Jackson et al., 1981; Usman et al., 2010; O'Shaughnessy and Evett, 2010). Irrigation's primary purpose is to relieve water stress effects that can reduce the number of bolls the plant can accommodate (Guinn, 1976; Pettigrew, 2004). However, irrigation can also minimize the effects of high temperature. Increasing transpiration rates reduce the temperature of the plant relative to the air temperature (Burke and Upchurch, 1989; Wanjura and Mahan, 1994; Mahan et al., 1995). The need for cooler leaves relative to the ambient air temperature is due to cotton's relative sensitivity to high temperature stress (Singh et al., 2007; Snider et al., 2009; Gür

et al., 2010). Under greenhouse conditions, cotton develops optimally in air temperatures of 20 to 30 °C (Reddy et al., 1991, 1992b). Field observations indicate an enzymatic optimal temperature range between 23.5 and 32 °C (Burke et al., 1988). Temperatures in the Mississippi River Valley routinely exceed these temperatures in the summer months, with maximal temperatures occasionally exceeding 38 °C (Boykin et al., 1995).

These increased temperatures occur during the most sensitive of cotton development, reproduction (Reddy et al., 1992a; Snider et al., 2011b). The developing ovary and boll is highly susceptible to high temperatures within the first 14 days after anthesis with homeostatic disruptions leading to the developing boll's abortion (Stewart 1986). Consequently, warmer temperatures have a negative impact of yield. Pettigrew (2008) noted that cotton exposed to a modest increase of 0.5 to 0.8 °C over the duration of the growing season negatively impacted yield by as much as 10%. Work by Katani et al. (2005) noted that cultivars that were bred for greater thermo-tolerance failed to increase pollen germination rates or develop longer pollen tubes. This suggests that pollen tolerance may be another limiting factor in heat related reproductive success. Snider et al. (2011a) observed that high temperature decreased growth rates of pollen tubes and subsequently decreased rates of reproductive success. Higher temperatures during reproductive development has a significant negative impact upon future yields (ur Rahman et al., 2004; Pettigrew, 2008; Zinn et al., 2010).

Planting earlier in the spring season can be a significant factor for yield response. Later planting dates producing both less yield and lower micronaire values than cotton planted at earlier dates (Bilbro and Ray, 1973; Wrather et al., 2008). For effective stands, proper planting is heavily dependent upon soil temperature. Soil temperatures must be 15.5 °C (60 ºF) for at least 10 days in the upper portion of the soil profile being the minimum accepted value for planting

(Gipson, 1986). At lower temperatures, rates of radicle tip death, and root cortex disintegration increase considerably (Christiansen, 1968), leading to the condition often referred to as "nub root". Work from Wanjura et al. (1967) indicated that if soil temperatures decreased from 20 to 12 °C, then the number of hours needed for seedling emergence increased from 100 to approximately 425 hours. McMichael and Burke (McMichael and Burke, 1994) demonstrated that increasing soil temperatures between 20 °C and 32 °C was ideal for root growth development and outside of these temperatures root length decreased to near zero. This coincides with previous work that in more northern sites of cotton production, including the southern Missouri and northern Arkansas counties, that temperatures in the upper soil must be at least 20 °C for several days to account for sporadic cooler weather (McQuigg, James et al., 1965). For areas of central Arkansas, the University of Arkansas Lon Mann Research Station has on average (1985-2014) a cotton planting date about the 20th week of the year (Data not shown), and identified as optimal time for planting within the region (Ballard and Simpson, 1925).

Cotton requires a certain period of heat to produce a productive crop. The amount of accumulated heat units (HU) and developmental growth rates are closely associated to productivity. In the Mid-south regions of the United States these HU's are calculated simply by the daily mean temperature minus the lower growth temperature threshold of the crop, and commonly referred to as a DD_{60} (Arnold, 1960; Baskerville and Emin, 2015):

$$\left(\frac{Maximum\ Temperature + Minimum\ Temperature}{2}\right) - 15.5\ °C$$

Silvertooth et al. (1991) reported that in Arizona climates of Pima cotton (*Gossypium barbadense* L.) production, HU requirements were dependent upon the fruiting habit of the particular cultivar selected. For instance, full-season cotton cultivars needed on average 350 more HU than short season cultivars for fruiting. The HU calculations based upon an adjustment

of 30 and 12.8 °C, as upper and lower temperature limits, which is not what the majority of cotton growers utilize. In Tifton, Georgia which is more representative of the Mississippi Delta region, first squaring averages after 550 HU is accumulated, or 38 days following planting, first flower occurs at 950 HU or about 60 days, and the first open boll corresponds to 2150 HU or a little more than 115 days based upon the DD_{60} (Ritchie et al., 2004).

Understanding that fruiting and final yield are firmly based upon temperature effects and subsequent irrigation strategies, we hypothesized that historical yield response would be correlated with irrigation, temperature, and growing regions of the state. To our knowledge, no previous research exists of this type in the Mississippi Delta. We wanted to investigate the response of yield to temperature during the months of reproductive development and determine when heat stress would negatively impact yield. Identification of the months that heat stress has the greatest impact, it may be possible to optimize irrigation strategies for the region. Moreover, since the vast majority of cultivars planted today are genetically modified (Dill et al., 2008), we sought to examine if these newer cultivars were better tolerant of temperatures and precipitation stress than of decades past.

Materials and Methods

Cotton yields in lbs. / acre was acquired from the United States Department of Agriculture's (USDA) National Agricultural Statistics Service (NASS) for both irrigated and non-irrigated fields within the state of Arkansas and three Arkansas agricultural districts. The Northeast agricultural district was comprised of the following counties: Clay, Craighead, Greene, Independence, Jackson, Lawrence, Mississippi, Poinsett, Randolph, and White. The East Central agricultural district was comprised of the following counties: Arkansas, Crittenden, Cross, Lee,

Lonoke, Monroe, Phillips, Prairie, Saint Francis, and Woodruff. The Southeast agricultural district was comprised of the following counties: Ashley, Chicot, Desha, Drew, Jefferson, and Lincoln. Yields were converted into conventional SI units of kg / ha, and all data analyses performed on the transformed datasets. Cotton yields were available from 1980 to 2009 for the East Central and Southeast Districts, from 1980 to 2012 for the Northeast district, and 1980 to 2014 for the state of Arkansas.

Daily maximum and minimum temperatures and precipitation (cm) totals were collected from as centrally located a station as possible within each district. Data was collected from 1980 until 2014 from weather station daily reports collected by the National Oceanic and Atmospheric Administration. Specifically, the Northeast district weather data came from the Jonesboro 2 (GHCND: USC0003373) weather station. Missing datasets were collected from the adjacent weather station located at the Jonesboro Municipal Airport (GHCND: USW00003953). East Central district weather data was acquired from the Marianna, AR weather station (GHCND: USC00034638), missing values were gathered from the Arkabutla Dam, MS weather station (GHCND: USC00220237). The Southeast district weather data was acquired from the Rohwer 2 NNE weather station (GHCND: USC00036253), missing values were input from the Dumas, AR weather station (GHCND: USC00032148).

Data Setup and Statistical Analysis

Taking the average days of development as a guide (Ritchie et al., 2004), and that cotton is sown on average in the 20th week in Marianna, AR averages for anthesis were calculated. The average date of squaring occurred on the 25th week of the year, and first flowering on the 28th week of the year. This places reproductive development firmly within the summer months of June, July, and August, and limited this study to those months.

Due to yearly variations in yield, decadal trends analyses was performed to investigate increasingly modern cultivars and response to temperature and precipitation. Certain assumptions that producers would have provided proper management such as pesticides, nematicides, and herbicides during the growing season and not included as confounding factors. We also predicted that growers would plant the optimal cultivar for the region for the time; making the maximal yields dependent upon the irrigation and temperature of that year. Factors analyzed included the district, irrigation type, period, month, maximum and minimum temperatures, and precipitation. All regression analyses were performed in JMP 12.1 (SAS Institute, Cary, NC) at $\alpha = 0.05$ level using the above cofactors.

Results and Discussion

Arkansas irrigation practices have changed significantly since 1980. Figure 3-1 indicates the hectares of irrigated and non-irrigated fields to the total hectares planted from 1980 to 2014. It shows that irrigated fields have become the overwhelming dominant field type in Arkansas. Irrigated hectares surpassed non-irrigated hectares in the mid-1990's and continue to dominate non-irrigated trends. In the past 5 years, total planted hectares have been declining. Flander et al. (2014) attributed this decline to markets shifting from cotton to other crops in the region as prices for cotton decreased.

The disparity between irrigated and non-irrigated yields has remained steady over the years while irrigation has increased in the region. The state and agricultural district breakdown for irrigated and non-irrigated yields from 1980-2010 (Figure 3-2) indicate that irrigated and non-irrigated fields rise and fall in tandem. There is a distinct rise, fall, and stagnation of yields from both irrigated and non-irrigated fields across all districts that indicate a broad environmental effect. This is especially true when examining the cubic splines (lambda of 0.05)

of the state.

State yield data illustrates that the greatest disparity between irrigation types occurred during the 1980-1989 period, as more fields began to incorporate irrigation. Non-irrigated fields also experienced increased yield, though not to the same extent as irrigated fields. On average, the disparity between irrigated and non-irrigated fields has remained similar since the early 1980's (Table 3-1). This suggests that the region received a fair amount of rainfall that maintained a consistent 21-25% average difference between the two irrigation strategies. A multifactor analysis of precipitation to yield for each district, month, and period indicate only one significant relationship in the Southeast agricultural district of non-irrigated fields from 1980-1989 during August ($p = .0460$). Only a limited number of precipitation effects were significant, with non-irrigated fields experiencing greater interaction with temperatures. However, the East Central agricultural district did not have any significant effect associated with precipitation by yield, indicating that there is enough rainfall in the region (Table 3-2). Moreover, across the state the largest influence of precipitation is in the month of August, with significant interactions occurring for irrigated cotton in 1980-1989 ($p = .0175$), and non-irrigated cotton in 1980-1989 ($p = .0118$) and for the duration of analyses, 1980-2014 ($p = .0445$). Whereas for the districts of the Northeast and Southeast the majority of the interactions occur in July.

Modern cultivars appear to have become more sensitive to precipitation effects since 2000 (Table 3-2). This sensitivity to minor rainfall events hints at the constriction of genetic robustness in the cotton plant at the expense of faster flowering and greater yield potentials. Typically, cotton's innate tolerance to drought conditions are due to deeper taproots, greater lateral root branching, and higher root-to-shoot ratios (Cook, 1985; Pace et al., 1999). However, frequent watering cycles often increase yields (Radin et al., 1992; Hunsaker et al., 1998). Overall

yields of non-irrigated fields are increasing and could be linked to better management practices such as pesticide (Malo et al., 2000; Oerke, 2006) and weed management changes (Werth et al., 2013).

There was an extended period of yield stagnation and slow decline for all agricultural districts from the mid-1980's until near 2000 (Figure 3-2). This extended period was prevalent even as newer cultivars were introduced (Malo et al., 2000; Meredith Jr., 2002). This stagnation and decline was not an isolated incident in Arkansas as was summarized by Paterson et al. (2004) who explained that the entire United States cotton growing region was affected by declining yields through this time. The accredited reasoning was that cotton had achieved a genetic bottleneck (Iqbal et al., 2001, 2005), and therefore cultivars at the time had achieved their maximum genetic potential for the environment.

Significant Arkansas state and agricultural district yields of all decadal trends were summarized in Table 3-3. In total, the state observed an insignificant cotton yield decline of 3.5 kg/ha/yr for irrigated ($p = .1232$), but a statistically significant decline of near 7.1 kg/ha/yr for non-irrigated fields ($p = .0237$). This is in partial accordance to Paterson et al. (2004), since there was no distinction in their study between irrigated or non-irrigated yields. However, we do believe that reduced genetic variability had a significant role in the yield stagnation at the time.

Yields increased after the introduction of genetically modified cotton in the early 2000's (Figure 3-2). Nevertheless, by the end of data reporting irrigation fields in the Southeast district experienced either yield stagnation or decline. Non-irrigated yields in the Southeast ceased yield growth towards the end of 2000's. Irrigated fields in the Northeast district experienced the greatest yield increase of 18.9 kg/ha/yr ($p = <.0001$, Table 3-3), while the Northeast district non-irrigated fields were statistically insignificant ($p = .4441$) by the end of data collection in 2012.

The East Central district after the year 2000 experienced the most rapid increase in yield non-irrigated fields; increasing by 21.5 kg/ha/yr ($p = 0.0025$). Irrigated fields in the East Central district increased at a significant 14.9 kg/ha/yr ($p = .0341$) until data collection cessation in 2009. The state experienced a significant increase in yields for both irrigated ($p = <.0001$) at 13.4 kg/ha/yr and for non-irrigated fields ($p = .0137$) at 9.4 kg/ha/yr.

Examinations of more recent yield trends indicate that from 2010 until 2014 state trends experienced a rapid increase in yields of both irrigation strategies (Figure 3-2). Irrigated fields across the state experiences a significant increase of near 29.5 kg/ha/yr ($p = .0070$, Table 3-3) while non-irrigated fields increased at a 56.9 kg/ha/yr ($p = .0018$). Tables 3-4 and 3-5 show the monthly averages for the maximum and minimum temperatures for the state of Arkansas for the agricultural districts and respective periods. There were no weather related trends found in the average monthly maximum or minimum temperatures, suggesting that temperatures in the region have remained consistent over the long term.

Quadratic analyses of yield to temperature indicated significant trends between the different agricultural districts in relation to temperature and month (Figure 3-3). Across all agricultural districts of the state, June minimum temperatures did not possess any correlated effects on yield to irrigation type. The effects of July minimum temperatures were pronounced across the state. July minimum temperatures had decreases of 67.7 kg/ha/°C ($R^2 = 0.23$, $p = .0195$), and the state as a whole had irrigated yield decreases of 51.6 kg/ha/°C ($R^2 = 0.19$, $p = .0335$). Increased minimum temperatures in July on non-irrigated fields had significant decreases in yield for the Northeast district with a decrease of 81.2 kg/ha/°C ($R^2 = 0.39$, $p = .0001$), as well as for the state as a whole with decreases of near 71.9 kg/ha/°C ($R^2 = 0.32$, $p = .0006$). In August, increased minimum temperatures had an unusual response in the Southeast

district with warmer temperatures having a parabolic response of 47.0 kg/ha/°C (R^2 = 0.31, p = .0076) on either side of a 20.9°C threshold. Earlier planting of cotton in the southeast district, and subsequently greater accumulation of heat units could explain this unusual response. Any additional heat units accumulated is transferred into additional flower and boll development leading to greater yields (Bilbro and Ray, 1973).

Maximum temperature quadratic analyses of yield indicated significant trends between the different agricultural districts in relation to temperature and month (Figure 3-3). The influence of maximum temperatures in June had a significant impact on irrigated yields in the Southeast district with a decrease of 49.6 kg/ha/°C (R^2 = 0.17, p = .0298), though no other districts of either irrigation strategy were significantly affected by increased maximal June temperatures. Increased maximum temperatures in July had a significant response with near all agricultural districts. The exception being irrigated fields in the Northeast district, which although a decreasing trend, was not significant. Irrigated fields in the East Central district were highly affected by increased daily temperatures with decreases of 57.1 kg/ha/°C (R^2 = 0.44, p =.0004). Irrigated fields in the Southeast district had decreases of 44.3 kg/ha/°C (R^2 = 0.33, p =.0051). Across the entire state, irrigated yields were significantly affected and decreased 47.6 kg/ha/°C (R^2 = 0.31, p =.0027). Non-irrigated yields appeared more significantly affected by increased maximal temperatures. The East Central district experienced significant decreases of 66.5 kg/ha/°C (R^2 = 0.55, p = <.0001), while the Northeast district decreased yields by 44.0 kg/ha/°C (R^2 = 0.21, p =.0320). Fields in the Southeast district had highly significant decreases of 48.3 kg/ha/°C (R^2 = 0.46, p =.0002). Overall, non-irrigated yields decreased significantly by 56.9 kg/ha/°C (R^2 = 0.39, p =.0004) of July maximum temperatures. August maximum temperatures experienced fewer significant correlations to yield (Figure 3) compared with July,

where no significant correlations for irrigated fields were identified. Non-irrigated fields were significantly decreased in the East Central district with decreases of 35.0 kg/ha/°C (R^2 = 0.23, p =.0274).

Since decadal trends were identified within each agricultural district (Figure 3-2), we investigated the relationship between yield, temperature, and month as categorized according to decades. Figure 3-4 indicates the effect temperature had on yield by month for decadal periods of the state. In the 1980's, irrigated and non-irrigated cotton were not affected by either maximum or minimal temperatures in June. In July irrigated fields showed decreased yields of 38.3 kg/ha/°C of maximum temperatures (R^2 =.65, p =.0241), and similarly non-irrigated fields also decreased by 39.0 kg/ha/°C (R^2 = 0.73, p =.0096). Although there was high correlation of July minimum temperatures to yield it was not significant (R^2 =.51, p =.0804). Higher temperatures in August did not have any significant effect on yield for both irrigated and non-irrigated fields of the 1980's.

During the 1990's, there was a stagnation or decline in cotton yield (Figure 3-2) for all agricultural districts in Arkansas. June temperatures did not have any significant impact on yield for either irrigated or non-irrigated cotton. There was greater correlation of maximal temperatures in July than in June, though irrigated cotton was not significantly influenced by high temperatures during the 1990's (R^2 =.52, p =.0781). Non-irrigated yield, though, was significantly influenced by greater maximum temperatures decreasing 69.7 kg^{-1} ha for each 1°C increase (R^2 = 0.60, p =.0399). In August, maximum temperatures of irrigated cotton was nearly significant in influencing yield decreasing 38.0 kg/ha/°C (R^2 = 0.57, p =.0522), but increasing minimum temperatures greatly affected yields decreasing by 60.2 kg/ha/°C (R^2 = 0.78, p =.0051). August non-irrigated yields decreased significantly by 59.8 kg/ha/°C (R^2 = 0.77, p

=.0058) of maximum temperature and decreased by 73.2 kg/ha/°C (R^2 = 0.62, p =.0327) of minimum temperature.

From 2000-2014, yields increased across the state for all agricultural districts and irrigation strategies. However, temperatures did not have a significant influence on decreasing yields across the state. The only significant interaction occurred in June for non-irrigated crops, decreasing yields by 35.2 kg/ha/°C (R^2 = 0.53, p =.0108) of minimum temperatures. Table 3-5 summarizes similar trends identified in the agricultural districts.

One unusual finding was that irrigated cotton of the 1980's appeared to benefit from warmer minimum temperatures in August. State analysis identified that from 1980–2014 yields increased by 47.01 kg/ha/°C (R^2 = 0.313, p =.008). This appears to be buoyed by the Southeast district which had significantly increased yields with warmer minimum temperatures from 2000-2009 with increases of 64.5 kg/ha/°C (R^2 = 0.712, p =.0128) and over the long-term with increases of 21.2 kg/ha/°C (R^2 = 0.313, p =.0076). Non-irrigated cotton did not exhibit any increases, nor did any other district. The Southeast district generally plants at an earlier time than the more northern districts and thus could have increased yields from the longer growing season. Boll maturation periods shortens by about 7 days per degree increase in average temperature (Reddy et al., 1997) which can translate into a longer growing season with more nodes and bolls (Reddy et al., 1991). Thus, in the Southeast district with earlier plantings compared to the more northern districts, a longer season coupled with a greater accumulation of heat units may be why higher minimum August temperatures increased yields.

Conclusions

Cotton yields in Arkansas are increased by as much as 25% over non-irrigated fields.

However, because historical trends indicate that irrigated and non-irrigated fields parallel in their historical trends of yield, precipitation effects did not appear to be a limiting factor to yield in the region. This is in part to the region receiving significant rainfall amounts to recharge soil moisture levels early in the season (Bengtson et al., 1995), plus cotton's inherent drought tolerance (Nepomuceno et al., 1998; Basal et al., 2005). Thus, temperature was determined to be the overriding impact factor that causes large swings in yield production from year to year.

Historical evidence indicates that in the state of Arkansas precipitation events in July increase yields significantly more so for non-irrigated fields. This analysis has demonstrated that increased maximal and minimal temperatures in July are the most damaging times for cotton yield across all agricultural districts, and increased precipitation during this time can increase yields. The timing of planting can make a significant impact on when flowering will occur, therefore the time of flowering, heat stress severity, and low precipitations must coincide for the greatest negative impact on yield.

There is also a difference in the decadal trends across the growing region, with a long period of yield stagnation during the 1980's through the 1990's. This unproductivity has been attributed to the genetic "bottlenecking" of cotton genome yield capacity (Iqbal et al., 2001, 2005). Though better yielding cultivars and better field management practices have led to significantly greater yields within the past decade. Nonetheless, these improved cultivars still possess the same sensitivity to heat and precipitation during flowering as older cultivars.

This study has demonstrated that increased temperatures have a greater impact on yield in the Mississippi Delta compared to precipitation, but it also offers some possible insights for future research. Firstly, if irrigation timings focused to the weeks surrounding flowering, final yields will generally increase. Secondly, heavy irrigation in the month of squaring did not

improve yields. Thirdly, planting earlier maturing cultivars, or seeding as early in the season as possible can offset the damage to yield, by altering the timing of the reproductive period when deleterious effects occur. In conclusion, though yields of both irrigated and non-irrigated fields have been increasing, the yearly volatility suggests that current cultivars in the region remain vulnerable to both heat and drought stress.

References

Arnold, C.Y. 1960. Maximum-minimum temperatures as a basis for computing heat units. Proc. Am. Soc. Hortic. Sci. 76: 682–692.

Ballard, W.W., and D.M. Simpson. 1925. Behavior of cotton planted at different dates in weevil-control experiments in Texas and South Carolina. U.S. Dept. of Agriculture, Washington, D.C.

Basal, H., C.W. Smith, P.S. Thaxton, and J.K. Hemphill. 2005. Seedling drought tolerance in upland cotton. Crop Sci. 45: 766–771.

Baskerville, G.L., and P. Emin. 1969. Rapid estimation of heat accumulation from maximum and minimum temperatures. Ecology 50: 514–517.

Bengtson, R.L., C.E. Carter, J.L. Fouss, L.M. Southwick, and G.H. Willis. 1995. Agricultural drainage and water quality in Mississippi Delta. J. Irrig. Drain. Eng. 121: 292–295.

Bilbro, J.D., and L.L. Ray. 1973. Effect of planting date on the yield and fiber properties of three cotton cultivars. Agron. J. 65: 606–609.

Boykin, D.L., R.R. Carle, C.D. Ranney, and R. Shanklin. 1995. Weather data summary for 1964-1993 Stoneville, MS. Tech. Bull. 201 Mississippi Agric. For. Exp. Station. Mississippi State.

Burke, J.J., J.R. Mahan, and J.L. Hatfield. 1988. Crop-specific thermal kinetic windows in relation to wheat and cotton biomass production. Agron. J. 80: 553–556.

Burke, J.J., and D.R. Upchurch. 1989. Leaf temperature and transpirational control in cotton. Environ. Exp. Bot. 29: 487–492.

Christiansen, M.N. 1968. Induction and prevention of chilling injury to radicle tips of imbibing cottonseed. Plant Physiol. 43: 743–746.

Cook, C.G. 1985. Identifying root traits among MAR and non-MAR cotton, *Gossypium hirsutum* L. cultivars that relate to performance under limited moisture conditions. (Doctoral dissertation, Texas A&M University).

Dill, G.M., C.A. Cajacob, and S.R. Padgette. 2008. Glyphosate-resistant crops: adoption, use and future considerations. Pest Manag. Sci. 64: 326–331.

Flanders, B.A., B. Coats, and C. Dunn. 2014. Change in Arkansas cotton acreage during 2002-2010. J. ASFMRA: 14–24.

Gipson, J.R. 1986. Temperature effects on growth, development, and fiber properties. p. 47–56. *In* Mauney, J.R. and McD. Stewart, J. (eds.), Cotton Physiology. The Cotton Foundation.

Guinn, G. 1976. Water deficit and ethylene evolution by young cotton bolls. Plant Physiol. 57: 403–5.

Gür, A., U. Demirel, M. Özden, A. Kahraman, O. Opur, and O. Çopur. 2010. Diurnal gradual heat stress affects antioxidant enzymes, proline accumulation and some physiological components in cotton (*Gossypium hirsutum* L.). African J. Biotechnol. 9: 1008–1015.

Hake, K., and J. Silvertooth. 1990. High temperature effects on cotton. Cott. Physiol. Today 1: 1–4.

Hunsaker, D., A.. Clemmens, and D.. Fangmeier. 1998. Cotton response to high frequency surface irrigation. Agric. Water Manag. 37: 55–74.

Iqbal, M.J., M.Z. Iqbal, R.S.A. Khan, and K. Hayat. 2005. Comparison of obsolete and modern varieties in view to stagnancy in yield of cotton (*G. hirsutum* L.). Asian J. Plant Sci. 4: 374–378.

Iqbal, M.J., O.U.K. Reddy, K.M. El-Zik, and a. E. Pepper. 2001. A genetic bottleneck in the 'evolution under domestication' of upland cotton *Gossypium hirsutum* L. examined using DNA fingerprinting. TAG Theor. Appl. Genet. 103: 547–554.

Jackson, R.D., S.B. Idso, R.J. Reginato, and P.J. Pinter. 1981. Canopy temperature as a crop water stress indicator. Water Resour. Res. 17: 1133–1138.

Kakani, V.G., K.R. Reddy, S. Koti, T.P. Wallace, P.V. V. Prasad, V.R. Reddy, and D. Zhao. 2005. Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Ann. Bot. 96: 59–67.

Mahan, J.R., B.L. McMichael, and D.F. Wanjura. 1995. Methods for reducing the adverse effects of temperature stress on plants: A review. Environ. Exp. Bot. 35: 251–258.

Malo, J.P., L.D. Parsch, and N.P. Tugwell. 2000. Arkansas cotton: Stagnant yield and increased risk? Spec. Rep. Arkansas Agric. Exp. Stn. 198: 275–278.

Maupin, M.A., and N.L. Barber. 2000. Estimated withdrawals from principal aquifers in the United States. US Geological Survey Circular, 1279.

McMichael, B.L., and J.J. Burke. 1994. Metabolic activity of cotton roots in response to temperature. Environ. Exp. Bot. 34: 201–206.

McQuigg, James, D., O.H. Calvert, and W.L. Decker. 1965. Using weather information to cut the cost of getting a good stand of cotton in southeast Missouri. Univesity of Missouri, Agri. Exp. Stat. Circular, 835.

Meredith Jr., W.R. 2002. Factors that contribute to the lack of genetic progress. p. 8–12. *In*

Proceedings of the Beltwide Conference. Atlanta, GA, NCC, Memphis, TN.

Nepomuceno, A.L., D.M. Oosterhuis, and J.M. Stewart. 1998. Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol. Environ. Exp. Bot. 40: 29–41.

O'Shaughnessy, S.A., and S.R. Evett. 2010. Canopy temperature based system effectively schedules and controls center pivot irrigation of cotton. Agric. Water Manag. 97: 1310–1316.

Oerke, E.C. 2006. Crop losses to pests. J. Agric. Sci. 144: 31–43.

Pace, P.F., H.T. Cralle, S.H.M. El-halawany, J.T. Cothren, and S.A. Senseman. 1999. Drought-induced changes in shoot and root growth of young cotton plants. Journal Cott. Sci. 187: 183–187.

Paterson, A.H., R.K. Boman, S.M. Brown, P.W. Chee, J.R. Gannaway, A.R. Gingle, O.L. May, and C.W. Smith. 2004. Reducing the genetic vulnerability of cotton. Crop Sci. 44: 1900–1901.

Pettigrew, W.T. 2004. Moisture deficit effects on cotton lint yield, yield components, and boll distribution. Agron. J. 96: 377–383.

Pettigrew, W.T. 2008. The effect of higher temperatures on cotton lint yield production and fiber quality. Crop Sci. 48: 278–285.

Radin, J.W., L.L. Reaves, J.R. Mauney, and O.F. French. 1992. Yield enhancement in cotton by frequent irrigations during fruiting. Agron. J. 84: 551–557.

Reddy, R.K., H.F. Hodges, and J.M. Mckinion. 1997. Crop modeling and applications: A cotton example. Adv. Agron. 59: 225–290.

Reddy, K.R., H.F. Hodges, J.M. McKinion, and G.W. Wall. 1991. Temperature effects on Pima cotton growth and development. Agron. J. 84: 237–243.

Reddy, K.R., H.F. Hodges, and V.R. Reddy. 1992a. Temperature effects on cotton fruit retention. Agron. J. 84: 26–30.

Reddy, K.R., V.R. Reddy, and H.F. Hodges. 1992b. Temperature effects on early season cotton growth and development. Agron. J. 84: 229–237.

Ritchie, G.L., C.W. Bednarz, P.H. Jost, and S.M. Brown. 2004. Growth and development of the cotton plant. The University of Georgia College of Agricultural and Environmental Sciences, Athens, GA.

Silvertooth, J.C., P.W. Brown, and J.E. Malcuit. 1991. Basic cotton crop development patterns.

Cott. A Coll. Agric. Rep.: 43–49.

Singh, R.P., P.V.V. Prasad, K. Sunita, S.N.N. Giri, K.R. Reddy, and Donald L. Sparks. 2007. Influence of high temperature and breeding for heat tolerance in cotton: a review. Adv. Agron. 93: 313–385.

Snider, J.L., D.M. Oosterhuis, and E.M. Kawakami. 2011a. Diurnal pollen tube growth rate is slowed by high temperature in field-grown *Gossypium hirsutum* pistils. J. Plant Physiol. 168: 441–448.

Snider, J.L., D.M. Oosterhuis, D.A. Loka, and E.M. Kawakami. 2011b. High temperature limits in vivo pollen tube growth rates by altering diurnal carbohydrate balance in field-grown *Gossypium hirsutum* pistils. J. Plant Physiol. 168: 1168–75.

Snider, J.L., D.M. Oosterhuis, B.W. Skulman, and E.M. Kawakami. 2009. Heat stress-induced limitations to reproductive success in *Gossypium hirsutum.* Physiol. Plant. 137: 125–138.

Snipes, C.E., and S.P. Nichols. 2005. Current Agricultural Practices of the Mississippi Delta. Bulletin 1143.

Sullivan, M.E., and W.M. Delp. 2007. How a systems approach to irrigation promotes sustainable water use. p. 145–159. *In* NBAC Report 24: Water Sustainability in Agriculture.

ur Rahman, H., S.A. Malik, and M. Saleem. 2004. Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane thermostability. F. Crop. Res. 85: 149–158.

Usman, M., M. Arshad, A. Ahmad, N. Ahmad, M. Zia-Ul-Haq, A. Wajid, T. Khaliq, W. Naseem, Z. Hasnain, H. Ali, and S. Ahmad. 2010. Lower and upper baselines for crop water stress index and yield of *Gossypium hirsutum* L. Under variable irrigation regimes in irrigated semiarid environment. Pakistan J. Bot. 42: 2541–2550.

Wanjura, D.F., and G.L. Barker. 1985. Cotton lint yield accumulation rate and quality development. Field. Crop. Res. 10: 205–218.

Wanjura, D.F., E.B. Hudspeth, and J.D. Bilbro. 1967. Temperature-emergence relations of cottonseed under natural diurnal fluctuation. Agron. J. 59: 217–219.

Wanjura, D.F., and J.R. Mahan. 1994. Thermal environment of cotton irrigated using canopy temperature. Irrig. Sci. 14: 199–205.

Wendel, J.F., C.L. Brubaker, and A.E. Percival. 1992. Genetic diversity in *Gossypium hirsutum* and the origin of upland cotton. Am. J. Bot. 79: 1291–1310.

Werth, J., L. Boucher, D. Thornby, S. Walker, and G. Charles. 2013. Changes in weed species since the introduction of glyphosate-resistant cotton. Crop Pasture Sci. 64: 791–798.

Wrather, J.A., B.J. Phipps, W.E. Stevens, A.S. Phillips, and E.D. Vories. 2008. Cotton planting date and plant population effects on yield and fiber quality in the Mississippi Delta. J. Cott. Sci. 7: 1–7.

Zinn, K.E., M. Tunc-Ozdemir, and J.F. Harper. 2010. Temperature stress and plant sexual reproduction: Uncovering the weakest links. J. Exp. Bot. 61: 1959–1968.

Tables

Table 3-1: The percent yield difference between irrigated and non-irrigated crops for each period and district investigated.

Time Period	East Central	Northeast	Southeast	State
1980-1989	21.4%	24.4%	21.0%	25.9%
1990-1999	23.1%	22.4%	27.1%	25.4%
2000-2009	20.5%	-	24.8%	-
2000-2012	-	22.9%	-	-
2000-2014	-	-	-	22.4%
1980-Current	21.7%	23.2%	24.3%	24.6%

Table 3-2: Agricultural, irrigation type, and period that were significant according to precipitation at or below the 0.05 level.

Agricultural District	Irrigation	Period	Month	Slope Coefficient (kg/ha/cm)	R^2	P-Value
State	Irrigated	1980-1989	August	-7.14	.685	.0175
	Non-Irrigated	1980-1989	August	11.48	.719	.0118
		1980-2014	August	19.76	.177	.0445
East Central	Irrigated	NS				
	Non-Irrigated	NS				
Northeast	Irrigated	1980-2012	July	14.47	.305	.0051
	Non-Irrigated	2000-2012	July	13.67	.561	.0247
		1980-2012	June	9.21	.232	.0220
			July	15.43	.434	.0003
Southeast	Non-Irrigated	1980-1989	August	21.01	.518	.0293
		2000-2009	July	12.70	.575	.0500

Table 3-3: Agricultural district, irrigation type, period, and temperature combinations that were significant at or below the 0.05 level.

Agricultural District	Irrigation	Time Period	Month	Temperature	Slope Coefficient (kg / ha/ ºC)	R^2	P-Value
State	Irrigated	1980-1989	July	Maximum	-38.3	.655	.0241
		1990-1999	August	Maximum	-60.25	.779	.0051
				Minimum	-60.17	.773	.0008
		1980-2014	July	Maximum	-47.64	.309	.0027
				Minimum	-51.61	.191	.0335
			August	Minimum	47.01	.313	.0080
	Non-Irrigated	1980-1989	July	Maximum	-39.00	.735	.0096
		1990-1999	August	Maximum	-59.78	.770	.0058
				Minimum	-73.21	.623	.0327
		2000-2014	June	Minimum	-35.22	.530	.0108
		1980-2014	July	Maximum	-56.92	.388	.0004
				Minimum	-71.94	.322	.0020
Northeast	Irrigated	1980-1989	August	Maximum	-50.43	.794	.0040
		1990-1999	August	Minimum	-65.69	.620	.0339
		1980-2012	July	Minimum	-67.71	.231	.0195
	Non-Irrigated	1980-1989	August	Maximum	-34.97	.155	.0235
		1990-1999	August	Maximum	-87.68	.691	.0165
		1980-2012	July	Maximum	-44.03	.205	.0320
				Minimum	-81.17	.391	.0006
East Central	Irrigated	1980-1989	July	Minimum	-101.1	.560	.0128
		1990-1999	August	Maximum	-64.3	.618	.0345
		1980-2009	July	Maximum	-56.51	.436	.0004
				Minimum	-51.42	.221	.0345
	Non-Irrigated	1980-1989	July	Maximum	-42.5	.653	.0247
				Minimum	-85.4	.706	.0137
		1990-1999	August	Maximum	-86.8	.768	.0060
		2000-2009	August	Maximum	-37.2	.417	.0436
		1980-2009	July	Maximum	-66.87	.549	.0001
				Minimum	-54.4	.240	.0245
			August	Maximum	-49.5	.308	.0070
Southeast	Irrigated	2000-2009	August	Minimum	64.5	.712	.0128
		1980-2009	August	Minimum	21.16	.313	.0076
	Non-Irrigated	1980-1989	July	Maximum	-27.20	.623	.0330
		1990-1999	August	Maximum	-61.35	.778	.0052
				Minimum	-64.75	.601	.0402
		2000-2009	July	Maximum	-52.21	.369	.0004
		1980-2009	July	Maximum	-48.34	.463	.0002

Table 3-4: Decadal maximum temperatures for each month using the daily maximum temperatures for each agricultural district in Arkansas spanning from 1980 - 2014.

Time Period	Agricultural District											
	Arkansas State			Northeast			East Central			Southeast		
	June	July	August	June	July	August	June	July	August	June	July	August
1980-2014	31.6	33.2	33.0	31.3	33.4	32.6	31.1	32.8	32.4	31.6	33.2	33.1
1980-1989	31.6	33.3	32.6	31.3	33.6	32.7	31.6	33.5	32.6	31.9	33.4	33.3
1990-1999	31.3	33.4	32.7	30.8	32.8	32.1	30.9	32.9	32.1	31.3	33.4	32.7
2000-2009	31.7	33.2	33.7	31.1	32.5	32.6	30.8	31.8	32.5	31.0	32.4	33.1
2010-2014	32.4	32.5	33.1	32.0	32.5	33.1	32.6	32.5	33.1	30.9	31.8	33.0

Table 3-5: Decadal minimum temperatures for each month using the daily minimum temperatures for each agricultural district in Arkansas spanning from 1980 - 2014.

Time Period	Agricultural District											
	Arkansas State			Northeast			East Central			Southeast		
	June	July	August	June	July	August	June	July	August	June	July	August
1980-2014	20.7	22.0	21.2	19.4	21.7	20.7	20.1	21.7	20.9	20.5	22.0	20.9
1980-1989	20.2	22.1	21.2	19.5	22.2	21.2	20.0	21.7	20.8	20.3	21.8	20.9
1990-1999	20.9	22.6	20.8	19.7	21.7	20.2	20.1	21.7	20.0	20.5	22.1	20.2
2000-2009	20.5	21.5	21.1	18.8	20.8	20.7	20.2	21.8	21.8	20.8	22.0	21.7
2010-2014	21.3	22.0	21.8	17.9	21.3	22.5	22.9	24.0	23.4	20.6	21.6	23.3

Figures

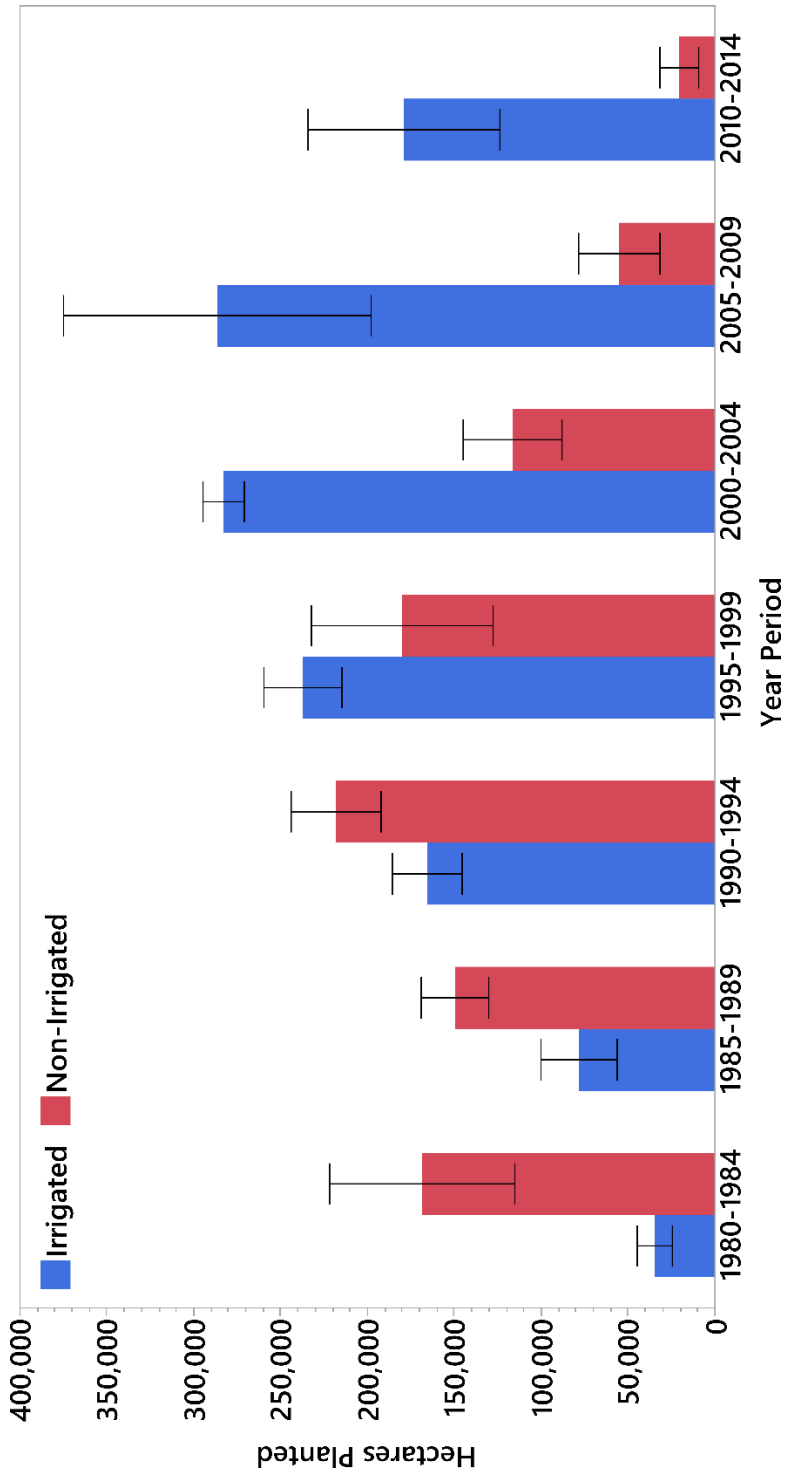

Figure 3-1: Irrigated and non-irrigated hectares of cotton planted in the state of Arkansas acquired from NASS and grouped into 5-year periods from 1980 to 2014. Error bars indicate the confidence interval at $\alpha = 0.05$.

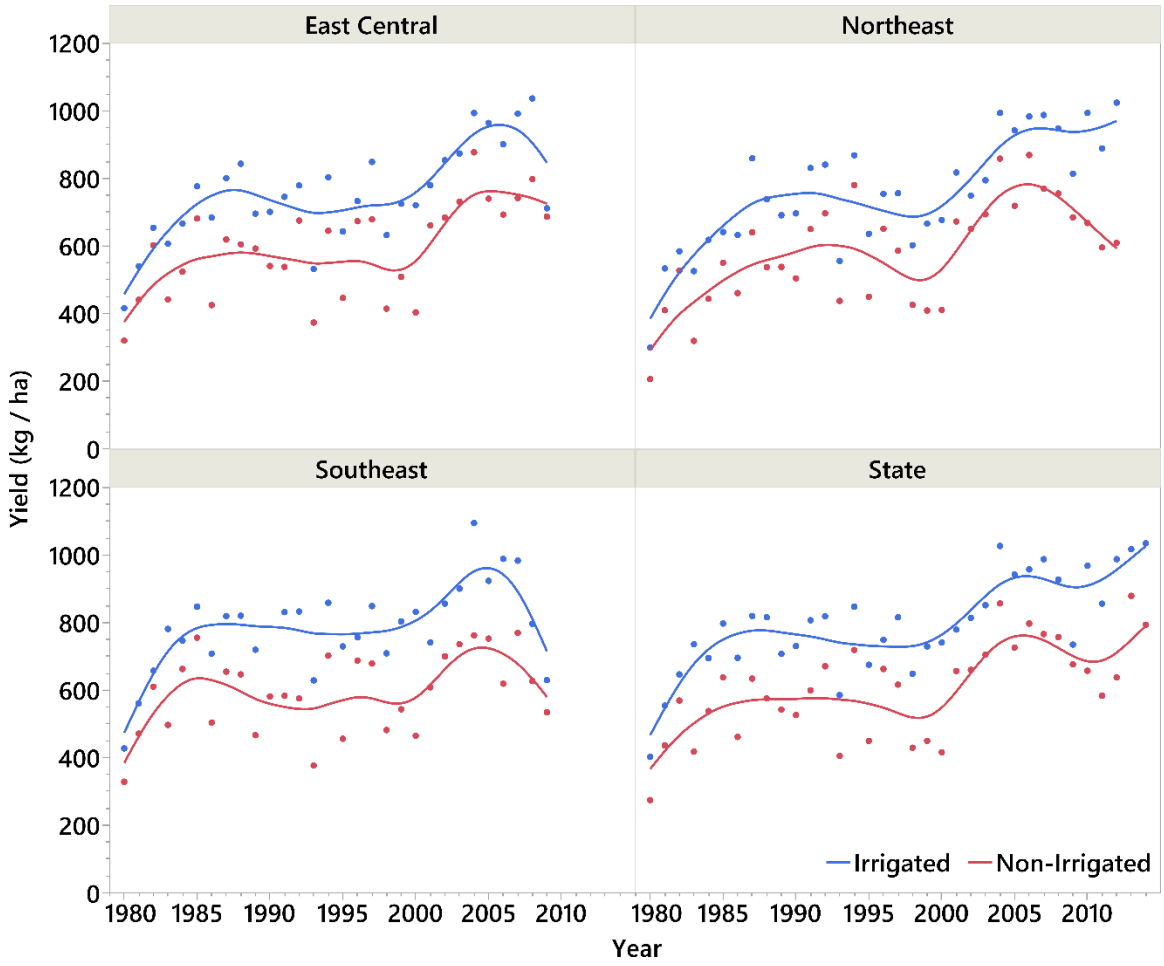

Figure 3-2: Irrigated and non-irrigated hectares of cotton planted in the state of Arkansas from 1980 to 2014. The smoothed line indicates that smoothed cubic spline at a lambda of 0.05. Error bars indicate the confidence interval at $\alpha = 0.05$.

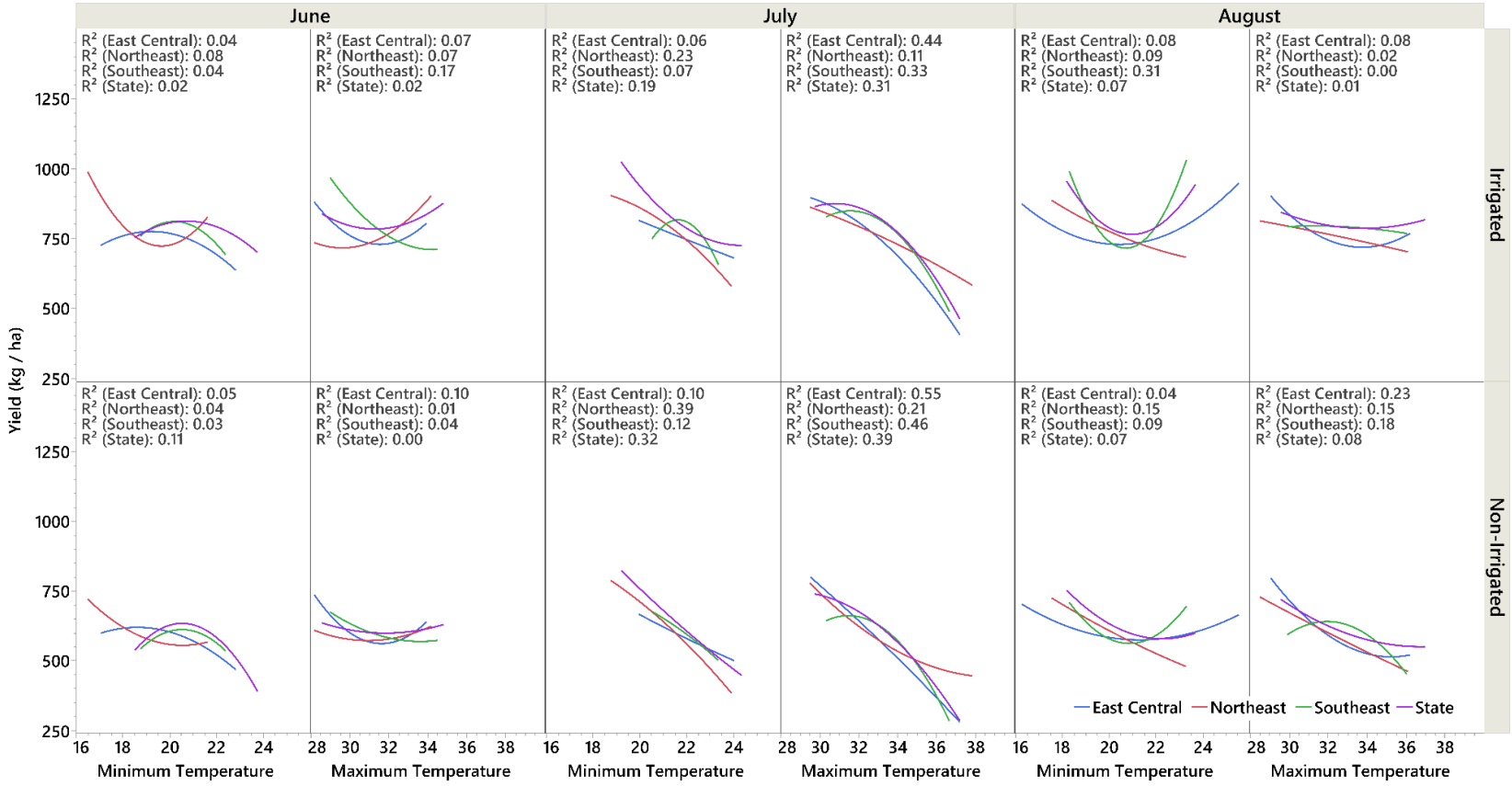

Figure 3-3: Quadratic trend line and associated R^2 value of the effect of temperature on both irrigated and non-irrigated fields of each agricultural district and the state of Arkansas for each month of reproductive development.

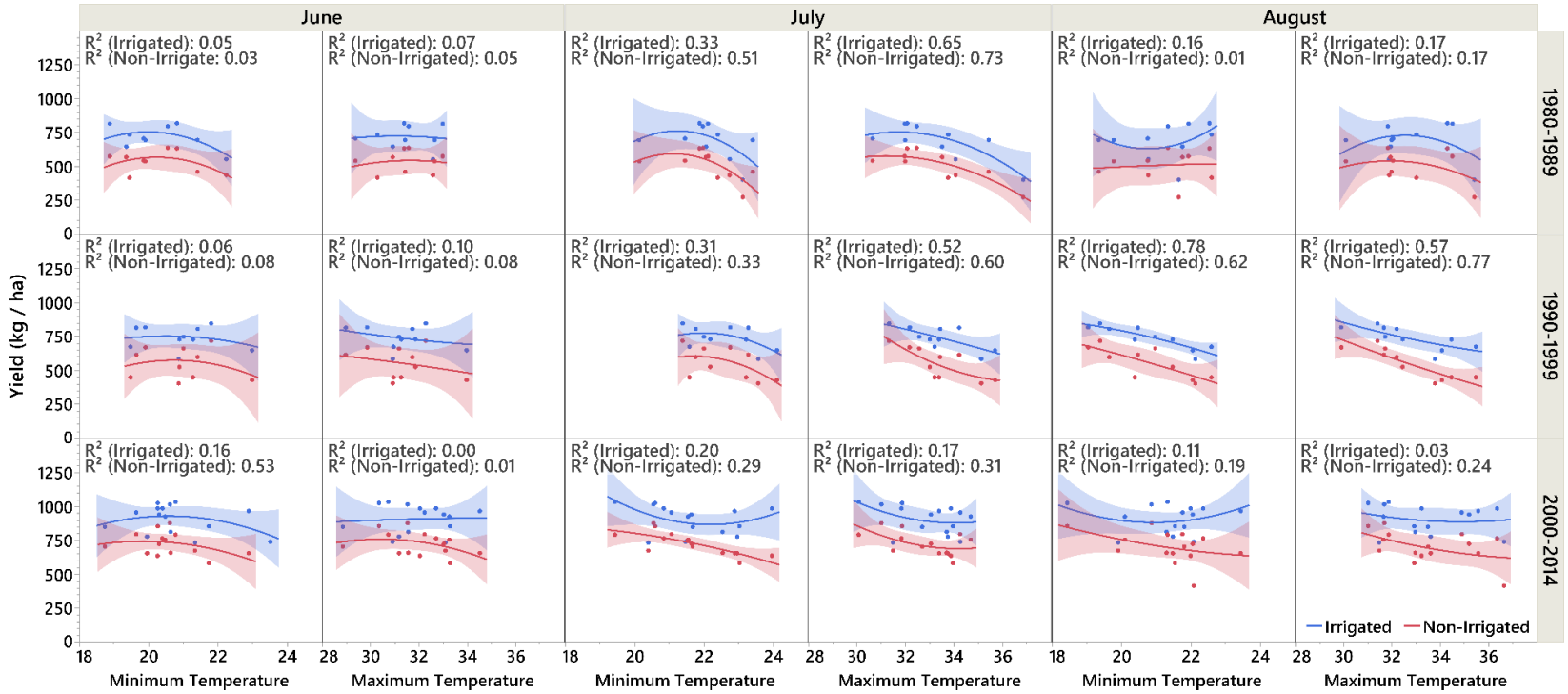

Figure 3-4: Quadratic analyses of decadal trends of temperature on irrigated and non-irrigated fields in the state of Arkansas by each month of reproductive development. Shaded areas represent the confidence region of the fitted line at 0.05.

CHAPTER IV

Effects of High Temperature on Carbohydrate Concentrations Within the Developing Ovary and Subtending Leaf of Cotton Before, During, and After Anthesis

Abstract

High temperatures affect the proper balance of sugar dynamics in cotton (*Gossypium hirsutum* L.). However, the affects that these changes have in the days surrounding anthesis has not been extensively investigated in cotton. In this study, the impact high temperatures have on soluble carbohydrate concentrations within the subtending leaf and ovaries were monitored within an environmental chamber, the day prior to anthesis (DBA), the DOA (DOA), and one day after anthesis (DAA). High temperature significantly affected concentrations of each carbohydrate, with the greatest fluctuations occurring in starch and sucrose. Subtending leaves under heat stress exhibited 32.1% lower starch concentrations DAA compared to the control leaves. Heat stressed ovary tissues had 18.4% and 32.3% lower starch concentrations DBA and DAA, respectively. Sucrose levels in heat-stressed leaves were 14.8% greater at DAA. Ovary concentrations were 23.7%, 13.6%, 26.3% greater during heat stress for DBA, DOA, and DAA respectively. This suggests that heat significantly affects carbohydrate pools within reproductive tissues, which may be a significant factor of heat related reproductive shedding.

Introduction

The period of anthesis is of the highest importance for cotton, as the commercial value lies in the reproductive fruit rather than in the vegetative component. Modern breeding has produced cultivars capable of greater yields than from generations past. Still, cotton's

reproductive units are highly susceptible to abscission within the first 14 days after anthesis (Stewart, 1986) from a wide variety of environmental factors including: high fruit load (Guinn, 1976), insect damage (Holman and Oosterhuis 1999), water deficits (Guinn, et al. 1981), high night temperatures (Loka et al., 2011), and heat stress (Reddy et al., 1992; Zhao et al., 2005). The competition for available photoassimilate resources to cotton boll sinks leads to higher abscission rates when too much sink demand is placed on available photoassimilate sources (Lieth et al., 1986).

Cotton has a maximum temperature for leaf area development near 26 ^{o}C (Reddy et al., 1997), with an optimal growth curve residing between 20-30 ^{o}C (Reddy et al., 1991; Bibi et al., 2008). Many regions of the southern of the US regularly experience greater temperatures than these optima, negatively affecting yields. The impacts have been well documented (Ashraf et al., 1994; Faver et al., 1996; Law and Crafts-Brandner, 1999; Snider et al., 2009) demonstrating cotton's acute sensitivity to temperature outside of its optimum. Of the development stages, anthesis is the more sensitive of stages (Reddy, Reddy, et al. 1992). Unfortunately, anthesis occurs during the peak of the summer months when temperatures are at their highest. The temperature affects productivity due to disruption of the flowering and fruiting periods (Wullschleger and Oosterhuis, 1990b), affecting final fiber quality and quantity and greater shed (Azhar et al., 2009).

Carbon is still being fixated in the canopy leaves, and most of which is used for the developing boll comes from the subtending leaf (Ashley, 1972; Wullschleger and Oosterhuis, 1990b). The primary export is sucrose and cotton stores high levels of sucrose in its leaves with no ill effects to the leaf tissue (Goldschmidt and Huber, 1992). Leaf sucrose concentrations during heat stress are dependent upon the species with too much sucrose having an adverse effect

such as in mulberry (*Morus alba* L.) (Chaitanya et al., 2001), or a positive effect as in potato (*Solanum tuberosum* L.) (Lafta and Lorenzen, 1995). Additionally, sucrose export rates from the subtending leaf to the developing embryos is negatively affected as temperatures increase (Aloni et al. 1991).

Zhao et al. (2005) reported that heat-stressed cotton ovaries following anthesis had sucrose concentrations increase, while concentrations of glucose and fructose declined. However, the earliest reproductive measurements in that study was 10 DOA. This leaves a substantial gap of soluble sugar partitioning effects due to heat-stress up to 10 DBA. In other species, heat stress disrupts carbon partitioning in maize (*Zea mays* L.*)* leading to kernels with carbon being directed towards sucrose and hexoses as opposed to starch (Cheikh and Jones, 1995). Starch granules in immature rice (*Oryza sativa* L.) grains were noted to be smaller and the granules of starch depleted faster under increased temperatures (Zakaria et al., 2002). Carbohydrate availability is negatively affected by heat stress in cotton styles reducing overall fertilization efficiency (Snider et al., 2011).

The purpose of this study was to investigate carbohydrate relationships of the subtending leaf and ovary of cotton the DBA, the DOA, and the DAA in response to heat stress. Information of carbohydrate dynamics within this narrow window of development is either limited or absent for many flowering species. However, since carbohydrate assimilate transport and its use in the developing fruit is so critical for its survival, the need to understand the rapid carbon flux influencing anthesis may hold greater insight into higher flower abscission rates during heat stress. Thus, we hypothesize that heat stress effects will significantly alter carbohydrate pools within the developing flower and associated subtending leaf.

Materials and Methods

All studies were conducted at the Altheimer Laboratory, Arkansas Agricultural Research and Extension Center in Fayetteville, AR. Environmental chamber studies were performed October 2012 and repeated in May 2013. Cotton (*Gossypium hirsutum* L.) cultivar ST5288BRF was grown in sixty 4 L pots filled with nutrient free potting soil mix (Conrad Fafard, Agawam, MA, USA). Pots were split evenly between two environmental chambers (Model PGW36, Conviron, Winnipeg, Canada) set for a 14 hour day/night cycle of 32 / 20 ^{o}C, with first light beginning at 6 am, with approximately 300 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) increasing to 500 µmol m^{-2} s^{-1} by hour two of daylight. Nutrition supplementation with quarter-strength Hoagland's solution was supplied daily until first true leaf then with half-strength Hoagland's solution watering every day thereafter. Plants were randomized weekly within both chambers to minimize growth variations. The day prior to measurements, temperatures were increased in one environmental chamber to a 40 / 24 ^{o}C day night with maximal and minimum temperatures comprising 6 hours each per day. All samples collections were made between 1200 to 1400 hrs when temperatures and PAR was at their peak.

Tissues collected for carbohydrate analysis were submerging in liquid nitrogen and placed into an ultra-deep freezer at -80 ^{o}C. Tissue samples were lyophilized the following day (Model 18DX485SA, Botanique Preservation Equipment, Inc., Phoenix, AZ) at -15 ^{o}C, until a stable pressure of at least 50 mTorr was maintained for 72 hours. Samples were promptly removed and ground by hand in a porcelain mortar cooled with liquid nitrogen. Tissues were powdered and transferred into a 2 ml centrifuge tube, labeled, and either used immediately for analysis or capped for storage at -80 ^{o}C.

The soluble sugars of glucose, fructose, and sucrose were extracted by heating 40 mg of dried powdered tissue in 1 ml of 80% ethanol (EC 200-578-6) at 90 ^{o}C. The supernatant was collected into a separate tube after centrifugation at 10,000 g for 15 minutes. Ethanol and centrifugation was repeated three times, adding the resultant supernatant to the previous. The residual pellet after the three washings was reserved for later starch analysis. The combined supernatant was adjusted to a final volume of 3 ml with 80% ethanol. Activated charcoal (EC 231-153-3, 60 mg) was added to each extract, vortexed, and allowed to remain undisturbed for 5 minutes at room temperature to remove interfering compounds. The extract was vortexed again and then centrifuged at 10,000g for 30 minutes and 1.5 ml of supernatant was reserved for soluble sugar analysis.

For soluble sugar analysis, 20µl of extracted sugar supernatant was added to a 96-well microplate. The microplate was dried under vacuum to remove remaining ethanol. The wells of each sample were then filled with 20µl of a working solution containing: HEPES (EC 230-907-9) 50mM at a pH of 7.2, 20 mM ATP (Adenosine triphosphate, EC 213-579-1), and 20 mM NAD (Nicotinamide adenine dinucleotide) dissolved in double distilled water. All wells received 100µl of glucose assay reagent (Glucose Assay Kit, GAHK-20; Sigma Chemical Company, St. Louis, MO), then incubated in the dark at room temperature for 15 min. Absorbance was measured as the change of glucose-6-phosphate (EC 3.1.3.9) to 6-Phosphogluconate (EC 1.1.1.44) at 340 nm with an Ascent Multiskan microplate reader (Molecular Devices Corporation, Sunnyvale, CA). For fructose concentrations, an additional 10µl of phosphoglucoseisomerase (EC 5.3.1.9, 0.25 units, Sigma P-9544) was added to each well of sample. The samples were incubated for 15 minutes at room temperature and absorbance read again at 340 nm. For sucrose concentrations, 20 µl of invertase (EC 3.2.1.20, 83 units, Sigma I-

4504) was added to each well, incubated at room temperature for 60 minutes, and absorbance measured at 340 nm. Carbohydrate concentrations were calculated using a standard curve of known glucose concentrations and expressed as mg / g DW (dry weight).

The remaining starch pellet following the three 80% ethanol washings was dried in a vacuum to remove any residual ethanol. Pellets were removed and 0.5 ml of 1.0 M KOH added to each tube, vortexed until thoroughly mixed, and placed into a dry-bath at 90 ^{o}C for 60 minutes. The pH of the samples were adjusted between 6.5 and 7.5 using 0.2 M acetic acid. 100µl of 0.1 M TRIS-HCl buffer (pH 7.2). To each tube, 100 µl of α-amylase (EC 3.2.1.1, Sigma P-9544) was added, vortexed, and set into a dry-bath at 65 ^{o}C for 60 minutes. Then the pH of each sample was decreased to below 4.5 with 1.0 M acetic acid. Following the pH reduction, 0.25 ml of amyloglucosidase (EC 3.2.1.3, Sigma A-3402) was added to each sample. Sample volumes were adjusted to 1.5 ml with double deionized water and incubated at 55^{o}C for 60 minutes. Temperatures were increased to 95 ^{o}C and maintained for 5 minutes to ensure all enzyme activity had ceased. Tubes were centrifuged at 20,000g for 20 minutes, and the supernatant from each sample was transferred into another tube for storage at -80 ^{o}C or used for immediate analysis. Methods to calculate glucose concentrations present within the tubes of extracted starch was identical to that used to identify glucose from previous soluble glucose extraction assay. The concentration of each starch sample was multiplied by 0.9 to account for water loss when glucose units are linked to form starch (Zhao et al. 2010).

Statistical analyses were performed using JMP 11.1 (SAS Institute, Cary, NC) using an analysis of variance at an alpha level of 0.05. Differences within the means were identified using a student's t-test at an alpha level of 0.05. Factors for analyses included the collection time

(DBA, DOA, DAA), the temperature of the chambers (heat and control), and different plant tissues (leaf or ovary).

Results

Significance

Significant interactions were identified among all soluble carbohydrates between both leaves and ovaries indicating an effect of tissue type upon the concentrations of soluble sugars during each collection time (Table 4-1). However, the effects of temperature upon tissue type were only significant for sucrose and starch. Concentrations of glucose and fructose were not significant for a temperature and tissue combination, suggesting that the effects of temperature did not have an effect on their concentrations within the tissue. Temperature did have a highly significant effect on the concentrations of glucose, sucrose, and starch dependent upon the day of measurement. Fructose concentrations however, were not significantly different from each day at either control or heat conditions.

Single factor significance of soluble carbohydrate concentrations were all highly significant dependent upon tissue type (Table 4-1). Concentrations of glucose, fructose, and sucrose were all highly significant when examining concentrations at each stage ($p < 0.0001$). Starch concentrations during the different collection days ($p = 0.0253$) were not as significant as values from the other sugars. Fructose, sucrose, and starch concentrations were all highly significant ($p = 0.0088$, $p < 0.0001$, $p < 0.0001$, respectively) indicating a substantial change in concentration due to temperature.

Sugar Concentration Surrounding Anthesis

The quantity of sugar was significantly greater at DBA, while DOA and DAA were both statistically similar to each other (Table 4-2). Total sugar concentrations exhibited large

variations dependent upon the tissue type examined and collection time. There were 26.1% more soluble sugars present within the ovaries (77.68 mg / g DW) than leaves (59.74 mg / g DW, $p < .0001$). Additionally, heat stress increased the amount of total sugars significantly by 9.9% compared to control tissues. Concentrations in the subtending leaves for total sugars remained statistically similar between temperature profiles. Total sugar concentrations had significant differences between both leaves and ovaries, although the values for each stage differed for collection and tissue type. Total sugar concentrations were highest among ovaries collected DBA and were 19.3% greater than DAA which was 22.0% greater than concentrations DOA. Total sugar concentrations were greatest among leaves collected DOA, possessing 5.2% more than DAA and 6.1% more than DBA. Concentrations from DAA and DBA were significantly similar to each other.

Glucose

Collectively, glucose concentrations were not significantly distinct due to temperature ($p = 0.6076$), with 1.3% difference in concentrations between the control and heat stress. Likewise, temperature did not show any significant change of glucose concentrations within either leaves or ovaries (Table 4-1). Total concentrations present in leaves were 123% higher than concentrations identified in the ovary (Table 4-2).

Temperature and collection day interactions were highly significant ($p = 0.0027$, Table 4-1). Control DAA tissues had a significantly higher concentration of glucose, 14.8%, than DAA heat-stressed tissues. DBA and DAA heat-stressed tissues were both statistically similar. Heat-stressed DBA tissues were no different from control DBA and heat-stressed tissues. Glucose concentrations of control tissues DOA were among the lowest, but shared significance with control DBA and heat-stressed tissues.

A significant interaction was identified between tissue types and sampling periods for glucose concentration ($p = 0.0154$, Table 4-1). Leaves possessed greater amounts of glucose for all days sampled, however both leaves and ovaries followed the same patterns of significance. Leaves had the highest glucose concentrations DAA, followed by a 4.9% decrease DBA, and DOA with DBA a 6.5% decrease (Table 4-2). Leaf glucose concentrations DBA and DOA shared significance. Ovaries possessed the highest concentration of glucose DAA, followed by DBA, and the lowest concentrations at anthesis, DOA shared significance with DBA.

Fructose

Fructose concentrations present within disparate tissues were influenced by temperature with 4.7% greater concentrations under higher temperatures (Table 4-2). Increased fructose concentrations of 5.5% were present in leaves compared to ovary tissues, regardless of stage or temperature. Concentrations of fructose were significantly higher DBA, which was 8.8% and 10.7% greater than concentrations observed at either DOA or DAA. Fructose concentrations in the ovaries of the control were the lowest (18.44 mg / g DW) of factor combinations between temperature and tissue type. Concentrations of fructose was highly significant dependent upon the stage and the tissue. The greatest concentrations of fructose were present DBA in ovaries and leaves DOA. Leaves at DOA were statistically similar at DBA and DAA. Ovaries DAA and DOA had the lowest concentrations of fructose and shared statistical similarity.

Sucrose

Sucrose concentrations were highly significant ($p < 0.0001$, Table 4-1) with increased temperatures possessing 20% more than the control. Sucrose was heavily influenced by day, with highest concentrations in DBA, followed by DOA, and then DAA, which had 13.1% and 20.1%

less sucrose, respectively (Table 4-2). A highly significant ($p < 0.001$) difference between tissues was observed, with ovaries possessing 125.3% more sucrose than that of leaves.

The interaction of collection day with temperature was highly significant ($p = 0.0008$). The highest concentration of sucrose were in heat-stressed tissues DBA (34.61 mg / g DW). Lesser amounts were found in tissues DAA and DOA at 18.1% and 18.7% less than heat-stressed DBA (Table 4-2). Control tissues held the lowest values, although sucrose in the control at DBA was significantly similar to levels of DAA in the heat. Control tissues DOA were statistically similar to control tissues DBF, with a 4.3% difference. Control DAA tissues were the least significant, having a 15.1% difference from control tissues DOA, and concentrations were 22.8% lower than that of the higher values found in heat-stressed DAA.

The tissue type to temperature interaction was highly significant ($p < 0.0001$, Table 4-1) with ovaries possessing the largest concentration of sucrose. Increased temperatures increased sucrose concentrations compared to the control ovaries by 27.5%, and both the heat-stressed and the control ovaries were significantly different from one another. Concentrations of sucrose displayed significance between leaves and ovaries, with 17.88 and 16.09 mg / g DW from heat-stressed and control leaves, respectively (Table 4-2). Ovaries possessed 88.1% more sucrose per gram DW than the highest concentrated heat-stressed leaves.

The interaction of sampling day to tissue type was also highly significant ($p < 0.0001$, Table 4-1) with the greatest concentration of sucrose in DBA ovaries (44.61 mg / g DW, Table 4-2). Concentrations of sucrose from the ovaries at DOA and DAA ovaries were 19.2% and 23.6% less and statistically similar to each other. Sucrose concentrations in leaves did not possess any statistical difference from one another between days.

Starch

Starch present within the tissues was strongly influenced by temperature ($p < 0.0001$, Table 4-1) with control tissues possessing 16.0% greater concentrations than heat-stressed tissues (Table 4-2). Starch concentration was significant with different tissues; ovaries possessed 27.3% more starch than leaves, regardless of temperature or sampling date. Collection dates were also significant ($p = 0.0253$) with starch concentrations highest in DBA tissues, followed by tissues DOA, and DAA.

Strong significance was found in the interaction of temperature to the collection day ($p = 0.0001$, Table 4-1). The highest concentrations of starch were found in DAA control tissues and were statistically similar to control tissues DBA, though at a 6% reduction (Table 4-2). Starch concentrations in heat-stressed tissues DBA were similar to that found in DAA ovaries, but heat-stressed DBA tissues were indistinguishable from either control or heat-stressed tissues at anthesis. The lowest concentrations were in heat-stressed tissues DAA, which were 20.9% less than found in heat-stressed DOA tissue and 32.3% less than in the control DAA tissue.

The interaction of temperature to tissue concentration of starch was strongly significant ($p = 0.0079$, Table 4-1). Concentrations of starch were highest in control ovaries (17.03 mg / g DW). Starch decreased 17.4% in ovary tissue under heat stress. Leaf concentrations possessed significantly less starch than the ovaries at either temperature (Table 4-2). Leaves under control conditions had 9.9% more starch (12.8 mg / g DW) than in heat-stressed leaves (11.65 mg / g DW).

Interactions between collection date and tissue type were highly significant ($p < 0.0001$, Table 4-1) DBA, ovaries possessed the highest concentrations of starch (18.31 mg / g DW, Table 4-2). Concentrations were lower in the statistically similar DAA ovaries and DOA leaves, at 19%

and 24.1% respectively, compared to DBA ovaries. Starch in the leaves and ovaries at DOA were statistically similar to each other (13.89 and 13.52 mg / g DW). Leaves DAA had lower concentrations (12.01 mg / g DW), followed by the lowest concentrations in the leaves DBA, which were 41.2% less than the DBA ovaries.

Sugar Concentration Relationships

Subtending leaves indicated differences in sugar concentrations dependent upon the temperature (Figure 4-1). Fructose concentrations of control subtending leaves were similar at DBA (20.09 mg / g DW) and DOA (20.43 mg / g DW, $p = 0.3054$), however control leaves DAA had a significant 5% decrease (19.09 mg / g DW, $p < 0.0001$). Leaf fructose concentrations during heat stress did not show any significant differences between any of the collection days. Glucose concentrations were significantly different for all combinations within the control temperatures, with the highest concentrations DAA (11.71 mg / g DW) followed by DBA and DOA which contained 12.3% and 21.5% less. Leaf sucrose concentrations of the control were greatest at DOA (18.2 mg / g DW). No statistical differences existed between DBA and DAA control sucrose concentrations within the leaves that were 16.6% and 18.2% less than concentrations found on the DOA. Leaf starch concentrations in the control DAA (9.91 mg / g DW) were significantly less ($p < 0.0001$) than levels observed DOA (14.17 mg / g DW) or DAA (14.31 mg / g DW).

Under heat stress, leaves for all periods exhibited less dynamic adjustment to the soluble carbohydrate concentrations when compared to control leaves (Figure 4-1). Fructose exhibited no significant differences in concentrations between the collection times ($p = 0.8416$). Glucose also failed to show significance in concentrations between collection times ($p = 0.8974$). Likewise, sucrose did not display any significant difference between the different collections (p

= 0.5805), although sucrose concentrations DBA (18.61 mg / g DW) were 6.0% greater than the lowest concentrations at DAA (17.48 mg / g DW). Starch concentrations levels, however were significantly different ($p < 0.0001$). The highest starch levels were found during DOA (13.61 mg / g DW), which was 14.6% an 28.7% greater than levels found at DBA and DAA.

Ovary soluble sugar concentrations differed significantly for all levels within control tissues (Figure 4-2). Concentrations of fructose were significantly different, with the highest levels found DBA (22.56 mg / g^{-1} DW), which was 13.6% less at DAA and 17.8% less than at anthesis. Glucose concentrations had highly significant ($p < 0.0001$) differences between the collections with DAA having the greatest amounts (6.78 mg / g^{-1} DW, Table 4-2) followed by DBA and anthesis which had 65.0% and 75.6% less, respectively. Sucrose had a highly significant ($p < 0.0001$) linear decrease in concentration with DBA having the highest concentrations (38.6 mg / g DW) followed by DOA and DAA, possessing 13.6% and 25.1% less sucrose. Significant levels of starch were identified within control ovaries with temperature ($p < 0.0001$). Greatest amounts were found DBA (20.16 mg / g DW), and was 12.3% lower DAA, and 34.3% lower at DOA.

Ovaries under the influence of high temperature demonstrated highly significant ($p < 0.0001$) differences in their glucose concentrations, with the highest amounts at DAA (5.85 mg / g DW) followed by DBA and DOA which had 24.1% and 34.7% less (Figure 4-2). Levels of fructose in stressed ovaries were significantly different ($p = 0.0011$) with the greatest concentrations occurring at DBA (22.56 mg / g DW). Fructose concentrations at DOA and DAA were both statistically similar to each other possessing at 17.6% and 20.3% less fructose than at DBA. Sucrose concentrations under heat stress were highly significant ($p < 0.0001$) at DBA (50.62 mg / g DW) having almost 29.0% more sucrose than DAA and 30.7% more than

concentrations found at DOA. Levels of sucrose at DOA and DAA were both statistically similar. Heat affected the levels of starch significantly ($p < 0.0001$) and was different for all times of collection, with DBA possessing the greatest concentrations (16.46 mg / g DW), followed by DOA and DAA which were 16.2% and 27.3% lower, respectively.

Discussion

Major translocation of the primary photoassimilate, sucrose, occurs until growth reaches the 12th main-stem node when leaf photosynthetic capacity of the subtending leaf is enough to maintain sucrose levels (Wullschleger and Oosterhuis, 1990a). Additionally, the subtending leaf provides the largest majority of carbon assimilates for the developing fruit (Ashley, 1972; Wullschleger and Oosterhuis, 1990b). The impacts that heat stress had upon our experimental flower carbohydrate pools were significant. In leaves, heat stress appears to affect starch levels most significantly DAA, though at DOA the levels of starch were reduced compared to the control. Sucrose concentrations were greater the DBA and DAA compared to the control.

Rates of photosynthesis within the canopy structure can be reduced substantially as a byproduct of shading (Zhao and Oosterhuis, 1998a). A PAR penetration impairment of 30% diminishes micronaire and quality of cotton lint (Pettigrew, 1994), which was attributed to a reduction in photosynthesis and carbon export. Subtending leaves in our experiment were exposed to PAR values of about 100 µmol m^{-2} s^{-1}. This constitutes a decrease of 80% in the transmitted PAR available for photosynthesis. PAR reductions of 63% at the upper canopy have been shown to reduce photosynthetic capacity by the main-stem leaves by as much as 55% (Zhao and Oosterhuis, 1998b), impacting floral development by decreasing photoassimilate production. Cotton within growth chambers have net photosynthesis rates of near half that of field-grown plants due to reduced chloroplast densities (Patterson et al. 1977), though the plants in our

chambers had adapted to light levels in the chamber, thus we expect similar results in the field, if it were duplicated there.

Transmitted PAR values in field-grown cotton have a large variation dependent on canopy structure and position that can be as low as 13% the PAR values within the canopy compared to above it (Zhi et al., 2014). With a reduction in photosynthesis capacity affected by light, the addition of heat disrupts carbon fixation or starch related gene expression leading to the starch declines as seen in Figure 4-1. Additionally, in tomatoes (*Solanum lycopersicum* L.) under heat stress, diminished sucrose transport rates occur from the subtending leaf to the fruit (Aloni et al., 1991). Recent work suggests this may be the result of impaired sucrose transporter gene transcription (Phan et al., 2013). Leaf tissue in our study maintained similar levels of fructose and glucose levels in both temperature regimes, which was in contention of the findings from Zhao et al.(2005), although their work focused on upper canopy leaves. What was significant was the stability of sucrose levels during the experiement. Likewise, starch levels decreased during the same periods, suggesting that to maintain sucrose concentrations starch in the leaf tissues were consumed at a greater rate than in the controls.

Proper fertilization requires a ready supply of carbohydrate for maximum efficiency (González et al., 1996; Snider et al., 2009). Sucrose levels in heat-stressed ovaries were significantly higher than levels found in control ovaries (Figure 4-2). It has been reported that in bell pepper (*Capsicum annum* L.) that an overabundance of sucrose within ovary tissue could lead to ovarian tissue swelling, fruit deformities, and abscission (Darnell, 2013). Cotton however does not store the majority of its energy reserves as sucrose, but as starch (Goldschmidt and Huber, 1992). Thus, excessive sucrose should promote storage into starch. In our case, heat-stressed flowers had decreased starch concentrations the DBA and the DAA, but concentrations

at DOA were similar to the control. However, sucrose levels were significantly higher in the flowers under heat stress compared to the controls.

Cotton bolls are absent of active xylem vessels for three weeks after fertilization while boll expansion occurs (Van Iersel et al., 1994) and the majority of sugars for the developing ovules are derived from the apoplast (Buchala, 1987). The high levels of sucrose and decreased levels of starch in the ovaries could be caused by difficulties in importing sucrose from the apoplastic space. The leaf sucrose levels in heat stressed flowers and subtending leaves the DAA were increased, but starch levels continued to decrease. In heat-stressed sorghum (*Sorghum bicolor* L.), microspores have reduced cell wall invertase hydrolysis activities leading to a starch deficiency within the pollen grain (Jain et al., 2007). Acid invertases within the cell wall have been shown to be the dominant invertase type in *Lilium* styles (Sturm and Tang, 1999). In combination with reduced style carbohydrate concentrations affecting reproductive success in cotton (Snider et al., 2009), research suggests that either cell wall invertase or the apoplastic sucrose importer mechanisms may be impaired by heat stress. In our case, we did not examine the sucrose importers into the ovary, however past research plus the decrease of ovarian starch compared to control flowers suggests that sucrose import deficiencies may be a plausible reason for increased sucrose levels.

Ovaries metabolize a majority of available sucrose into starch (Aloni et al., 1996). Both leaf and ovary starch concentrations were impaired at higher temperatures DAA. The decrease in starch concentration is similar to past work on main-stem leaves (Reddy et al., 1998). Levels of starch in heat-stressed ovaries DAA were significantly lower than levels observed in control ovaries (Figure 2). This finding is similar to results obtained by Bhullar and Jenner (1986) in temperature stressed wheat endosperm. Higher temperatures have also been shown to repress

starch synthesis related transcription significantly (Phan et al., 2013), providing a possibility for decreased starch concentrations due to high temperatures. Thus, higher temperatures may also be negatively affecting the manufacture of starch within the ovary, limiting energy reserves.

Conclusions

We have identified high temperatures impacts upon carbohydrate balance in the leaves and ovaries the days surrounding anthesis (Figures 4-1 and 4-2). Steep increases in sucrose concentrations and significant decreases in starch production in the days surrounding anthesis suggest that high temperature places vast constraints on proper carbon partitioning. However, these constraints lie in the possible export and import of sugars. Manufacture of hexoses from sucrose remained unchanged, indicating that hexose manufacture was not impacted by increasing temperatures. This research suggests that multiple sources of stress may be working in concert within the canopy to facilitate shed. Under normal conditions, the reduced PAR transmission within the canopy hamper photosynthetic rates and sucrose synthase activities of the subtending leaf, but not sufficiently enough to hinder boll development. Yet, heat stress places an excessive burden onto this delicate dynamic of carbon partitioning, in particular at DAA when fertilization is most susceptible to carbohydrate imbalance. Our results indicate that carbohydrate is rapid and dynamic in response to high temperature stress, and these changes on the day of flowering may significantly affect successful flower development.

References

Aloni, B., L. Karni, Z. Zaidman, and A.A. Schaffer. 1996. Changes of carbohydrates in pepper (*Capsicum annuum* L.) flowers in relation to their abscission under different shading regimes. Ann. Bot. 78: 163–168

Aloni, B., T. Pashkar, and L. Karni. 1991. partitioning of [14C] sucrose and acid invertase activity in reproductive organs of pepper plants in relation to their abscission under heat stress. Ann. Bot. 67: 371–377.

Ashley, D. 1972. 14C-labelled photosynthate translocation and utilization in cotton plants. Crop Sci. 12: 69–74.

Ashraf, M., M.M. Saeed, and M.J. Qureshi. 1994. Tolerance to high temperature in cotton (*Gossypium hirsutum* L.) at initial growth stages. Environ. Exp. Bot. 34: 275–283.

Azhar, F., Z. Ali, M. Akhtar, A. Khan, and R. Trethowan. 2009. Genetic variability of heat tolerance, and its effect on yield and fibre quality traits in upland cotton (*Gossypium hirsutum* L.). Plant Breed. 128: 356–362.

Bhullar, S.S., and C.F. Jenner. 1986. Effects of temperature on the conversion of sucrose to starch in the developing wheat endosperm. Aust. J. Plant Physiol. 13: 605–615.

Bibi, A.C., D.M. Oosterhuis, and E.D. Gonias. 2008. Photosynthesis, quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes. J. Cotton Sci. 12: 150–159.

Buchala, A.J. 1987. Acid β-fructofuranoside fructohydrolase (invertase) in developing cotton (gossypium arboreum l.) fibres and its relationship to β-glucan synthesis from sucrose fed to the fibre apoplast. J. Plant Physiol. 127: 219–230.

Chaitanya, K.V., D. Sundar, and A.R. Reddy. 2001. Mulberry leaf metabolism under high temperature stress. Plant Sci. 44: 379–384.

Cheikh, N., and R.J. Jones. 1995. Heat stress effects on sink activity of developing maize kernels grown in vitro. Physiol. Plant. 95: 59–66.

Darnell, R. 2013. Night temperature and source-sink effects on growth, leaf carbon exchange rate, and carbohydrate accumulation in bell pepper ovaries. J. Am. Soc. Hortic. Sci. 138: 331–337.

Faver, K.L., T.J. Gerik, P.M. Thaxton, and K.M. El-Zik. 1996. Late season water stress in cotton: II. Leaf gas exchange and assimilation capacity. Crop Sci. 36: 922–928.

Goldschmidt, E.E., and S.C. Huber. 1992. Regulation of photosynthesis by end-product accumulation in leaves of plants storing starch, sucrose, and hexose sugars. Plant Physiol. 99: 1443–1448.

González, M.V., M. Coque, and M. Herrero. 1996. Pollen-pistil interaction in kiwifruit (*Actinidia deliciosa*; *Actinidiaceae*). Am. J. Bot. 83: 148–154.

Guinn, G. 1976. Water deficit and ethylene evolution by young cotton bolls. Plant Physiol. 57: 403–405.

Guinn, G., J.R. Mauney, and K.E. Fry. 1981. Irrigation scheduling and plant population effects on growth, bloom rates, boll abscission, and yield of cotton. Agron. J. 73: 529–534.

Holman, E.M., and D.M. Oosterhuis. 1999. Cotton photosynthesis and carbon partitioning in response to floral bud loss due to insect damage. Crop Sci. 39: 1347–1351.

Van Iersel, M.W., D.M. Oosterhuis, W.M. Harris, and M.W. Van lersel. 1994. Apoplastic water flow to cotton leaves and fruits during development. J. Exp. Bot. 45: 163–169.

Jain, M., P.V.V. Prasad, K.J. Boote, A.L. Hartwell, and P.S. Chourey. 2007. Effects of season-long high temperature growth conditions on sugar-to-starch metabolism in developing microspores of grain sorghum (*Sorghum bicolo*r L. Moench). Planta 227: 67–79.

Lafta, A.M., and J.H. Lorenzen. 1995. Effect of high temperature on plant growth and carbohydrate metabolism in potato. Plant Physiol. 109: 637–643.

Law, R., and S. Crafts-Brandner. 1999. Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant Physiol. 120: 173–182.

Lieth, J.H., G.K. Arkin, A.B. Hearn, and B.S. Jackson. 1986. Modeling cotton fruiting form abscission. Agron. J. 78: 730–735.

Loka, D.A., D.M. Oosterhuis, C.J. Fernandez, and B.A. Roberts. 2011. The effect of water-deficit stress on the biochemistry of the cotton flower. Summ. Arkansas Cott. Res. AAES Research Series. 602: 51–56.

Patterson, D.T., J.A. Bunce, R.S. Alberte, and E. Van Volkenburgh. 1977. Photosynthesis in relation to leaf characteristics of cotton from controlled and field environments. Plant Physiol. 59: 384–387.

Pettigrew, W.T. 1994. Source-to-sink manipulation effects on cotton lint yield and yield components. Agron. J. 86: 731–735.

Phan, T.T.T., Y. Ishibashi, M. Miyazaki, H.T. Tran, K. Okamura, S. Tanaka, J. Nakamura, T. Yuasa, and M. Iwaya-Inoue. 2013. High temperature-induced repression of the rice sucrose

transporter (OsSUT1) and starch synthesis-related genes in sink and source organs at milky ripening stage causes chalky grains. J. Agron. Crop Sci. 199: 178–188.

Reddy, K., H. Hodges, and J. McKinion. 1997. Modeling temperature effects on cotton internode and leaf growth. Crop Sci. 37: 503–509.

Reddy, K.R., H.F. Hodges, and V.R. Reddy. 1992a. Temperature effects on cotton fruit retention. Agron. J. 84: 26–30.

Reddy, V.R., K.R. Reddy, and D.N. Baker. 1991. Temperature effect on growth and development of cotton during the fruiting period. Agron. J. 83: 211–217.

Reddy, K.R., V.R. Reddy, and H.F. Hodges. 1992b. Temperature effects on early season cotton growth and development. Agron. J. 84: 229–237.

Reddy, A.R., K.R. Reddy, and H.F. Hodges. 1998. Interactive effects of elevated carbon dioxide and growth temperature on photosynthesis in cotton leaves. Plant Growth Regul. 26: 33–40.

Snider, J.L., D.M. Oosterhuis, D.A. Loka, and E.M. Kawakami. 2011. High temperature limits in vivo pollen tube growth rates by altering diurnal carbohydrate balance in field-grown *Gossypium hirsutum* pistils. J. Plant Physiol. 168: 1168–1175.

Snider, J.L., D.M. Oosterhuis, B.W. Skulman, and E.M. Kawakami. 2009. Heat stress-induced limitations to reproductive success in *Gossypium hirsutum*. Physiol. Plant. 137: 125–138.
.

Stewart, J. 1986. Integrated events in the flower and fruit. p. 261–300. *In* Mauney, J. and Stewart, J.M. (eds.), Cotton Physiology. The Cotton Foundation, Memphis, TN.

Sturm, A., and G.Q. Tang. 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. Trends Plant Sci. 4: 401–407.

Wullschleger, S.D., and D.M. Oosterhuis. 1990a. Photosynthetic carbon production and use by developing cotton leaves and bolls. Crop Sci. 30: 1259–1264.

Wullschleger, S.D., and D.M. Oosterhuis. 1990b. Photosynthetic and respiratory activity of fruiting forms within the cotton canopy. Plant Physiol. 94: 463–469.

Zakaria, S., T. Matsuda, S. Tajima, and Y. Nitta. 2002. Effect of high temperature at ripening stage on the reserve accumulation in seed in some rice cultivars. Plant Prod. Sci. 5: 160–168.

Zhao, D., C.T. MacKown, P.J. Starks, and B.K. Kindiger. 2010. Rapid analysis of nonstructural carbohydrate components in grass forage using microplate enzymatic assays. Crop Sci. 50: 1537–1545.

Zhao, D., and D. Oosterhuis. 1998a. Influence of shade on mineral nutrient status of field-grown cotton. J. Plant Nutr. 21: 1681–1695.

Zhao, D., and D. Oosterhuis. 1998b. Cotton responses to shade at different growth stages: Nonstructural carbohydrate composition. Crop Sci. 38: 1196–1203.

Zhao, D., K.R. Reddy, V.G. Kakani, S. Koti, and W. Gao. 2005. Physiological causes of cotton fruit abscission under conditions of high temperature and enhanced ultraviolet-B radiation. Physiol. Plant. 124: 189–199.

Zhi, X., Y. Han, S. Mao, G. Wang, L. Feng, B. Yang, Z. Fan, W. Du, J. Lu, and Y. Li. 2014. Light spatial distribution in the canopy and crop development in cotton. PLoS One 9: e113409.

Tables

Table 4-1: Factor combinations of each soluble carbohydrate according to temperature, flowering stage, and tissue type at the 0.05 level.

	Glucose	Fructose	Sucrose	Starch	Total
Temperature	0.6508	0.0088	<.0001	<.0001	<.0001
Stage	<.0001	<.0001	<.0001	0.0253	<.0001
Tissue	<.0001	0.0021	<.0001	<.0001	<.0001
Temperature x Stage	0.0027	0.8940	0.0008	<.0001	0.0073
Temperature x Tissue	0.6076	0.3019	<.0001	0.0079	0.0010
Stage x Tissue	0.0154	<.0001	<.0001	<.0001	<.0001

Table 4-2: Soluble carbohydrate concentration means (mg / g DW) ± the 95% CI of tissues for each factor and associated combinations (p = 0.05).

Interaction	Factors		Glucose	Fructose	Sucrose	Starch	Total
Temperature	Control		7.65 ± 0.15	19.15 ± 0.24	24.86 ± 0.43	14.92 ± 0.23	66.58 ± 1.27
	Heat		7.55 ± 0.16	20.07 ± 0.25	30.37 ± 0.44	12.86 ± 0.24	70.84 ± 1.32
Day	DBA		7.36 ± 0.2	20.91 ± 0.31	30.75 ± 0.55	14.54 ± 0.3	73.55 ± 1.62
	Anthesis		6.8 ± 0.18	19.14 ± 0.28	26.96 ± 0.49	13.71 ± 0.27	66.58 ± 1.47
	DAA		8.65 ± 0.2	18.79 ± 0.31	25.13 ± 0.56	13.42 ± 0.31	66 ± 1.65
Tissue	Leaf		10.39 ± 0.16	20.15 ± 0.24	16.98 ± 0.44	12.22 ± 0.24	59.74 ± 1.29
	Ovary		4.81 ± 0.16	19.07 ± 0.24	38.25 ± 0.44	15.55 ± 0.24	77.68 ± 1.29
Day x Tissue	DBA	Leaf	10.44 ± 0.54	20.23 ± 0.86	16.89 ± 1.53	10.77 ± 0.84	58.33 ± 2.3
		Ovary	4.27 ± 0.54	21.58 ± 0.86	44.61 ± 1.53	18.31 ± 0.84	88.77 ± 2.3
	Anthesis	Leaf	9.76 ± 0.49	20.52 ± 0.77	17.87 ± 1.38	13.89 ± 0.76	62 ± 2.07
		Ovary	3.84 ± 0.49	17.76 ± 0.77	36.04 ± 1.38	13.52 ± 0.76	71.16 ± 2.07
	DAA	Leaf	10.98 ± 0.55	19.7 ± 0.87	16.19 ± 1.55	12.01 ± 0.85	58.88 ± 2.33
		Ovary	6.32 ± 0.55	17.89 ± 0.87	34.08 ± 1.55	14.83 ± 0.85	73.12 ± 2.33
Temperature x Tissue	Control	Leaf	10.39 ± 0.42	19.87 ± 0.67	16.09 ± 1.19	12.8 ± 0.65	59.14 ± 1.79
		Ovary	4.92 ± 0.42	18.44 ± 0.67	33.63 ± 1.19	17.03 ± 0.65	74.01 ± 1.79
	Heat	Leaf	10.4 ± 0.44	20.43 ± 0.7	17.88 ± 1.24	11.65 ± 0.68	60.33 ± 1.86
		Ovary	4.7 ± 0.44	19.71 ± 0.7	42.87 ± 1.24	14.07 ± 0.68	81.35 ± 1.86
Temperature x Day	Control	DBA	7.19 ± 0.53	20.35 ± 0.84	26.89 ± 1.49	15.04 ± 0.82	69.46 ± 2.24
		Anthesis	6.53 ± 0.5	18.68 ± 0.78	25.78 ± 1.39	13.71 ± 0.76	64.69 ± 2.09
		DAA	9.24 ± 0.53	18.44 ± 0.84	21.9 ± 1.49	16 ± 0.82	65.59 ± 2.24
	Heat	DBA	7.53 ± 0.56	21.47 ± 0.88	34.61 ± 1.57	14.04 ± 0.86	77.65 ± 2.35
		Anthesis	7.07 ± 0.49	19.6 ± 0.77	28.14 ± 1.36	13.7 ± 0.75	68.47 ± 2.05
		DAA	8.06 ± 0.57	19.14 ± 0.9	28.36 ± 1.61	10.84 ± 0.88	66.4 ± 2.42

Figures

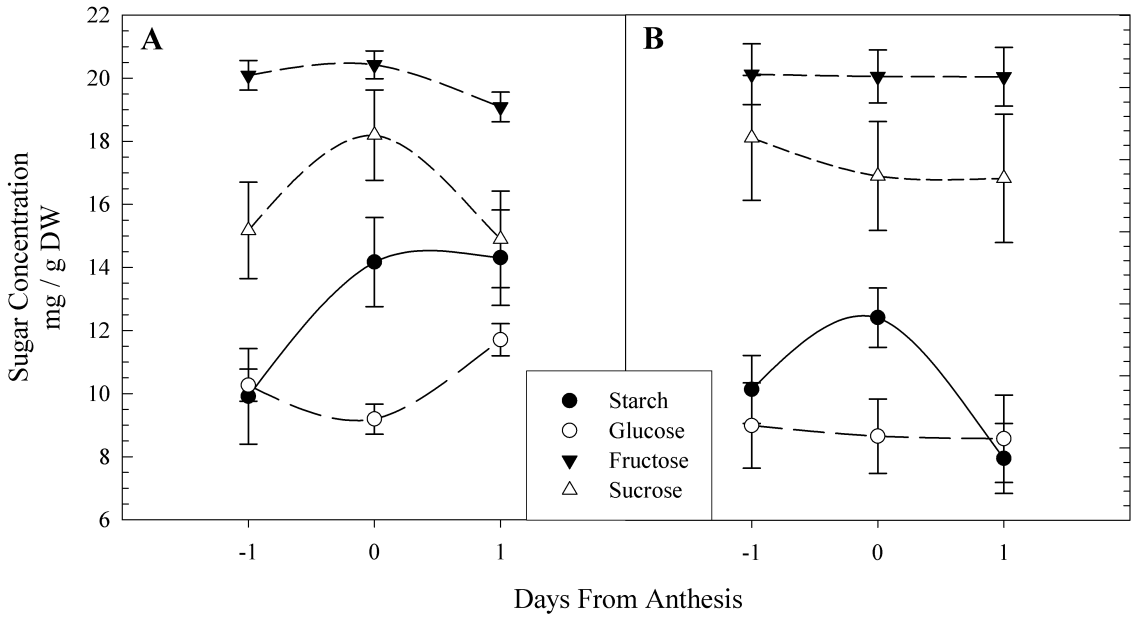

Figure 4-1: Sugars concentrations in mg / g of dry weight (DW) of the subtending leaves of flowers for each collection time during both control (A) and heat-stressed (B) conditions. Error bars indicate the 95% confidence interval at p = 0.05.

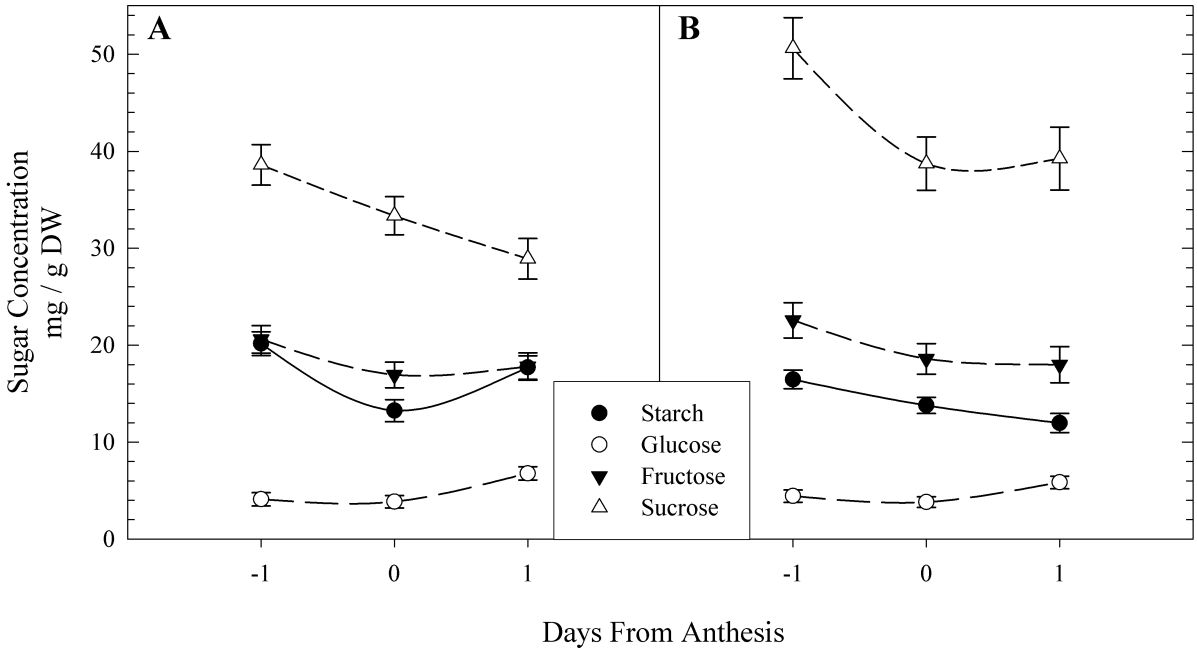

Figure 4-2: Sugars concentrations in mg / g of dry weight (DW) of the ovaries of white flowers for each collection time during both control (A) and heat-stressed (B) conditions. Error bars indicate the 95% confidence interval at p = 0.05.

CHAPTER V

Cotton's Acclimation Response to Repeated Periods of Heat Stress during Anthesis

Abstract

Cotton (*Gossypium hirsutum* L.) is sensitive to heat stress, but is capable of mitigating several negative effects over time. However, acclimation studies in cotton in regards to heat stress are limited. The aim of this study was to examine several known biochemical markers of stress to identify the extent that cotton was capable of acclimating to heat stress. The experiment consisted of controlled environmental chamber studies under day / night conditions of 32 / 20 ºC until flowering. At flowering, temperatures were increased in one growth chamber to 40 / 24 ºC for one week. Temperatures in the environmental chamber were then returned to control temperatures for one week, and back to elevated temperature conditions for another week. Increased temperatures resulted in higher leaf temperatures compared to the control, but more than 10 ºC cooler than the ambient air temperatures due to transpiration effects. Leaf plasma membranes had the least integrity during the first heat cycle, but were similar to membrane integrities of the control after three days. The second week cycle reduced membrane damage compared to week one, but still required three days to be similar to the control. Electron transport rates increased significantly compared to control plants throughout the duration of stress application. Antioxidants such as glutathione reductase (GR) and peroxidase (POX) differed significantly dependent upon tissue type, cycle of stress, and day. We conclude that cotton is capable of rapidly adjusting its response to heat stress that changes the physiological response of the plant following an initial period of heat stress. The subsequent acclimation affects both the intensity and timing of the biochemical markers used by other researcher for stress which can significantly affect future researcher's interpretations.

Introduction

Abiotic stresses such as high temperatures have a substantial impact on the growth, development, and yield of a plant. Plants have developed complex mechanisms to respond to rapidly changing environmental conditions to maximize reproductive success. In some cases, these changes occur within minutes at the cellular level (Cooper and Ho, 1983; Ruelland and Zachowski, 2010), or develop within days at the tissue and organism levels that can persist for some time (Chen et al., 1982; Kozlowski and Pallardy, 2002; ur Rahman et al., 2004). Recent work has demonstrated that even short term environmental stresses can produce changes within the DNA that are subsequently passed onto successive generations (Molinier et al., 2006; Verhoeven et al., 2010).

In cotton producing areas of the United States, weather conditions fluctuate markedly across the different growing regions. While a tropical plant of origin, cotton has an optimal growth temperature of 30 ^{o}C (Burke et al., 2004), for photosynthesis at 33 ^{o}C (Bibi et al., 2008), and for maximal boll development at 30 ^{o}C (Reddy et al., 1991). However, temperatures across much of the growing region may have mid-season maximum daily temperatures in excess of 45 ^{o}C in arid regions of production, and in excess of 40 ^{o}C in more temperate regions. The effects of high temperature has been well documented on reduced lint yields and quality (Faver et al., 1996; Azhar et al., 2009; Snider et al., 2009).

However, little work has been introduced demonstrating the acclimation potential on cotton. Cotton is capable of modulating its temperature by more than 10 degrees by evaporative cooling provided low humidity and adequate water are available mitigating yield losses (Burke and Upchurch, 1989; Hake and Silvertooth, 1990). Though the leaves may be cooled substantially under less humid, higher temperature conditions, many growing regions often have

high humidity and temperatures in concert limiting the effectiveness of evaporative cooling and ultimately leading to increased tissue temperatures (Radin et al., 1994).

Photosynthesis is extremely sensitive to the effects of increased temperatures. Reductions in the photosynthetic rate have been consistently shown in cotton to decrease available carbohydrate supply to the developing bolls, leading to increased rates of fruit shed (Guinn, 1982; Hake and Silvertooth, 1990; Sadras, 1995; Brown and Oosterhuis, 2010). Photorespiration increases under heat stress at faster rates than carbohydrate production, even if the leaf is maintained at cooler temperatures via evapotranspiration (Schuster and Monson, 1990). This photosynthetic sensitivity appears to be most likely the result of the aggregation of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) activase at moderately increased temperatures (> 35 ^{o}C) (Feller et al., 1998). This hypothesis was further substantiated with work performed by Salvucci and Crafts-Brander (2000) who reported steep declines in cotton and tobacco Rubisco activity due to aggregated Rubisco activase as temperatures exceeded 37 ^{o}C. They also noted that moderate increases in temperate also stimulated ATP / ADP ratios sufficiently to offset the effects of reduced activase efficiencies. In creeping bentgrass (*Agrostis stolonifera* L.) the photosynthetic apparatus was observed to possess significantly higher photosynthesis and Rubisco activation states after heat stress when compared to plants that had not been stressed prior (Liu and Huang, 2008).

Photosynthesis and its corresponding electron transport rates (ETR) are often dependent upon the effects induced by the growing conditions. ETR efficiency is dependent upon the pool of available plastiquinone molecules available on the reducing side of photosystem II, with greater decreases in plastiquinone reduction capabilities as temperatures increased (Pshybytko et al., 2008). This limits the capacity of quantum energies transmitted to photosystem I, decreasing

the effectiveness of photosynthesis. Acclimation, though can permit plants to be much more robust at elevated temperatures due to adjustments of the plastiquinone pool (Yamasaki et al., 2002).

Additionally, the plasma membrane needs to maintain its integrity under high temperature stress, particularly in the chloroplast. Under heat stress, lipid peroxidation within the membrane negatively affects the efficiency of the photosystem complex by permitting increased fluidity of the thylakoid membranes, thereby disrupting the photosystem (Schrader et al., 2004; Sharkey, 2005). Under continued heat stress, cyclic photophosphorylation is increased to dissipate excess energies and preserve the more sensitive photosystem II complex (Schrader et al., 2004). The ability of cotton to adjust its membrane structure under heat-stressed conditions has been recognized as a significant physiological adaptation to heat stress (ur Rahman et al., 2004).

The protective nature of antioxidants cannot be understated when high temperature stress occurs. Reactive oxidative species (ROS) increase significantly with increased levels of heat stress (Wahid et al., 2007). Normal levels of ROS act as signaling markers that precipitate a cascade of downstream pathways encouraging positive effects on plant growth and development (Schroeder et al., 2001; Foreman et al., 2003; Arasimowicz and Floryszak-Wieczorek, 2007). Increases in ROS also activate enzymatic pathways used to initiate stress response (Dat et al., 1998; Foyer and Noctor, 2005). Though, if stress conditions exceed that of the protective measures then ROS can also initiate programmed cell death (Gechev et al., 2006). Therefore, an otherwise healthy tissue exposed to heat stress must increase antioxidant responses to mitigate excessive damage caused by excessive ROSs. Sufficient antioxidant pools are necessary to mitigate heat related responses for proper growth and continued development of cotton during

stressful periods (Snider et al., 2011). If plants are exposed ROSs at sub-lethal levels and later exposed to a severe stress event, a greater likelihood of survival occurs due to increased antioxidant production (Lopez-Delgado et al., 1998; Karpinski et al., 1999; Larkindale and Huang, 2004). In some cases, this acclimatizing effect from stress was identified for more than a month following the initial stress response (Dat et al., 1998; Lopez-Delgado et al., 1998).

The objective in this study was to determine if there existed an acclimation response within the antioxidant concentrations, ETR response, and membrane permeability of heat-stressed cotton leaves and reproductive units. We hypothesized that due to the survival mechanisms inherent in a plant's genome, heat stress acclimation would occur within a short period by adjusting several biochemical markers related to stress. Additionally, these acclimations may occur at different rates and may change from one period of stress to another.

Materials and Methods

Two environmental control chamber experiments using cotton (*Gossypium hirsutum* L.) cv. ST5288B2F planted were performed in 2013 at the Altheimer Laboratory, University of Arkansas. Twenty-five 4 L pots filled with nutrient free potting soil mix (Conrad Fafard, Agawam, MA, USA) were placed into two environmental control chambers (PGW36, Conviron Inc., Winnipeg, Canada) and set for a 14-h photoperiod beginning at 6:00 am with a photosynthetically active radiation (PAR) of 500–550 μmol / m^2 s^{-1} and a relative humidity of 60%. Cotton in both chambers was grown under normal day/night temperatures of 32 / 20°C and received one-quarter strength Hoagland's nutrient solution daily until the first true leaf after which the solution was adjusted to one-half strength Hoagland's solution for every day thereafter. Plants were randomly distributed within each chamber as well as across chambers to minimize growth variations.

High Temperature Studies

At the first flower stage, temperatures within one chamber were increased to a maximum daily temperature of 40 ^{o}C and a nightly low of 24 ^{o}C. All measurements were made between 1300 h and 1500 h on a daily basis. Measurements of leaf temperature, fluorescence, and membrane leakage were taken daily (10 replications from each chamber). White flowers and associated subtending leaves were collected for subsequent antioxidant analysis. Collected tissues were immersed in liquid nitrogen and stored at -80^{o}C. Following five days of high temperatures, temperatures within the stress chamber were returned to control day / night temperatures of 32 / 20 ^{o}C for one week. Temperatures were raised in the heat stress chamber in the same manner as before for another five days, and measurements repeated as before.

Membrane Leakage Measurements

Avoiding both major and secondary veins, three 1 cm^2 discs were excised from the upper fourth main-stem leaf. The collected vials were capped and placed in the dark at room temperature for 24 hours. Electrical conductivity (EC) of the water was measured with an EC meter (Primo 5, HANNA Instruments, USA) and recorded as the initial ionic leakage. Tubes were capped and autoclaved for 20 minutes to dissociate all cellular cytosols into solution. After cooling to room temperature, the EC was again measured as total ionic leakage. Calculations were performed as an injury index percentage as shown below, with greater initial ionic leakage giving a higher value than lesser initial ionic leakage:

$$1 - \left(\frac{Final - Initial}{Initial}\right) * 100\%$$

Fluorescence

Efficacy of the photosystem II complex was estimated via the electron transport rate (ETR) efficiency using light-adapted techniques as described by Flexas et al. (1999) using a OS5p Modulated Fluorometer (Opti-Science, Tyngsboro, MA). Three locations of the light exposed fourth upper main-stem leaf were sampled for each plant and averaged together. Ten plants were sampled from each chamber per day. The change in irradiance was measured under actinic light conditions. The adaxial surface of the leaf was illuminated stepwise with increasing intensity (2850, 5700, and 8550 µmol m^{-2} s^{-1}) for approximately 0.95 s, which provided the estimate of Fm' (maximal fluorescence) when all reaction centers have been fully occupied. The ETR estimate was calculated via the following:

$$\left(\frac{Fv}{Fm'}\right) * (PAR) * 0.5 * 0.84$$

Where PAR is the photosynthetically active radiation at the leaf's surface, Fv / Fm' corresponds to the quantum efficiency of photosynthesis under ambient light conditions, where *Fv* is the variable fluorescence rate. The 0.5 corresponds to the excitation energy being divided amongst both photosystems I and II, and 0.84 is a common leaf absorbance coefficient in C3 plants.

Leaf Temperature

Upper fourth main-stem leaf temperatures were measured using an embedded K-type thermocouple in the OS5p Modulated Fluorometer (Opti-Science, Tyngsboro, MA) light wand that was used for ETR data collection. Leaf temperatures were collected from three different locations on the adaxial surface and averaged together. A total of 10 plants per chamber per day were sampled.

Antioxidant Analysis

Leaf and ovary antioxidant extractions were performed according to Anderson et al. (1992) with modification. Collections included the daily collections of 10 leaves and ovaries collected between 1100 h and 1300 h, frozen, and stored at -80 ^{o}C. Approximately 100 mg of tissue, ovary weights were recorded and used whole as their weights seldom reached 100 mg, was transferred to a mortar and pestle pre-chilled with liquid nitrogen. The tissue was ground into a fine powder with additional liquid nitrogen as needed to maintain cold conditions. The homogenate was transferred to a polypropylene test tube on ice to vent off remaining nitrogen. An ice cold solution comprised of 50 mM PIPES (1,4-piperazinediethanesulfonic acid) buffer (pH 6.8), 6 mM cysteine hydrochloride, 10 mM D- isoascorbate, 1mM ethylenediaminetetraacetic acid, 0.3% Triton X-100 and 1% (w/v) soluble polyvinylpyrrolidone (PVP) at a ratio of 10x the recorded tissue weight was added to each sample tube. Samples were vortexed following the addition of the solution. Sample tubes remained undisturbed on ice for 5 minutes then centrifuged at 5,000 g for 20 min at 2 ^{o}C. The supernatant was collected and the volume recorded. Tubes were either analyzed immediately or stored at −80 °C until further analysis.

Glutathione Reductase (GR)

Determination of GR content was performed according to Schaedle and Bassham (1977) with modification. To each well of a 96-well microtitration plate, a 15.7 µl aliquot of cold, thawed enzyme extract from each sample was added to 300 µl of reaction solution. The reaction solution was comprised of 50mM Tris–HCl buffer (pH= 7.5), 0.15mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 0.5 mM oxidized glutathione, and 3 mM $MgCl_2$. The activity of GR was indicated by the decrease in absorbance at 340 nm during a 1 min reaction

time. Microplates were measured using an Ascent Multiscan microplate reader (Molecular Devices Corporation, Sunnyvale, CA). Three replications were performed of each sample (n = 10 leaves and n = 10 ovaries, collected daily during the experiments) and the average GR activity was expressed as µKatal units per g of tissue.

Guaiacol Peroxidase (POX)

The oxidation of guaiacol to tetraguaiacol ($\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$) was used to determine POX activity. 50 µl of enzyme extract was diluted five times with 50 mM HEPES buffer (pH = 7.0). From the dilutant, 50 µl of enzyme extract was added to 950 µl of reaction solution comprised of 50 mM H_2O_2 and 50 mM HEPES buffered solution (pH = 7.0) in a 1.5 ml cuvette. Oxidation rates were monitored spectrophotometrically with a PharmaSpec UV-1700 (Shidmazu, Kyoto, Japan) at 470 nm over the course of 3 minutes. Two replications of the enzyme dilutant was made and the results averaged (n = 10 leaves and n = 10 ovaries, collected daily during the experiments). POX activity was expressed as µKatal units per g of tissue.

Statistical Analysis

The experimental design was comprised of the following factors of day, week, and temperature. The data of the two growth chamber studies were not significantly different and combined. Analysis of variance was performed (JMP 11.1 software, SAS Institute Inc., Cary, NC) and Tukey's goodness of fit test was used for the comparison of the means. Means were considered significantly different at a $p \leq 0.05$ level.

Results

Statistical significance

Significant two-way interactions were found between week and temperature for ovary POX, leaf and ovary GR, and membrane leakage (p = 0.0005, 0.0359, 0.0139, 0.0003, Table 5-

1). The two way interaction between the factors of day and temperature showed significance for ovary POX, leaf and ovary GR, leaf ETR, leaf temperature, and membrane leakage ($p = < 0.0001, < 0.0001, 0.0236, 0.0021, 0.0102, < 0.0001$). The final two-way interaction between day and week exhibited significance only with leaf POX, fluorescence, and membrane leakage ($p = 0.0186, 0.0221, < 0.0001$). There was highly significant differences amongst all tested parameters for the single factor of temperature ($p < 0.0001$). The single factor of week exhibited significant differences only between ovary POX, leaf ETR, and leaf temperatures ($p = 0.0014, < 0.0001, 0.0059$). Highly significant differences existed in the single factor of day for ovary POX, leaf and ovary GR, leaf ETR, and membrane leakage ($p = < 0.0001, < 0.0001, 0.0137, 0.0001, < 0.0001$).

Antioxidants

Glutathione reductase (GR)

Leaf GR rates were greatest on the first day of week one of heat stress with activities near 200% greater than activities found in the control leaves (Figure 5-1A). By day two of week one, activities in heat-stressed plants were only 65% greater than the control. GR activities in days three through five of week one remained higher than activities in the control plants. During week two of heat stress, activity levels of the first day did not differ significantly from the control leaves, however, the means were slightly higher. Day two activities of week two saw a 150% increase in activities when compared to the control, and was statistically similar to activities of day one of week one, although activity means were about 15% less of the second week. Heat-stressed activities on the third day of week two and forward were statistically similar to the control leaves.

Rates of ovary GR in control plants were statistically similar to each other across all days measured (Figure 5-1B). Heat-stressed activities were significantly greater in all days compared to the control plants; however the activities differed by day and week in their response. GR rates were approximately 305% higher than control plants on day one of week one. GR activities decreased slowly during the course of the week. During week two, GR activities of day one were similar to the activities from day five of week one. By day two of week two, GR activities rapidly increased to near 300% more than those found in the control. Heat-stressed plants on days four and five of week two were near 30% higher than heat-stressed levels from week one.

Guaiacol peroxidase (POX)

POX activities of the control leaves were significantly similar across all days and weeks. Heat-stressed plants had significantly increased levels of POX for all days and weeks, however activities of week two were significantly different than what was observed in week one (Figure 5-2A). For week one, levels of POX had maximal activity on the second day, with decreasing amounts thereafter. Similar means were identified for both days one and two, which were significantly less than temperatures recorded for days one and two of the first week. The greatest leaf peroxidase activities of week two occurred on day three, with s activities for the remaining days of measurement.

Heat-stressed ovarian POX activities were significantly higher when compared to control ovaries for all days of measurement (Figure 5-2B). Control POX activities remained similar across all days of measurement. The first day of week one of heat stress, POX levels increased, but had the highest amounts of activity on the second day when activities were near 475% greater than the control of the same day. Activities decreased to similar levels by days four and five. Heat stress during week two had similar first day means as week one. However, day two

means for week two were the highest amongst all days, being almost 470% greater than the controls recorded on that day and more than 23% greater than day two of week one. Activities quickly decreased on day three, but were significantly greater than means of activity for the same days of week one.

Leaf Temperature

Leaf temperatures were significantly higher in heat-stressed plants for any given week or day, averaging approximately 26 ^{o}C (Figure 5-3A). Temperatures of heat-stressed plants did not fluctuate significantly throughout the experiment, only differing by a few tenths of a degree. Control temperatures expressed slightly more variation in temperatures, but confined to within a 22 - 23 ^{o}C range.

Electron Transport Rates

Electron transport rates for control leaves remained relatively stable throughout the course of the experiments regardless of week with rates averaging under 100 µmol electrons m^{-2} s^{-1} (Figure 5-3B). Rates of the heat-stressed plants remained significantly higher than the control for most all days examined. Significant increases in ETR were identified the first day of heat stress, and continued increase until day three of week one. These rates subsided, but remained above the values of the control. Week two ETRs increased in similar fashion as week one, however rates reached a maximum on day two, with slight decreases in ETRs for the remaining days. Means on day five of week one were statistically similar to results of day three of week two.

Membrane Leakage

Control leaves exhibited little change in injury throughout the duration of the experiment; however, significant differences were identified in heat-stressed tissues (Figure 5-3C). During

week one, the greatest injury occurred upon the first day of the first week with an increased 52% injury index compared to the control. Day two week one of heat stressed cotton had a significant decrease in injury indexes. By day three on the first week, injuries ceased being significantly different from the control. Injuries to heat-stressed membranes remained similar to the control for the remainder of week one. In week two of heat-stress, day one had significantly less leakage compared to day one of the first week. Injuries were only 27% greater compared to the control and significantly similar to the week one day two measurements. Day two of week two had significantly lower injuries compared to day one, approximately 9% greater injury indices than of the control. However, the injuries were significantly less than values observed in day two of week one. No significant differences could be determined between the controls or heat-stressed leaves after day three of week two.

Discussion

Stressors if applied over time may be identified as a benefit for increased reproductive success (Kozlowski and Pallardy, 2002). When examining a plant's reactive response to a new change in its environment, either experimentally or unintentionally, acclimation responses are often not reported experimentally. Acclimation is the gradual introduction of moderate amounts of stress over time that often provides a buffer of protection when compared to plants exposed abruptly. In this study, by allowing a week of ideal temperatures in between two weeks of heat stress, we could potentially identify signals of an overlooked parameter of success, cotton's acclimation potential.

Exposure to temperatures of 40 ^{o}C increased leaf temperatures by about 4 ^{o}C on average to about 26 ^{o}C (Figure 5-3A). These temperatures were within the well-established optimum temperature range of cotton of 20 – 30 ^{o}C (Reddy et al., 1991; Bibi et al., 2008). This is due in

part to the transpirational cooling as discussed by Burke and Upchurch (1989) to be within 23.5 – 32 ºC if water stressed conditions are to be avoided as was done in this experiment. Although leaf temperatures remained within their optimal temperature range, heat-stressed leaf membranes had a significant 52% increase in leakage when compared to control leaf tissues. This heat-labile porosity of the plasma membrane has been investigated and been identified as the result of increased peroxidation of the lipid bilayer (Dhindsa et al., 1981). This is most likely due to absorption of the surrounding thermal energies of the air and its conversion into the evaporative cooling processes. Membrane injury peaked on day one of week one and declined thereafter to being insignificantly different from the control by day three (Figure 5-3C). In the second week of heat stress, membranes had less injury as opposed to week one. The increased stability of the membrane over time is related to the increase in saturated and monounsaturated lipids associated with higher temperatures to reduce its fluidity and deter peroxidation (Zhang et al., 2005).

The membrane porosity also extend to that of the chloroplast. Electron transport rates have been shown to be negatively affected as temperatures increase as been previously reported in other species such as maize (Xu et al., 2010), barley (Pshybytko et al., 2008), and cotton (Snider et al., 2013). Our experiments indicated a significant increase in ETR for heat-stressed plants over the course of the experiment (Figure 5-3B). Increased fluorescence in heat-stressed plants is a characteristic of photosystem disruption. It has been suggested that the electron transfer of plastiquinone between both linear and cyclic transport systems is disrupted (Bukhov and Carpentier, 2004). Thus, heat stress alters the rate of the reduction of the plastiquinone pool and induces an increase in cyclic electron transport (Xu et al., 2010), which is used as a mitigation of excess electron energy when the photosystem is damaged. The increase in fluorescence agrees with previous research indicating that high temperature stress can affect

ETRs. However, this research shows that even though the leaf is operating within the optimal temperature range due to increased transpiration. The increase in ETR has been associated with increased rates of photosystem I reductions, shifting the photosystem complex towards cyclic photophosphorylation (Zhang and Sharkey, 2009). As the membrane stabilizes, ETR rates decrease, suggesting a shift back towards non-cyclic photophosphorylation. In week two, the photosystem complex indicated some acclimation response as the increased fluorescence seen in week one was not as steep in week two of heat stress (Figure 5-3B).

The increase in POX and GR activity within the tissues functions as a proxy for increased oxidative damage potentials, as the result of increased formation of reactive oxygen species precursors, such as hydrogen peroxide. Activities of POX activity peaks after day two of week one when levels are near double that of values seen in the control temperatures (Figure 5-2A). Levels of POX remained significantly above activities of the control for the duration of the experiment. Week two POX activity of the heat-stressed leaf tissues following day two had no indication of decreasing activity as was seen on day five of week one. Activities of GR in leaf tissues for week one appeared to mimic the injury curve of membrane leakage of the same time (Figure 5-3C). Activity levels of GR under heat stress have been shown to be dependent upon the available gluthathione pool of reduced glutathione (GSH) and the oxidized form (GSSG) within the tissue the maintenance of a high GSH / GSSG ratio (May et al., 1998; Snider et al., 2009). Since the resource pool and its ratio fluctuates in accordance to the environment conditions, it effects the regulation of other antioxidant defenses (May et al., 1998). Thus, an enhanced GR activity is seen as a general feature of enhanced oxidation within a tissue (Foyer and Noctor, 2005). However, the activity of GR in week two of leaf tissue was only increased for day two compared to other days, possibly indicating that the increased GR pools of GSH / GSSG were

sufficient for day one and optimized for day two. Other antioxidants, such as POX, were sufficient to accommodate the increased oxidative influx for the remaining days. Leaf activities of POX increased substantially on day two of week one (Figure 5-2A) after GR activities increased on day one (Figure 5-1A), and POX activities again increased on day three following the increase of GR activities of week two day two with GR activities falling rapidly thereafter and POX activities remaining elevated.

Conversely, activities of POX and GR in ovaries did not follow the patterns identified in the leaves. Peak GR activities on day one week one, slowly reduced over the period of the first week (Figure 5-1B), which led to an increase in POX activities a day later (Figure 5-2B). Activities remained significantly elevated for both GR and POX during the first week, and again were elevated for week two, at significantly increased levels of response. The maintenance of GR and POX enzymatic activities in the ovaries can be attributed to the heat sensitivity of the flower compared to leaves (Snider et al., 2009). Increased antioxidant levels have been attributed to increased protection from the damaging effects of both biotic and abiotic factors (Bartosz, 1997; Blokhina et al., 2003; Wahid et al., 2007).

The presented results indicate cotton adjusts biochemically to heat-stress. More interestingly, this is true even if leaf temperatures were within thermodynamic limits due to transpirational cooling. The difference of antioxidant response between leaves and ovary tissues suggests the importance of understanding the relationship of antioxidants to tissue type. ETR indicated a significant difference between control leaves, supporting previous work that photosynthesis remains a heat-sensitive component with modest acclimation response. However, the increased rates under well-watered conditions may not be detrimental as, the plant is capable of shifting into other forms of phosphorylation as the membrane stabilizes.

References

Arasimowicz, M., and J. Floryszak-Wieczorek. 2007. Nitric oxide as a bioactive signalling molecule in plant stress responses. Plant Sci. 172: 876–887.

Azhar, F., Z. Ali, M. Akhtar, A. Khan, and R. Trethowan. 2009. Genetic variability of heat tolerance, and its effect on yield and fibre quality traits in upland cotton (*Gossypium hirsutum* L.). Plant Breed. 128: 356–362.

Bartosz, G. 1997. Oxidative stress in plants. Acta Physiol. Plant. 19: 47–64.

Bibi, A.C., D.M. Oosterhuis, and E.D. Gonias. 2008. Photosynthesis, quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes. J. Cotton Sci. 12: 150–159.

Blokhina, O., E. Virolainen, and K. V. Fagerstedt. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: A review. Ann. Bot. 91: 179–194.

Brown, R., and D. Oosterhuis. 2010. High daytime temperature stress effects on the physiology of modern versus obsolete cotton cultivars. Am. J. Plant Sci. Biotechnol. 4: 93–96.

Bukhov, N., and R. Carpentier. 2004. Alternative photosystem I-driven electron transport routes: Mechanisms and functions. Photosynth. Res. 82: 17–33.

Burke, J.J., and D.R. Upchurch. 1989. Leaf temperature and transpirational control in cotton. Environ. Exp. Bot. 29: 487–492.

Burke, J.J., J. Velten, and M.J. Oliver. 2004. In vitro analysis of cotton pollen germination. Agron. J. 96: 359–368.

Chen, H.H., Z.Y. Shen, and P.H. Li. 1982. Adaptability of crop plants to high temperatures stress. Crop Sci. 22: 719-725.

Cooper, P., and T.H.D.H. Ho. 1983. Heat shock proteins in maize. Plant Physiol. 71: 215–222.

Crafts-Brandner, S.J., and M.E. Salvucci. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. Proc. Natl. Acad. Sci. U.S.A. 97: 13430–13435.

Dat, J.F., H. Lopez-Delgado, C.H. Foyer, and I.M. Scott. 1998. Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol. 116: 1351–1357.

Dhindsa, R.S., P. Plumb-dhindsa, and T. a. Thorpe. 1981. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. 32: 93–101.

Faver, K.L., T.J. Gerik, P.M. Thaxton, and K.M. El-Zik. 1996. Late season water stress in cotton: II. Leaf gas exchange and assimilation capacity. Crop Sci. 36: 922–928.

Feller, U., S. Crafts-Brandner, and M. Salvucci. 1998. Moderately high temperatures inhibit ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. Plant Physiol. 116: 539–546.

Flexas, J., J. Flexas, J.M. Escalona, J.M. Escalona, H. Medrano, and H. Medrano. 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. Plant Cell Environ. 22: 39–48.

Foreman, J., V. Demidchik, J.H.F. Bothwell, P. Mylona, H. Miedema, M.A. Torres, P. Linstead, S. Costa, C. Brownlee, J.D.G. Jones, J.M. Davies, and L. Dolan. 2003. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422: 442–446.

Foyer, C.H., and G. Noctor. 2005. Oxidant and antioxidant signalling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. Plant, Cell Environ. 28: 1056–1071.

Gechev, T.S., F. Van Breusegem, J.M. Stone, I. Denev, and C. Laloi. 2006. Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. BioEssays 28: 1091–1101.

Guinn, G. 1982. Causes of square and boll shedding. USDA Tech. Bull. 1672: 1–21.

Hake, K., and J. Silvertooth. 1990. High temperature effects on cotton. Cott. Physiol. Today 1: 1–4. National Cotton Council.

Karpinski, S., H. Reynolds, B. Karpinska, G. Wingsle, G. Creissen, and P. Mullineaux. 1999. Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. Science. 284: 654–657.

Kozlowski, T.T., and S.G. Pallardy. 2002. Acclimation and adaptive responses of woody plants to environmental stresses. Bot. Rev. 68: 270–334.

Larkindale, J., and B. Huang. 2004. Thermotolerance and antioxidant systems in *Agrostis stolonifera*: Involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide, and ethylene. J. Plant Physiol. 161: 405–413.

Liu, X., and B. Huang. 2008. Photosynthetic acclimation to high temperatures associated with heat tolerance in creeping bentgrass. J. Plant Physiol. 165: 1947–1953.

Lopez-Delgado, H., J.F. Dat, C.H. Foyer, and I.M. Scott. 1998. Induction of thermotolerance in potato microplants by acetylsalicylic acid and H_2O_2. J. Exp. Bot. 49: 713–720.

May, M.J., T. Vernoux, C. Leaver, M. Van Montagu, and D. Inze. 1998. Glutathione homeostasis in plants: Implications for environmental sensing and plant development. J. Exp. Bot. 49: 649–667.

Molinier, J., G. Ries, C. Zipfel, and B. Hohn. 2006. Transgeneration memory of stress in plants. Nature 442: 1046–1049.

Pshybytko, N.L., J. Kruk, L.F. Kabashnikova, and K. Strzalka. 2008. Function of plastoquinone in heat stress reactions of plants. Biochim. Biophys. Acta - Bioenerg. 1777: 1393–1399.

Radin, J.W., Z. Lu, R.G. Percy, and E. Zeiger. 1994. Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. Proc. Natl. Acad. Sci. 91: 7217–7221.

Reddy, V.R., K.R. Reddy, and D.N. Baker. 1991. Temperature effect on growth and development of cotton during the fruiting period. Agron. J. 83: 211–217.

Ruelland, E., and A. Zachowski. 2010. How plants sense temperature. Environ. Exp. Bot. 69: 225–232.

Sadras, V.O. 1995. Compensatory growth in cotton after loss of reproductive organs. Field Crop Res. 40: 1–18.

Schaedle, M., and J.A. Bassham. 1977. Chloroplast glutathione reductase. Plant Physiol. 59: 1011–1012.

Schrader, S.M., R.R. Wise, W.F. Wacholtz, D.R. Ort, and T.D. Sharkey. 2004. Thylakoid membrane responses to moderately high leaf temperature in Pima cotton. Plant, Cell Environ. 27: 725–735.

Schroeder, J., G. Allen, V. Hugouvieux, J. Kwak, and D. Waner. 2001. Guard cell signal transduction. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52: 627–658.

Schuster, W.S., and R.K. Monson. 1990. An examination of the advantages of C3-C4 intermediate photosynthesis in warm environments. Plant Cell Environ. 13: 903–912.

Sharkey, T.D. 2005. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. Plant, Cell Environ. 28: 269–277.

Snider, J.L., D.M. Oosterhuis, G.D. Collins, C. Pilon, and T.R. FitzSimons. 2013. Field-acclimated *Gossypium hirsutum* cultivars exhibit genotypic and seasonal differences in photosystem II thermostability. J. Plant Physiol. 170: 489–496.

Snider, J.L., D.M. Oosterhuis, D.A. Loka, and E.M. Kawakami. 2011. High temperature limits in vivo pollen tube growth rates by altering diurnal carbohydrate balance in field-grown

Gossypium hirsutum pistils. J. Plant Physiol. 168: 1168–1175.

Snider, J.L., D.M. Oosterhuis, B.W. Skulman, and E.M. Kawakami. 2009. Heat stress-induced limitations to reproductive success in *Gossypium hirsutum*. Physiol. Plant. 137: 125–138.

ur Rahman, H., S.A. Malik, and M. Saleem. 2004. Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane thermostability. Field Crop Res. 85: 149–158.

Verhoeven, K.J.F., J.J. Jansen, P.J. van Dijk, and A. Biere. 2010. Stress-induced DNA methylation changes and their heritability in asexual dandelions. New Phytol. 185: 1108–1118.

Wahid, A., S. Gelani, M. Ashraf, and M. Foolad. 2007. Heat tolerance in plants: An overview. Environ. Exp. Bot. 61: 199–223.

Xu, Z., G. Zhou, G. Han, and Y. Li. 2010. Photosynthetic potential and its association with lipid peroxidation in response to high temperature at different leaf ages in maize. J. Plant Growth Regul. 30: 41–50.

Yamasaki, T., T. Yamakawa, Y. Yamane, H. Koike, and K. Satoh. 2002. Temperature acclimation of photosynthesis and related changes in photosystem II electron transport in winter wheat. Plant Physiol. 128: 1087–1097.

Zhang, M., R. Barg, M. Yin, Y. Gueta-Dahan, A. Leikin-Frenkel, Y. Salts, S. Shabtai, and G. Ben-Hayyim. 2005. Modulated fatty acid desaturation via overexpression of two distinct ω-3 desaturases differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and plants. Plant J. 44: 361–371.

Zhang, R., and T.D. Sharkey. 2009. Photosynthetic electron transport and proton flux under moderate heat stress. Photosynth. Res. 100: 29–43.

Table 5-1: Statistical p-values for all examined measurements and subsequent treatment combinations.

	Peroxidase		*Glutathione Reductase*		*Fluorescence*	*Leaf Temperature*	*Membrane Leakage*
	Leaf	Ovary	Leaf	Ovary	ETR	^{o}C	Injury Index
Day	0.9678	<.0001	<.0001	0.0137	0.0001	0.2818	<.0001
Week	0.1625	0.0014	0.5590	0.5037	<.0001	0.0059	0.0787
Temperature	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
*Day*Week*	0.0186	0.3036	0.1412	0.2443	0.0221	0.1332	<.0001
*Day*Temperature*	0.1530	<.0001	<.0001	0.0236	0.0021	0.0102	<.0001
*Week*Temperature*	0.3312	0.0005	0.0359	0.0139	0.1680	0.1477	0.0003

Figures

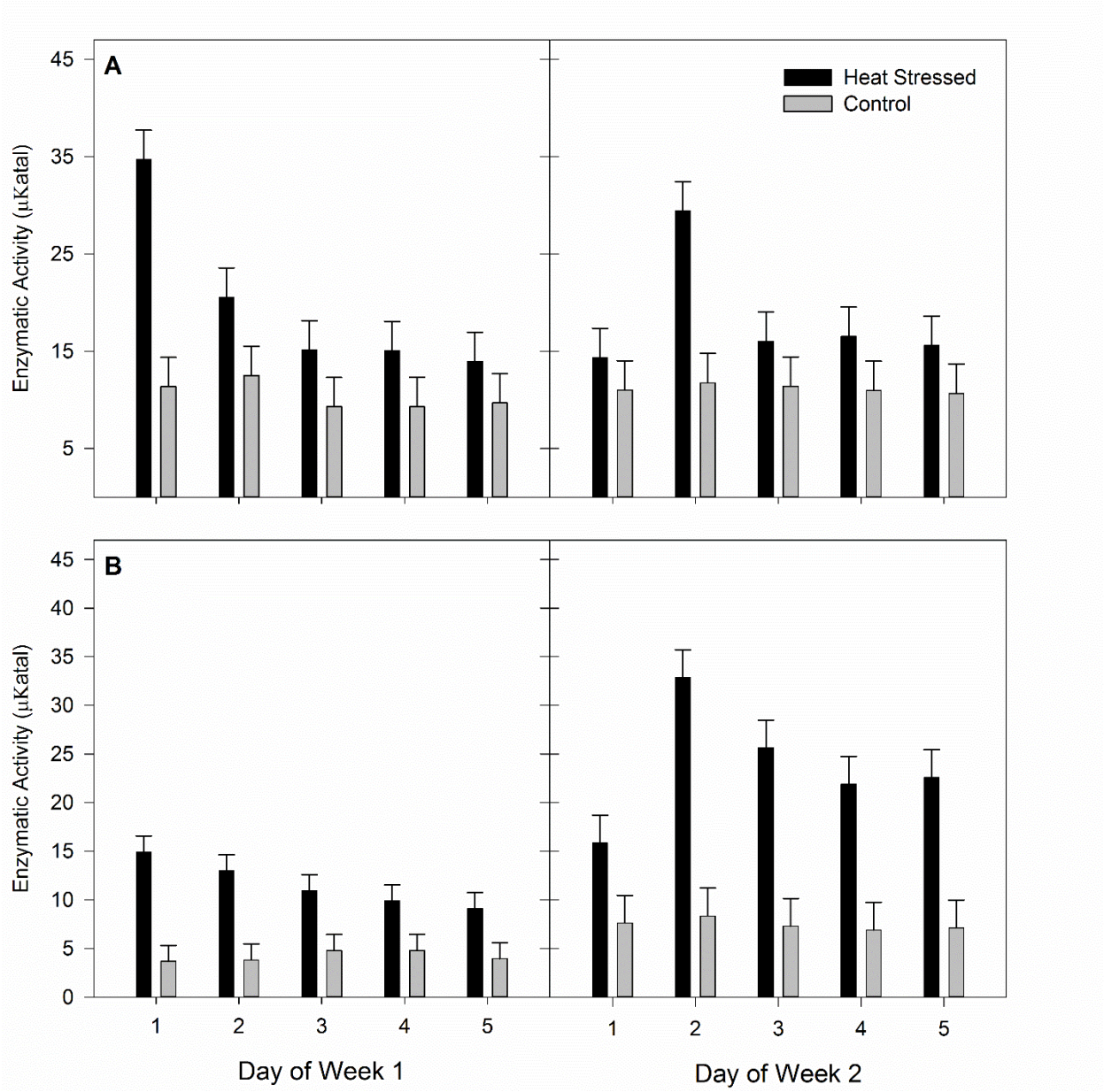

Figure 5-1: Enzymatic activity (µKatal) of GR of both (A) leaves and (B) ovaries for each day and week of heat stress (n = 10 daily measurements for both heat-stressed and control tissue types). Error bars represent the confidence interval at 95%.

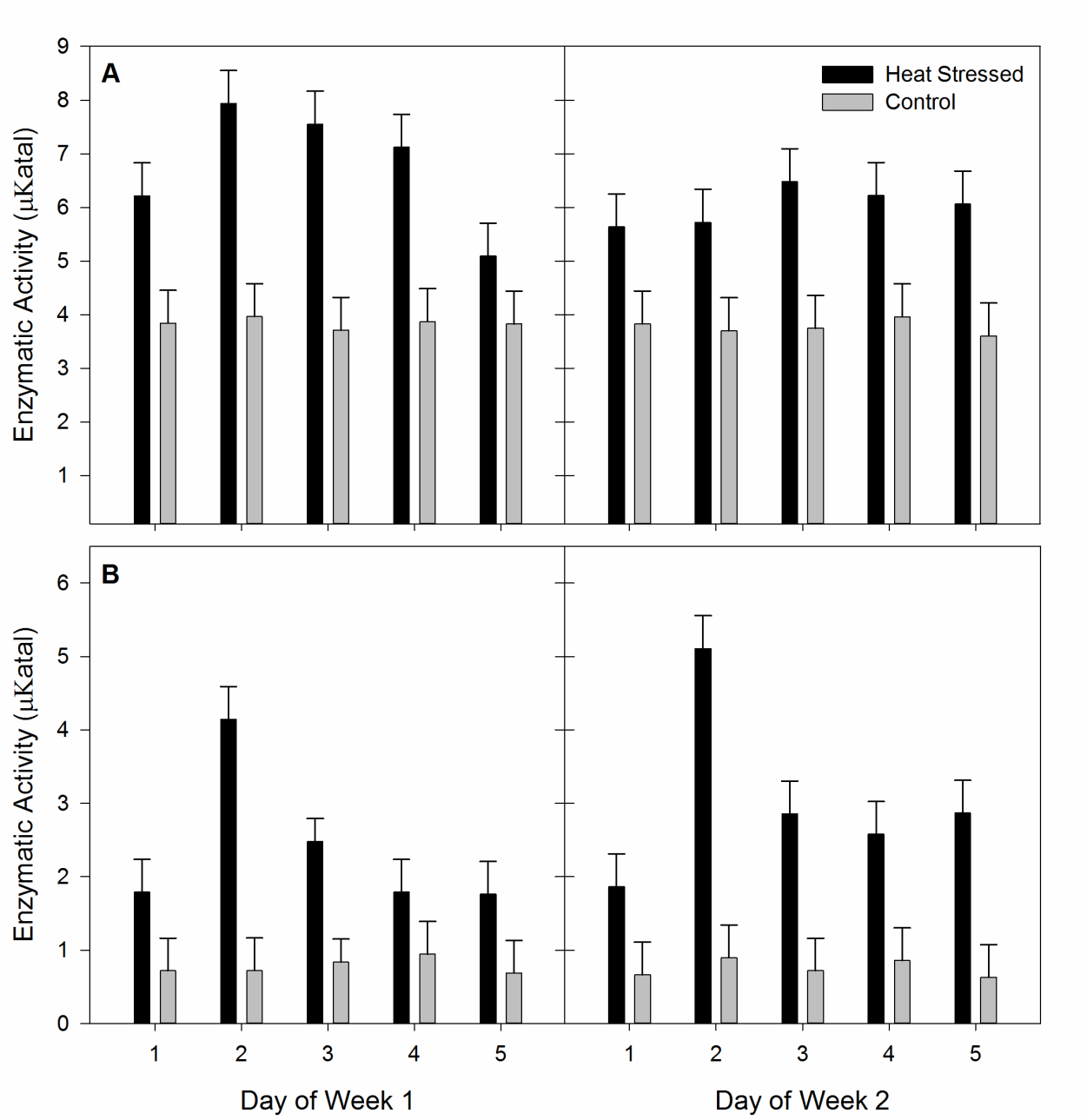

Figure 5-2: Enzymatic activity (µKatal) of POX of both (A) leaves and (B) ovaries for each day and week of heat stress (n = 10 daily measurements for both heat-stressed and control tissue types). Error bars represent the confidence interval at 95%.

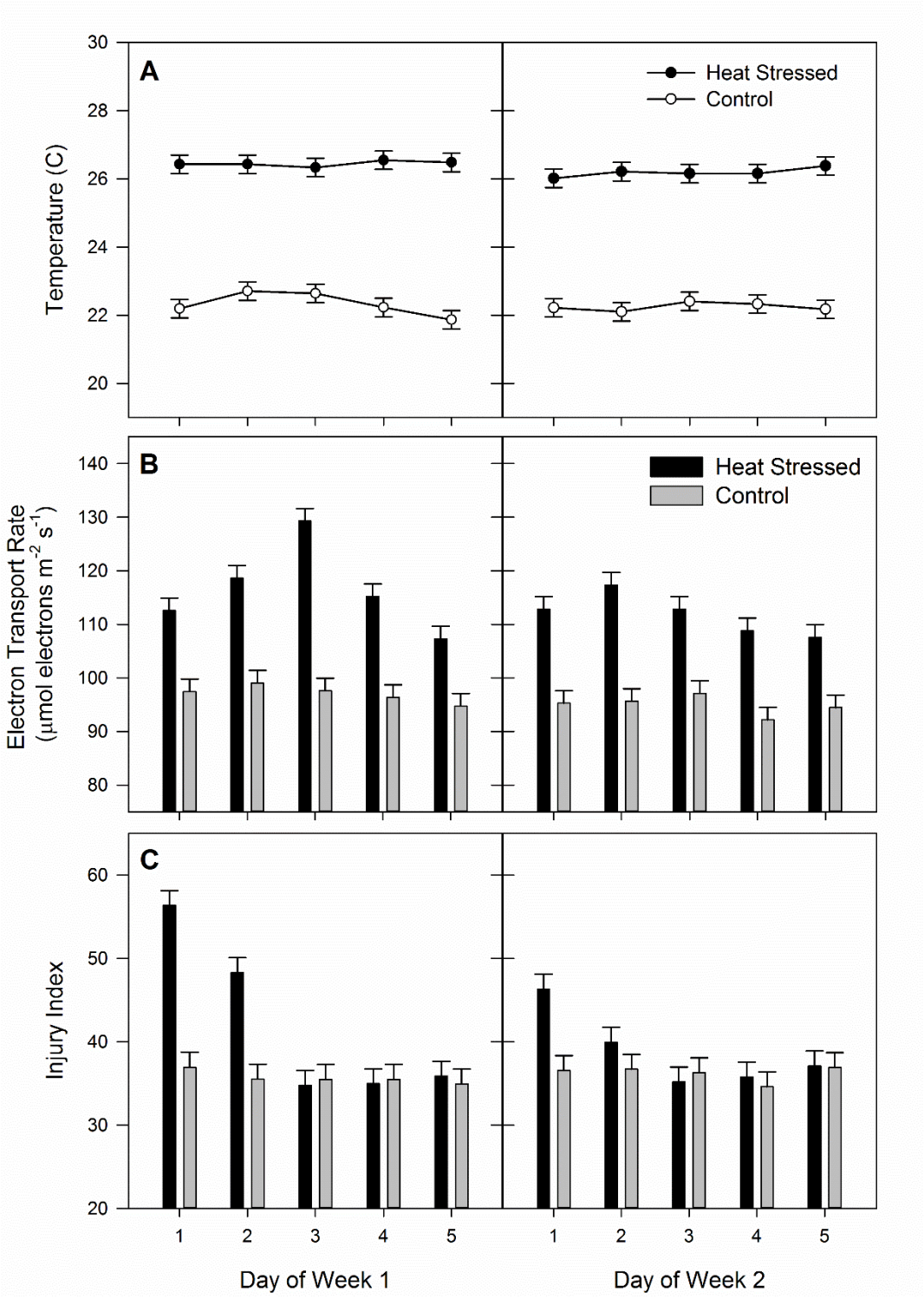

Figure 5-3: Daily measurements of the upper fourth main-stem leaf for (A) temperature, (B) ETR, and (C) Injury Index for each week of heat stress (n = 10 daily measurements for each parameter). Error bars represent the confidence interval at 95%.

CHAPTER VI

Heat Stress Negatively Affects Well-Irrigated Cotton's Leaf and Ovary Physiology Despite Cooler Canopy Temperatures

Abstract

A common alleviator for increasing canopy temperatures is to irrigate. This provides transpirational cooling in an effort to reduce the negative effects high temperature has upon yield. To analyze this compensatory measure, a study examining several biochemical effects under well-irrigated field conditions for heat related stress markers in cotton (*Gossypium hirsutum* L.) was initiated. Three data and tissue collections were made, prior to heat stress, during heat stress, and following heat stress. Canopy temperatures during heat stress were about 11 ^{o}C cooler than ambient air temperatures, however cell membrane porosity increased nearly 32% compared to membranes prior to heat stress. Electron transport rates (ETR) values also increased during heat stress indicating Photosystem II damage following heat stress. Carbohydrate concentrations changed significantly in leaf tissues with depressed starch content following heat stress, and increased hexose and sucrose concentrations during heat stress and maintained following heat stress. Ovaries of first position sympodial branch white flowers had increased sucrose concentrations both during and following heat stress, while hexose concentrations declined during heat stress but rebounded following stress, whereas starch concentrations continually fell throughout heat stress and following. Protein concentrations for both leaf and ovary tissues increased both during and following heat stress. The conclusions of this study provide evidence that transpirational cooling of the field canopy may not be fully capable of alleviating stress related physiological factors in field-grown cotton.

Introduction

Plants are frequently exposed to adverse weather conditions and must acclimate rapidly to the changing conditions to survive. If conditions are hostile for a significant period then plant growth may slow or cease until conditions improve. This is of particular concern agriculturally when significant impacts to yield are predicated on an extremely difficult factor to evade such as heat stress. Cotton (*Gossypium hirsutum* L.) is a perennial crop grown as an annual across the southern United States. For cotton, growth rates diminish when temperatures exceed 33 ^{o}C (Burke et al., 1988), with a significant portion of the southern United States where cotton is grown having summer averages far exceeding that optimal threshold. Additionally, cotton's most temperature sensitive stage of anthesis (Reddy et al., 1992) occurs during the warmest months of the year. Hence the development of more heat resistant cotton has been a primary focus for breeders (Iqbal et al., 2005), geneticists (Azhar et al., 2009; Khan et al., 2011), and researchers examining heat stress (Snider et al., 2010) related effects with chemical ameliorants (Kawakami et al., 2013). Yet, development of heat tolerant cotton is limited in that the domestication of wild-type cotton into its current lineages has reduced the genetic diversity for thermotolerance (Iqbal et al., 2001; Khan et al., 2011). This has led to many growers to use irrigation and the natural transpirational cooling of leaves to facilitate cooler canopy temperatures (Burke and Upchurch, 1989).

Past studies have emphasized the effects that heat stress has upon cotton's physiology. As ambient air temperatures rise above the optimal threshold overall yields decrease (Reddy et al., 1991). The reasons for this decrease are diverse, but include such physiological changes such as increases in the porosity of cell membranes (ur Rahman et al., 2004), which causes a significant disruption to photosystem II and the electron transport chain (Thomas et al., 1986; Bukhov et al.,

1999). This disruption of photosynthesis forces an increase in cyclic photophosphorylation to mitigate excessive light energies and maintain ATP (adenosine triphosphate) production for the short term (Schrader et al., 2004). Additionally, as temperatures increase, the Calvin-cycle begins to be impaired as rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) activase becomes increasing impaired (Crafts-Brandner and Salvucci, 2000). This in turn leads to a reduction of the carbohydrate precursor glyceraldehyde 3-phosphate diminishing soluble carbohydrate concentrations (Geigenberger et al., 1998a). Additionally, protein concentrations within the plant begin to decrease (Gulen and Eris, 2004) as upregulation of heat shock proteins to function as chaperones mitigating for heat-labile enzymes is increased (Larkindale and Vierling, 2008).

There is substantial research to indicate that irrigation can be utilized to prevent drought yield reductions in the field (Lacape et al., 1998; Falkenberg et al., 2007), but inferences of irrigated cotton must be made in relation to high temperature stress. It is reasonable to assume that heat stress related effects could be diminished through the use of irrigation to maintain temperatures within the optimum thermal range (Burke et al., 1988). Additionally, many studies investigate the effect of temperature stress without examinations further in the season after temperatures cool to determine if heat related injury returns to levels prior to heat stress. Thus, this study was instigated to test the hypothesis that even well irrigated fields would be physiologically affected by high temperature stress.

Materials and Methods

Cotton (*Gossypium hirsutum* L.) cv. ST5258B2RF was planted in May of 2011 and 2012 at the University of Arkansas agricultural station in Fayetteville, AR, USA at a density of eight plants per meter. Plot dimensions were 4 m by 15 m with a two-row border between each

treatment. The experiment contained four replication plots per year of study. Additionally, a second planting was made adjacent to the first field two weeks after the initial plot planting to ensure one field having high temperature stress at anthesis. The fields were irrigated to field capacity weekly. Fertilizer, herbicide, and insecticide applications were performed according to state recommended rates and practices.

Three tissue and data collections were made according to the ambient air temperatures. Collections were made at three different times during flowering, prior to heat stress when temperatures were at or below 33 ^{o}C, during heat stress when temperatures were over 38^{o}C, and following heat stress when temperatures had returned below 33 ^{o}C. Sampling occurred between 1300 and 1500 hours of each sampling date. Collected sampling consisted of ten first position sympodial leaves and white flowers between the 9th and 12th main-stem node. Collected tissues were kept on dry ice before being transferred back to the laboratory to be stored at -80 ^{o}C. Tissue samples were lyophilized the following day (Model 18DX485SA, Botanique Preservation Equipment, Inc., Phoenix, AZ) until dry. Lyophilized tissues were ground by hand in a mortar and pestle until finely powdered, then transferred into centrifuge tubes, capped, and held in the -80 ^{o}C until analysis.

Carbohydrate Extractions and Quantification

Soluble Carbohydrates

Soluble carbohydrate concentrations were measured according to the protocol outlined by Hendrix (1993). Fresh leaves sampled from the fourth main-stem node from the apical terminal bud and first position open white flowers were used for analysis (n = 15 samples per plot). Forty mg of lyophilized ground tissue was extracted with 1 ml of 80 ^{o}C aqueous ethanol (80%), centrifuged at 10,000 g for 10 minutes, and supernatant collected. The aqueous ethanol

extraction was repeated three times per 40 mg tissue sample, the extractions fractions combined, and finally brought to 3 ml with aqueous ethanol. The remaining pellet after the third extraction was reserved for later starch analyses. The remaining starch pellet was lyophilized to remove remaining ethanol, capped, and stored at -80 ^{o}C. To the ethanol extractions, 50 mg of activated charcoal was added and agitated. A 2 ml sample of activated charcoal and soluble sugar extracts was transferred to a microcentrifuge tube and centrifuged at 20,000 g for 20 minutes. The supernatant was collected for either immediate analyses of sucrose and hexoses (glucose and fructose) or stored at -80 ^{o}C.

Prior to analysis, a glucose assay reagent (G3293 Sigma Chemical Company, St. Louis., MO) was rehydrated and stored on ice. For the subsequent solution additives, a 100 ml HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 50 mM, and pH 7.2) buffer was made and stored on ice. In 10 ml of the HEPES buffer, 20 mM each of ATP (adenosine triphosphate) and NAD (nicotinamide adenine dinucleotide) were added, hence referred to as the HEPES additive buffer, and stored on ice. PGI (phosphoglucose isomerase, EC 5.3.1.9, Sigma Chemical Company, St. Louis., MO) enzyme was prepared in an aliquot of the HEPES additive buffer at a concentration of 0.25 EU per 10 µl and stored on ice for later use. Invertase (EC 3.1.1.26, Sigma Chemical Company, St. Louis., MO) was prepared in another aliquot of HEPES additive buffer at a concentration of 83 EU per 10 µl and stored on ice. The HEPES buffer and the rehydrated glucose assay reagent were stored at 4 ^{o}C for no longer than one week, whereas all additive solutions were prepared daily.

For glucose analysis, 20 µl of each extracted sample was added to all but the first column of a 96-well microplate. To the first column of the plate, an addition of 20 µl of a prepared glucose standards ranging from 0 to 0.5 µg / µl were introduced into each well. Plates were

lyophilized for 30 minutes to remove ethanol and moisture from the samples and standards. To all standards and samples, 100 µl of glucose assay reagent was added and incubated in the dark for 15 minutes at room temperature. Absorbance was measured at 340 nm using a MultiScan Ascent Microplate Reader (Thermo Fisher Scientific Inc., Waltham, MA). Glucose concentrations were calculated dependent upon the standard curve of the prepared first column standards, the extract volume, and the mass of each sample.

Fructose measurement followed initial glucose measurements by adding 10 µl of HEPES additive solution containing PGI to the wells, incubated for another 15 minutes at room temperature, and again read at 340 nm. Concentrations calculations were identical to glucose. Sucrose concentrations followed the glucose and fructose measurements with the addition of 20 µl of HEPES additive solution containing invertase to the wells. Plates incubated for 1 hour for complete sucrose hydrolysis and the absorbance read again at 340 nm. Sucrose concentrations were calculated according to the methods from Zhao *et al*. (2010).

Starch

The lyophilized tissue pellet reserved from the ethanol extractions was performed similarly to that of Hendrix (1993), but with the following modifications. A digital dry block heater (VWR International, Randor, PA) for 2 ml microcentrifuge tubes was used rather than a water bath. Additionally, to prevent caps from popping open during heating cycles, each microcentrifuge cap was punctured with small hole to allow for gas escape.

One ml of 1.0 M KOH was added to each tube and mixed thoroughly by vortexing and heated in a dry block heater for 1 hour at 85 ^{o}C. After heating, the pH was adjusted to 6.5 – 7.5, using aqueous 0.25 M acetic acid and vortexed extensively to eliminate pH gradients within the microcentrifuge tube. To the tube 100 µl of TRIS-HCl (Tris(hydroxymethyl)aminomethane

hydrochloride, 0.1 M, pH 7.2) was added and the tubes vortexed. To each tube, 100 µl of α-amylase from *Bacillus licheniformis* (A3403, EC 3.2.1.1, Sigma Chemical Company, St. Louis., MO) was added and vortexed thoroughly then incubated at 55 ^{o}C for 1 hour. After one hour, the pH was decreased to 4.5 to 5.0 using 1.0 M acetic acid in double deionized (DDI) water. To each tube, 0.25 ml of amyloglucosidase (A1602, EC 3.2.1.3, Sigma Chemical Company, St. Louis., MO) was added and vortexed. Tubes were adjusted to 1.5 ml with DDI water, vortexed, and subsequently incubated at 55 ^{o}C for 1 hour. After one hour, the dry block temperature was increased to 95 ^{o}C, and maintained for 5 minutes. Tubes were removed and centrifuged while still warm at 20,000 g for 20 minutes. The collected supernatant was transferred to another microcentrifuge tube and stored at -80 ^{o}C. To determine starch concentration, the glucose assay was performed. To account for water loss when glucose units were linked the calculated concentration was multiplied by 0.9.

Fluorescence

Efficacy of the photosystem was estimated via the electron transport rate (ETR) efficiency using light-adapted techniques as described by Flexas et al. (1999) using a OS5p Modulated Fluorometer (Opti-Science, Tyngsboro, MA) with modifications. Three areas from fully expanded upper fourth upper main-stem leaves were sampled between 1300 h and 1500 h and averaged for statistical analysis. The adaxial surface of the leaf was illuminated stepwise with increasing intensity (2850, 5700, and 8550 µmol m^{-2} s^{-1}) for approximately 0.95 s. The ETR of each averaged fluorescence was calculated via the following:

$$\left(\frac{Fv}{Fm'}\right) * (PAR) * 0.5 * 0.84$$

Where PAR is the photosynthetically active radiation at the leaf's surface, Fv / Fm' corresponds to the quantum efficiency of photosynthesis under ambient light conditions where *Fv* is the variable fluorescence rate. The 0.5 corresponds to the excitation energy being divided amongst both photosystems I and II, and 0.84 is a common leaf absorbance coefficient in C3 plants.

Membrane Leakage

Membrane leakage measurements were sampled from each fully expanded fourth main-stem leaf from the apical using a leaf punch similar to one described by Wullschleger and Oosterhuis (1986). Three 1 cm^2 discs were excised from the leaf's surface during the day between 1300 h and 1500 h. Disc collections avoided both major and secondary veins. The discs were submerged in scintillation tubes filled with 10 ml of DDI (double deionized) water. Vials were stored in the dark at room temperature for 24 hours. The scintillation vial's water was quantified using an electro-conductivity (EC) meter (Primo 5, HANNA Instruments, Rhode Island USA) and recorded as the initial reading. The scintillation tubes were then capped and autoclaved for 20 minutes to dissociate all cellular cytosols. Once the vials were at room temperature, the EC was again measured. Membrane leakage was calculated as a percent change of the EC before and after autoclaving:

$$1 - \left(\frac{Autoclaved\ EC - Initial\ EC}{Autoclaved\ EC}\right) * 100$$

Where lower values indicate lessened membrane injury due to the environmental conditions.

Leaf Temperature

Recording of leaf temperatures occurred simultaneously alongside the fluorescence measurements using the embedded K-type thermocouple in the OS5p Modulated Fluorometer (Opti-Science, Tyngsboro, MA) light wand. To minimize temperature variance from leaf to leaf,

measurements occurred 3 to 5 seconds after the wand's application. The average of three measurements per leaf was utilized for statistical analyses.

Protein

Leaf and ovary protein extractions procedures according to Anderson et al. (1992) were followed with modification. The final concentrations of the extraction buffer was 50 mM of PIPES sodium salt (1,4-Piperazinediethanesulfonic acid sodium salt), 6 mM of cysteine hydrochloride, 10 mM D-Isoascorbic acid, 1mM EDTA (Ethylenediaminetetraacetic acid), 1% PVP (Polyvinylpyrrolidone) (w/v), and 0.3% Triton X-100. One hundred mg of ground, lyophilized tissue was transferred to a 10 ml centrifugation tube kept on ice. Extraction buffer was added to each tube at a ratio of 15 times the weight of the sample. The extracted sample and buffer solution was vortexed repeatedly and then centrifuged at 5,000 g for 15 minutes at 4 ^{o}C. Supernatant was collected and recorded. Protein was analyzed similarly to the methods outlined by Bradford (1976) using bovine albumin as the standard.

Statistics

All statistical analyses were performed using JMP 11.0 software (SAS Institute Inc., Cary, NC) using a p value of 0.05. There was no significant differences between years identified, thus the datasets from 2011 and 2012 were combined for analysis. All ANOVA tests and subsequent mean examinations using the Tukey goodness of fit test were considered significant at or below a p-value of 0.05.

Results

Leaf Temperatures

Field cotton were affected by the warmer ambient air temperatures. Warmer temperatures in the field (39.5 ^{o}C) elicited significantly cooler leaves compared to leaves (28 ^{o}C) measured

either before or after heat stress (Figure 6-1). The cooler heat-stressed leaves were highly significant ($p < .001$, Table 6-1) compared to leaf temperatures before and after heat stress which were not significantly different from each other.

Membrane Leakage

Temperatures greater than optimal significantly affected leaf electrolyte leakage ($p < .0001$, Table 6-1). Heat stress caused a 31.6% increase in leakage compared to leakages prior to heat stress. Following heat stress, membrane stability returned and the leakage was similar to that before heat stress, differing by only 1.3% (Figure 6-2).

Leaf Electron Transport Rate

ETR is associated with the stability of the photosystem complex. Higher ETR during heat stress indicates that absorbed photons are diverted from photosystem II to photosystem I, transitioning to a cyclic photophosphorylation to mitigate stress to the photosystem II complex (Bukhov et al., 2001). Heat stress significantly increased ($p < 0.0001$, Table 6-1) the amount of ETR associated with the photosystem complex by 44.7% when compared to ETR rates prior to heat stress (Figure 6-2). Once heat stress was relieved from the field, the ETR continued to remain statistically similar to that of heat stress though ambient air temperatures were lower than prior to heat stress.

Leaf Carbohydrate

Heat stress significantly affected the concentrations of soluble sugars within the leaf tissues (Table 6-1). At the onset of heat stress, hexose concentrations significantly increased by 57.7% compared to concentrations before heat stress and remained elevated after heat stress was relieved (Figure 3a, $p < .0001$, Table 6-1). Sucrose concentrations within the leaf increase significantly by 55.3% after the onset of heat stress. Following heat stress, leaf sucrose

concentrations remained statistically similar to concentrations measured during heat stress and significantly higher than concentrations found prior to heat stress (Figure 6-3A, $p = 0.006$, Table 6-1). Starch concentrations before heat stress were not significantly different from leaves undergoing heat stress ($p < .0001$). However, following heat stress, leaf starch concentrations decreased significantly by 55.3% compared to leaves during heat stress.

Leaf Protein Concentration

Protein concentrations (mg / g dry weight) increased significantly over the duration of the experiment (Figure 6-4, $p < .0001$, Table 6-1). Leaf protein concentrations increased by 15.0% during heat stress compared to concentrations prior to heat stress. Additionally, levels after heat stress were 19.2% greater than during heat stress and 37.1% greater than concentrations before heat stress occurred.

Ovary Carbohydrate Concentration

Heat stress significantly altered the concentrations of all sugars and starches (Figure 6-3B). Sucrose concentrations increased linearly throughout the experiment. Sucrose increased by 33.3% under heat stress conditions compared to conditions prior to heat stress, and increased a further 28.4% following heat stress. Hexose concentrations also declined significantly during heat stress, declining 40.0%, but rebounded following heat stress to the initial concentrations ($p < .0001$, Table 6-1). Starch, however, declined rapidly from initial concentrations. During heat stress, starch declined 23.7% and following heat stress, starch levels continued to decrease another 52.5%. In total, starch levels reduced by 63.8% from initial concentrations.

Ovary Protein Concentration

Ovary concentrations increased significantly over the duration of measurements following a linear trend (Figure 6-4, $p < .0001$, Table 6-1). During heat stress, concentrations of

protein increased by 46.2% compared to conditions prior to heat stress. Following heat stress, protein concentrations continued to increase a further 21.1%. In total, protein concentrations increased 77.0% compared to initial concentrations.

Discussion

Generally, under plentiful water conditions, as ambient temperatures increase so too do rates of transpiration. These increased transpiration rates affect leaf temperature by cooling it to temperatures much lower than the ambient (Burke and Upchurch, 1989). In this study, ambient temperatures during heat stress was 39 ^{o}C, and upper canopy leaf temperatures were close to 28 ^{o}C, an 11 ^{o}C difference which coincides with temperature differences identified by Burke and Upchurch (1989). As ambient air temperatures diminished, leaf temperatures increased to about 29.5 ^{o}C. This thermoregulation maintains the optimal enzymatic kinetics that many cellular processes require; which for cotton is between 23.5 and 32 ^{o}C (Burke et al., 1988). However, cooler tissue temperatures did not mitigate disruptions in photosynthetic capacity as indicated by the increased ETR during heat stress.

Heat stress effects upon membrane integrity strongly affects the homeostasis and functions of the cell (Ashraf et al., 1994; Bibi et al., 2008). Thylakoid sensitivity was demonstrated by Bukhov et al. (1999) showing that as leaf temperatures approached 40 ^{o}C electron flow through photosystem II was disrupted. This was compensated by increasing rates of cyclic respiration to provide sufficient ATP (adenosine 5'-triphosphate) for cellular functions (Schrader et al., 2004). Following heat stress, membrane leakages returned to injuries similar to membranes measured prior to heat stress; however, ETR values continued to remain high. Wise et al. (2004) suggested that photosynthetic efficiency reductions even in irrigated fields were due

to spikes of temperature preceding the leaf cooling which disrupts the electron transport chain, reducing rubisco activity as an adaptive response.

Wise et al. (2004) noted that the flow of electrons in the photosystem restricts carbon assimilation due to inhibition of rubisco regeneration. Their results indicated that electron transport capacity could be sufficiently limited when ETR values rise to between 200 to 300 μmol electrons m^{-2} s^{-1}, regardless of temperature. Increased levels of fluorescent remittance indicate an inefficiency of the photosystem due to reaction sites that are either closed or impaired (Maxwell and Johnson, 2000). Our results indicated a significant increase in the ETR of heat-stress field cotton. Since heat stress impedes photosystem II and ETR is related to the efficiency of photosystem II (Maxwell and Johnson, 2000; Flexas et al., 2004), we believe that the increased ETR values we identified were due to a disruption of the photosystem II complex. Additionally, the increased porosity of the membranes that we identified during heat stress would have further limited the efficiency of photosystem II. This is supported by the work of Bibi et al. (2008) who examined both modern and obsolete cotton cultivars relating membrane leakage to photosynthetic efficiency. They noted that as temperatures increased above 36 ^{o}C, the porosity of the membrane rapidly increased while the efficiency of photosystem II markedly decreased which coincides with our findings.

High temperatures impede proper carbohydrate metabolism within plant tissue (Lafta and Lorenzen, 1995). Additionally, heat stress enhances respiration activities of cotton tissues linearly as temperatures increase (Bednarz and van Iersel, 2001). Increases in respiration require increases in ATP production to satisfy cellular demands as photosystem II efficiency is decreased. Likewise, increased hexose concentrations, similar to our results following heat stress, can also decrease photosynthetic activity by interfering with transcriptional regulation of

photosynthesis (Sheen, 1990) through hexokinase activities (Moore et al., 2003) which regulate hexose concentrations. Thus, higher levels of hexose in photosynthesis provide a feedback mechanism to reduce photosynthetic activity. The higher levels of hexose present in the leaves following heat stress in our study may be an adaptive response to limit the observed photosynthetic activity to prevent further damage to the photosystem.

Sugars such as sucrose and glucose act either as substrates for cellular respiration or as osmolytes to maintain homeostasis under abiotic stresses (Gupta and Kaur, 2005). In field-grown cotton, post-high temperature stress increases the amount of sucrose and total soluble carbohydrate concentrations (Snider et al., 2011). Similarly, in *Arabidopsis*, under well-watered heat stress conditions sucrose production is upregulated and serves as the primary osmolyte (Rizhsky et al., 2004). A practical explanation for the increased sucrose concentrations following heat stress could be due to decreased respiration rates due to lower temperatures (Darnell, 2013). Additionally, flowers further up in the canopy would have the disadvantage of being weaker carbohydrate sinks then older bolls (Kerby and Buxton, 1981), therefore increasing sucrose levels in these ovaries following heat stress could provide better sink competition for available photoassimilates.

Heat stress conditions during wheat (*Triticum aestivum* L.) grain development indicated higher rates of soluble sucrose synthase and heat shock protein production as protective strategies (Sumesh et al., 2008). Developing cotton bolls are very strong carbohydrate sinks, and can increase their weight by as much as 15% per day (Schubert et al., 1986). The continual increase in sucrose that we identified may be explained by the increased competitive sink of young floral buds as the season progresses (Guinn, 1982).

Starch content has been associated as a proxy for successful flower development (Zhao and Oosterhuis, 2000). In our study, white flower starch content continued to decrease following heat stress, to levels far lower than what was previously reported in older white flowers by Zhao and Oosterhuis (2000). Though this effect has been reported in potato (*Solanum tuberosum* L.) tubers, where increasing temperatures lead to decreased rates of starch synthesis (Geigenberger et al., 1998b). This would correlate with our analysis of carbohydrates in white flowers following heat stress. Therefore, it is possible that the reproductive unit's carbohydrate imbalances will induce increased shedding rates after high temperatures had ceased. However, more investigations need to be performed to validate this hypothesis.

Proteins under high temperature stress tend to denature with increasing temperatures and as a result upregulate the amount of heat-shock proteins to function as molecular chaperones to maintain homeostasis (Feder and Hofmann, 1999; Kotak et al., 2007). The function of molecular chaperones are to bind and stabilize unstable conformers of proteins for proper oligomeric folding, function, transport, and facilitate in their aggregated disposal (Hendrick and Hartl, 1995). Zhang et al. (2003) identified that the *Glycine max* co-chaperones know as HOP proteins work in unison with other heat shock proteins. These HOP proteins were normally present in low levels, but their levels increased during stress. Our results are in contrast to other studies that have indicated decreases of both leaf (Chaitanya et al., 2001) and pistil protein concentrations. There is a shortage of literature explanation concerning increases in protein concentration following heat stress, particularly for field-grown cotton. Snider et al. (2011) examined soluble protein extractions taken over the course of the day in in-field cotton during and following heat stress. Their work indicated that during the afternoons of high temperature days soluble protein concentrations decreased during the afternoon hours, but increased once heat stress had subsided.

Their results coincides with ours suggesting an increase in protein concentrations following heat stress, yet no examinations of protein concentrations prior to heat stress were performed in Snider et al. (2011). Elucidation of our post-heat stress protein concentration increase cannot be adequately explained and requires further investigation.

Heat significantly affected cotton leaf and ovary physiology by affecting carbohydrate production and concentration ratios. Leaf photosynthesis efficiency declined under high temperature stress despite a decrease in leaf temperature compared to the ambient. However, increased membrane leakage also coincided with lower photosynthetic rates, indicating thylakoid and photosystem II disruption. Additionally, hexose concentrations in the leaf following heat stress were similar to levels present during heat stress, which may be related to hexokinase activity downregulation of photosynthetic activities though it has not been demonstrated in cotton. Additionally, higher temperatures affected ovary carbohydrate concentrations, with significantly decreasing levels of starch as the season progressed. This suggests that floral bud carbohydrate concentrations may have retained their heat-stressed carbohydrate influence into flowering; however, this too requires additional investigation. Further, our results suggest that irrigated cotton in the field may increase protein concentrations during and following heat stress, which is in contradiction with previous research. Yet, the parameters for many of those studies did not examine well-irrigated fields as was performed here. This research study demonstrate that despite a significant amount of transpirational cooling in the canopy, cotton is still affected by high air temperatures in the field.

References

Anderson, J. V., B.I. Chevone, and J.L. Hess. 1992. Seasonal variation in the antioxidant system of evidence for thermal dependence. Plant Physiol. 98: 501–508.

Ashraf, M., M.M. Saeed, and M.J. Qureshi. 1994. Tolerance to high temperature in cotton (*Gossypium hirsutum* L.) at initial growth stages. Environ. Exp. Bot. 34: 275–283.

Azhar, F., Z. Ali, M. Akhtar, A. Khan, and R. Trethowan. 2009. Genetic variability of heat tolerance, and its effect on yield and fibre quality traits in upland cotton (*Gossypium hirsutum* L.). Plant Breed. 128: 356–362.

Bednarz, C.W., and M.W. van Iersel. 2001. Temperature response of whole-plant CO_2 exchange rates of four upland cotton cultivars differing in leaf shape and leaf pubescence. Commun. Soil Sci. Plant Anal. 32: 2485–2501.

Bibi, A.C., D.M. Oosterhuis, and E.D. Gonias. 2008. Photosynthesis, quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes. J. Cotton Sci. 12: 150–159.

Bradford, M.M. 1976. A rapid method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

Bukhov, N.G., G. Samson, and R. Carpentier. 2001. Nonphotosynthetic reduction of the intersystem electron transport chain of chloroplasts following heat stress. Photochem. Photobiol. 74: 438–443.

Bukhov, N.G., C. Wiese, S. Neimanis, and U. Heber. 1999. Heat sensitivity of chloroplasts and leaves: Leakage of protons from thylakoids and reversible activation of cyclic electron transport. Photosynth. Res. 59: 81–93.

Burke, J.J., J.R. Mahan, and J.L. Hatfield. 1988. Crop-specific thermal kinetic windows in relation to wheat and cotton biomass production. Agron. J. 80: 553–556.

Burke, J.J., and D.R. Upchurch. 1989. Leaf temperature and transpirational control in cotton. Environ. Exp. Bot. 29: 487–492.

Chaitanya, K.V., D. Sundar, and A.R. Reddy. 2001. Mulberry leaf metabolism under high temperature stress. Plant Sci. 44: 379–384.

Crafts-Brandner, S.J., and M.E. Salvucci. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. Proc. Natl. Acad. Sci. U. S. A. 97: 13430–13435.

Darnell, R. 2013. Night temperature and source-sink effects on growth, leaf carbon exchange rate, and carbohydrate accumulation in bell pepper ovaries. J. Am. Soc. Hortic. Sci. 138:

331–337.

Falkenberg, N.R., G. Piccinni, J.T. Cothren, D.I. Leskovar, and C.M. Rush. 2007. Remote sensing of biotic and abiotic stress for irrigation management of cotton. Agric. Water Manag. 87: 23–31.

Feder, M.E., and G.E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61: 243–282.

Flexas, J., J. Bota, F. Loreto, G. Cornic, and T.D. Sharkey. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biol. 6: 269–279.

Geigenberger, P., M. Geiger, and M. Stitt. 1998a. High-temperature perturbation of starch synthesis is attributable to inhibition of ADP-glucose pyrophosphorylase by decreased levels of c in growing potato tubers. Plant Physiol. 117: 1307–1316.

Geigenberger, P., M. Geiger, and M. Stitt. 1998b. High-Temperature Perturbation of Starch Synthesis Is Attributable to Inhibition of ADP-Glucose Pyrophosphorylase by Decreased Levels of Glycerate-3-Phosphate in Growing Potato Tubers. Plant Physiol. 117: 1307–1316.

Guinn, G. 1982. Causes of square and boll shedding. USDA Tech. Bull. 1672: 1–21.

Gulen, H., and A. Eris. 2004. Effect of heat stress on peroxidase activity and total protein content in strawberry plants. Plant Sci. 166: 739–744.

Gupta, A.K., and N. Kaur. 2005. Sugar signalling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. J. Biosci. 30: 761–776.

Hendrick, J.P., and F.U. Hartl. 1995. The role of molecular chaperones in protein folding. FASEB J. 9: 1559–1569.

Hendrix, D.L. 1993. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. Crop Sci. 1311: 1306–1311.

Iqbal, M.J., M.Z. Iqbal, R.S.A. Khan, and K. Hayat. 2005. Comparison of obsolete and modern varieties in view to stagnancy in yield of cotton (*G. hirsutum* L.). Asian J. Plant Sci. 4: 374–378.

Iqbal, M.J., O.U.K. Reddy, K.M. El-Zik, and a. E. Pepper. 2001. A genetic bottleneck in the 'evolution under domestication' of upland cotton *Gossypium hirsutum* L. examined using DNA fingerprinting. TAG Theor. Appl. Genet. 103: 547–554.

Kawakami, E.M., D.M. Oosterhuis, J.L. Snider, and T.R. FitzSimons. 2013. High temperature and the ethylene antagonist 1-methylcyclopropene alter ethylene evolution patterns, antioxidant responses, and boll growth in *Gossypium hirsutum*. Am. J. Plant Sci.: 1400–

1408.

Kerby, T.A., and D.R. Buxton. 1981. Competition between adjacent fruiting forms in cotton. Agron. J. 85721: 867–871.

Khan, A.I., I.A. Khan, F.S. Awan, H.A. Sadaqat, and S. Bahadur. 2011. Estimation of genetic distance based on RAPDs between 11 cotton accessions varying in heat tolerance. Genet. Mol. Res. 10: 96–101.

Kotak, S., J. Larkindale, U. Lee, P. von Koskull-Döring, E. Vierling, and K.-D. Scharf. 2007. Complexity of the heat stress response in plants. Curr. Opin. Plant Biol. 10: 310–316.

Lacape, M.J., J. Wery, and D.J.M. Annerose. 1998. Relationships between plant and soil water status in five field-grown cotton (*Gossypium hirsutum* L.) cultivars. Field Crop Res. 57: 29–43.

Lafta, A.M., and J.H. Lorenzen. 1995. Effect of high temperature on plant growth and carbohydrate metabolism in potato. Plant Physiol. 109: 637–643.

Larkindale, J., and E. Vierling. 2008. Core genome responses involved in acclimation to high temperature. Plant Physiol. 146: 748–761.

Maxwell, K., and G.N. Johnson. 2000. Chlorophyll fluorescence -- A practical guide. J. Exp. Bot. 51: 659–668.

Moore, B., L. Zhou, F. Rolland, Q. Hall, W.-H. Cheng, Y.-X. Liu, I. Hwang, T. Jones, and J. Sheen. 2003. Role of the *Arabidopsis* Glucose Sensor HXK1 in Nutrient, Light, and Hormonal Signaling. Science. 300: 332–336.

Reddy, K.R., H.F. Hodges, J.M. McKinion, and G.W. Wall. 1991. Temperature effects on Pima cotton growth and development. Agron. J. 84: 237–243.

Reddy, K.R., H.F. Hodges, and V.R. Reddy. 1992. Temperature effects on cotton fruit retention. Agron. J. 84: 26–30.

Rizhsky, L., H. Liang, J. Shuman, V. Shulaev, S. Davletova, R. Mittler, Rizhsky L., Liang H., Shuman J., Shulaev V., Davletova S., and M. R. 2004. When defense pathways collide. The response of arabidopsis to a combination of drought and heat stress. Plant Physiol. 134: 1683–1696.

Schrader, S.M., R.R. Wise, W.F. Wacholtz, D.R. Ort, and T.D. Sharkey. 2004. Thylakoid membrane responses to moderately high leaf temperature in Pima cotton. Plant, Cell Environ. 27: 725–735.

Schubert, A.M., C.R. Benedict, and R.J. Kohel. 1986. Carbohydrate distribution in bolls. p. 311–324. *In* Mauney, J.R., Stewart, J.M. (eds.), Cotton Physiology. The Cotton Foundation,

Memphis, Tennessee.

Sheen, J. 1990. Metabolic repression of transcription in higher plants. Plant Cell 2: 1027–1038.

Snider, J.L., D.M. Oosterhuis, D.A. Loka, and E.M. Kawakami. 2011. High temperature limits in vivo pollen tube growth rates by altering diurnal carbohydrate balance in field-grown *Gossypium hirsutum* pistils. J. Plant Physiol. 168: 1168–1175.

Sumesh, K. V., P. Sharma-Natu, and M.C. Ghildiyal. 2008. Starch synthase activity and heat shock protein in relation to thermal tolerance of developing wheat grains. Biol. Plant. 52: 749–753.

Thomas, P.G., P.J. Quinn, and W.P. Williams. 1986. The origin of photosystem-I-mediated electron transport stimulation in heat-stressed chloroplasts. Planta 167: 133–139.

ur Rahman, H., S.A. Malik, and M. Saleem. 2004. Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane thermostability. F. Crop. Res. 85: 149–158.

Wise, R.R., A.J. Olson, S.M. Schrader, and T.D. Sharkey. 2004. Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature. Plant, Cell Environ. 27: 717–724.

Wullschleger, S.D., and D.M. Oosterhuis. 1986. A rapid leaf-disc sampler for psychrometric water potential measurements. Plant Physiol. 81: 684–685.

Zhang, Z., M.K. Quick, K.C. Kanelakis, M. Gijzen, and P. Krishna. 2003. Characterization of a plant homolog of HOP, a cochaperone of HSP90. Plant Physiol. 131: 525–535.

Zhao, D., C.T. MacKown, P.J. Starks, and B.K. Kindiger. 2010. Rapid analysis of nonstructural carbohydrate components in grass forage using microplate enzymatic assays. Crop Sci. 50: 1537–1545.

Zhao, D., and D.M. Oosterhuis. 2000. Dynamics of non-structural carbohydrates in developing leaves, bracts and floral buds of cotton. Environ. Exp. Bot. 43: 185–195.

Tables

Table 6-1: Consolidated Tukey goodness of fit connecting letters report and p-value for each measurement factor tested. Similar letters indicate no significant difference between sampling periods for each measurement factor and tissue type analyzed.

Measurement		Tissue	Before Heat	During Heat	After Heat	P-Value
Fluorescence		Leaf	B	A	A	<.0001
Membrane Leakage		Leaf	B	A	B	<.0001
Temperature		Leaf	A	B	A	<.0001
Carbohydrate	*Hexose*	Leaf	B	A	A	<.0001
	Starch		A	A	B	<.0001
	Sucrose		B	A	A	.006
	Hexose	Ovary	A	B	A	<.0001
	Starch		A	B	C	<.0001
	Sucrose		C	B	A	<.0001
Soluble Protein Concentration		Leaf	C	B	A	<.0001
		Ovary	C	B	A	<.0001

Figures

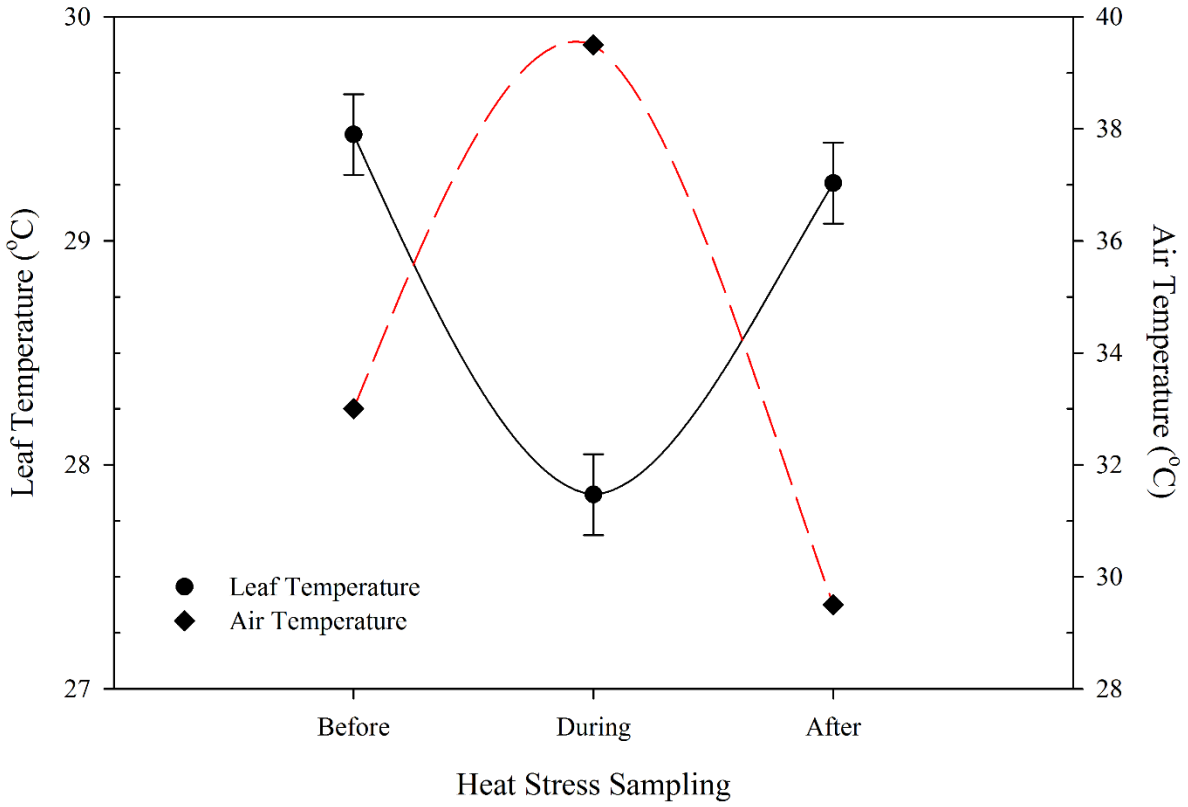

Figure 6-1: Average leaf and air temperatures during the sample collections. Error bars indicate the confidence interval for data of each point at $\alpha = 0.05$ level.

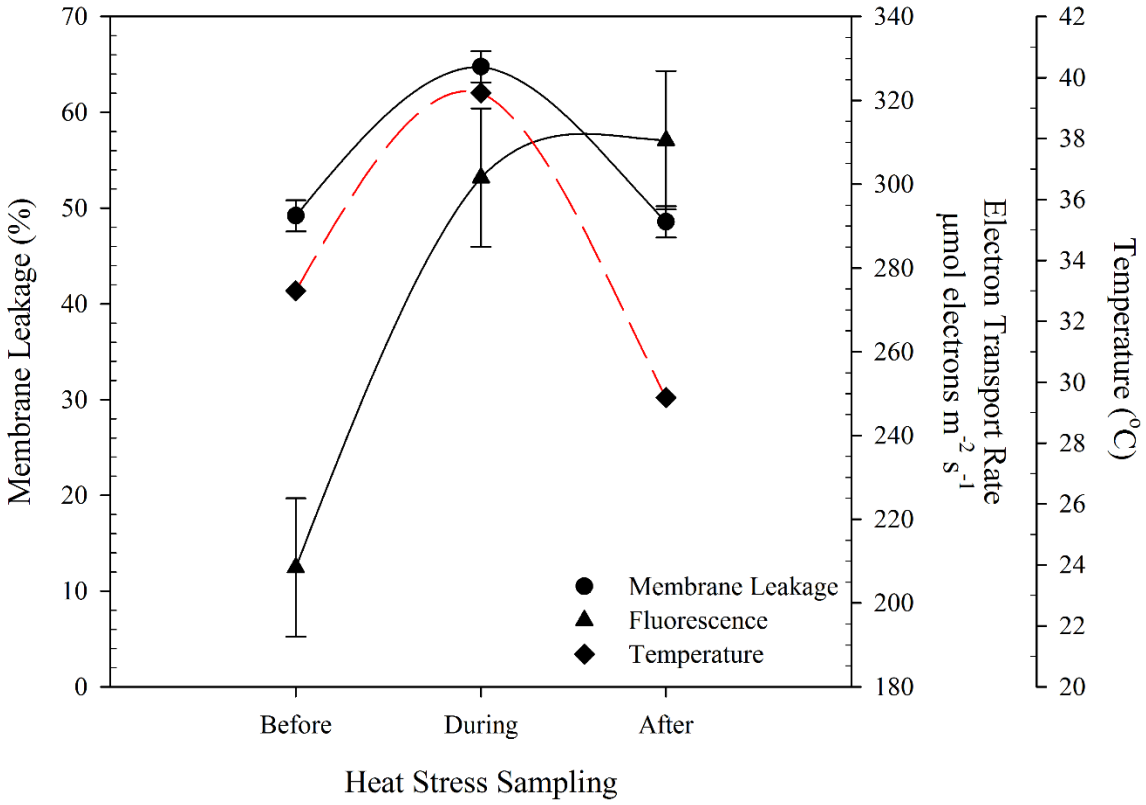

Figure 6-2: The membrane leakage percentage and fluorescence (µmol of electrons m^{-2} s^{-1}) of fully expanded fourth main-stem leaves collected from plots from each sampling period. A greater percentage of injury indicates a higher porosity of the membrane and a higher fluorescence value indicates damage to the photosystem as more electrons are fluoresced rather than incorporated into the electron pathway. The hashed line indicates the temperature of each sampling period. Error bars indicate the confidence interval of each point at $\alpha = 0.05$ level.

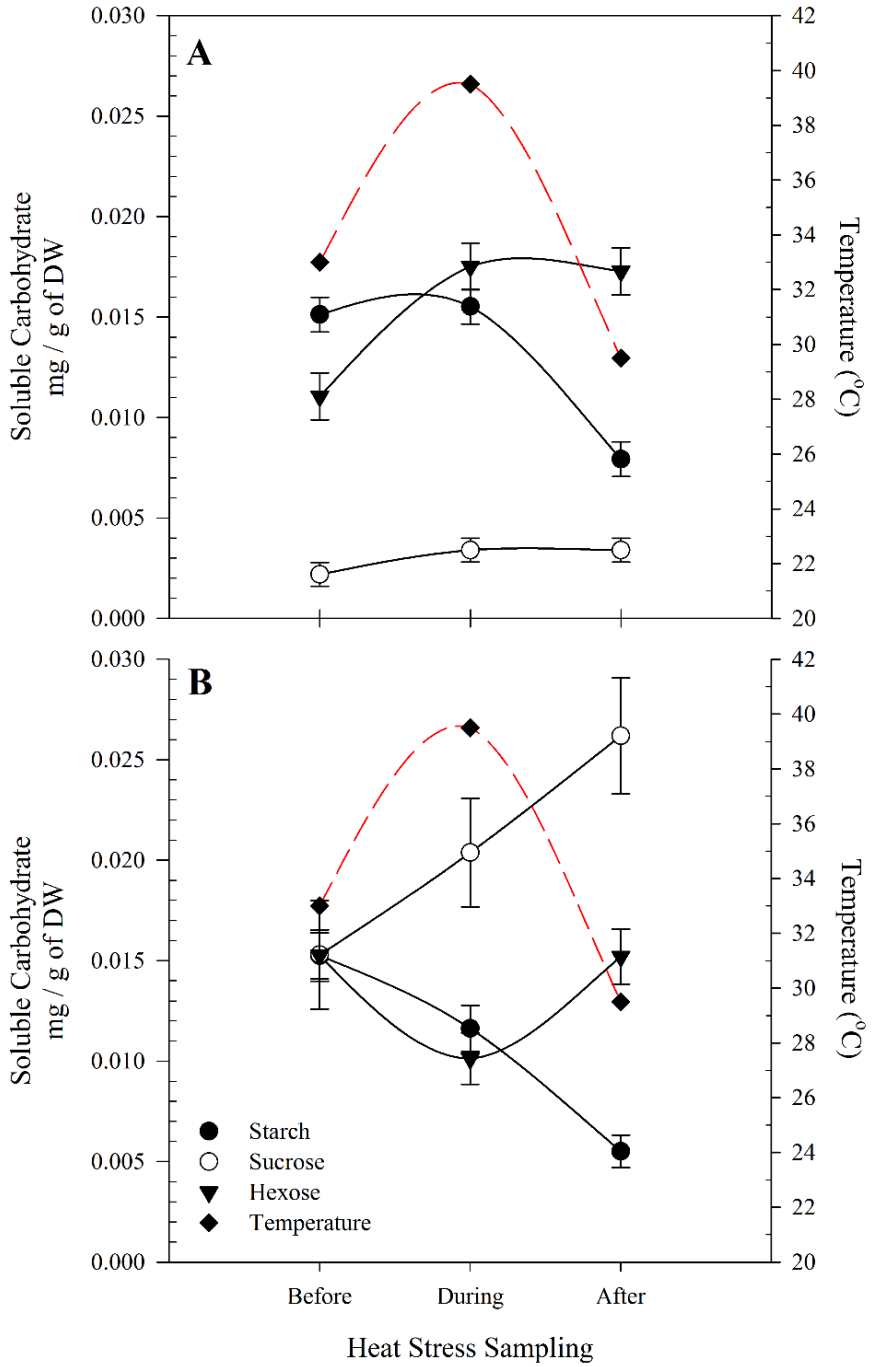

Figure 6-3: Carbohydrate concentrations (mg / g^{-1} of tissue dry weight (DW)) for both fourth main-stem leaves (A) and of ovaries (B) collected before, during, and after high temperature stress in the field. Error bars indicate the confidence interval for data of each point at $\alpha = 0.05$ level.

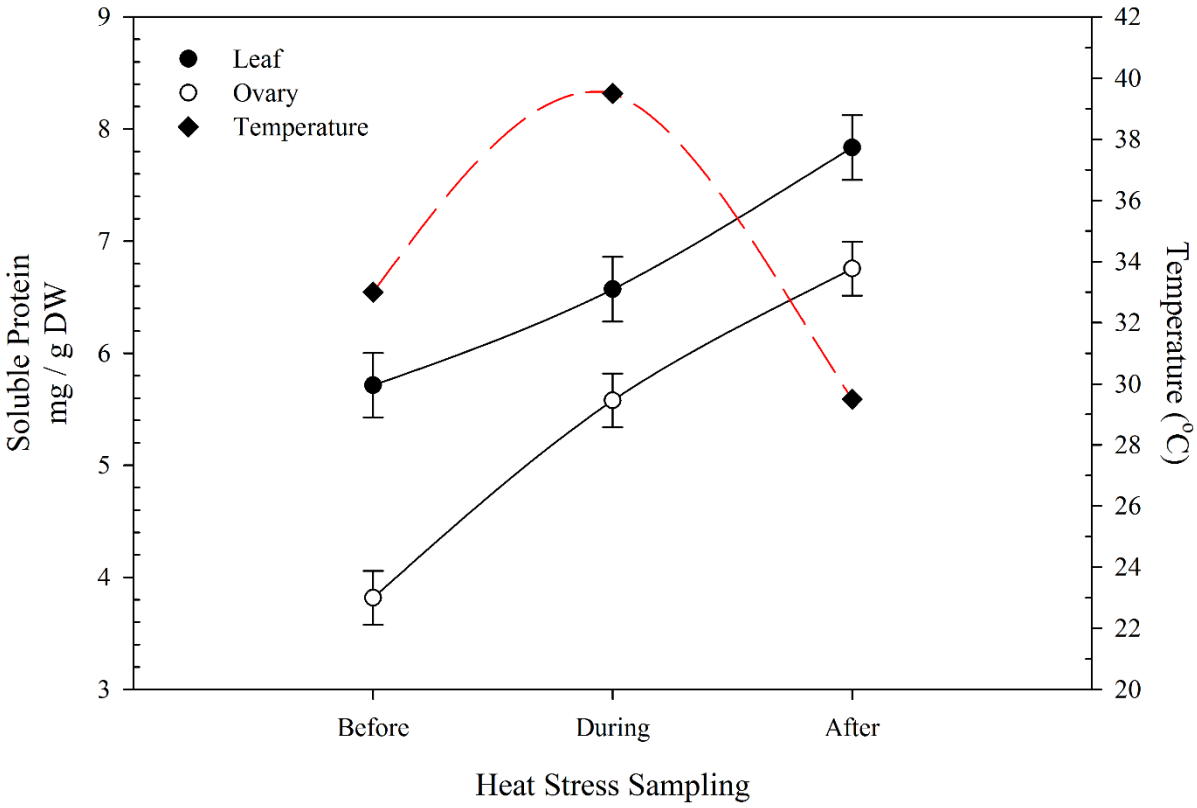

Figure 6-4: The dry weight soluble protein concentrations (mg / g^{-1} DW) of leaf and ovary tissues sampled during periods of heat stress. Temperatures indicate the ambient air temperature of the collected plants. Error bars represent the confidence interval of each point mean at α = 0.05 level.

Conclusions

High temperature heat stress is an endemic issue affecting most all cotton producers in the majority of the cotton growing regions of the world. Due to the thermal limits of cotton physiology coupled with systematic overarching ambient temperatures that occur most every year, mitigation of the negative effects of heat stress has been a priority. Producers have used irrigation as a primary to reduce temperature related yield losses, using transpiration to cool the canopy. In Arkansas, historical evidence in the region indicates that irrigation does provide a net increase in yield over non-irrigated fields, but irrigated fields suffer the same thermal limitations to heat stress during anthesis. This despite that canopy temperatures were well below warmer ambient temperatures, even under irrigation conditions. Research in the past has indicated that the obstacle inhibiting increased heat related yield is the reduction in photosynthetic capacity and carbohydrate partitioning. The carbon partitioning adjustments identified in the flower in the days surrounding anthesis indicate that carbohydrate ratios are rapidly disturbed due to heat stress. This readjustment is related to the stress response of the plant, and possibly relates to increased flower shed due to environmental stress. However, we determined that non-irrigated cotton producers in Arkansas have also experienced yield increases parallel to irrigated fields, though precipitation has on average not changed since 1980. By considering that the plant is able to adjust its fruit load according to a stressful condition, it is reasonable to assume that well-irrigated cotton that has not experienced significant stress will shed more fruiting structures than more acclimated plants.

Based upon our acclimation evidence, it is reasonable to assume that initiating a mild stress early could have a protective effect prior on shedding if initiated prior to flowering. A small stress initiated prior to reproductive development, such as heat stress or water stress may

mitigate damage later on in the season due to heat stress. Due to the increased shed that is experienced in fields following mid-season heat stress conditions, this small change in management may yield significant increases in cotton yield by better protecting carbon assimilation and distribution mechanisms. However, appropriate timings of this acclimatizing stress has not been well researched. Thus, future research should investigate whether irrigation timed to developmental milestones rather than general applications would benefit row crops of any significant value while preserving yield.