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High Daytime Temperature Stress Effects on the Physiology of Modern Versus Obsolete Cotton Cultivars

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ABSTRACT

Year-to-year variability in yield of modern cotton cultivars is a major concern in the cotton industry, and it is speculated that this variability is due to modern cultivars being more sensitive to environmental stress conditions compared to obsolete cultivars. However, research is lacking to explain physiological responses of contrasting cultivars in response to environmental stress conditions. Therefore, a study was designed in Fayetteville, AR to determine the physiological response of modern ('Stoneville 474' and 'Suregrow 747') versus obsolete ('Stoneville 213' and 'Deltapine 16') cultivars to daytime high temperature stress in a controlled growth chamber environment. Modern cultivars had improved physiological function at lower daytime temperatures, but obsolete cultivars had improved physiological function at elevated temperatures of 38°C. Furthermore, plant physiological functioning decreased with increasing temperatures. No significant differences existed between modern and obsolete cultivars at 30 or 34°C, however at 38°C, obsolete cultivars showed significant improvements ($P < 0.05$) in measured leaf photosynthesis, chlorophyll fluorescence, and membrane integrity of leaves. There were no differences between modern and obsolete cultivars with regards to total soluble leaf protein levels or leaf chlorophyll; however, the obsolete cultivars had a numerically greater protein concentration at 38°C, whereas the modern had more leaf protein at the lower temperatures. Overall, it appeared that modern cultivars had improved physiological responses under ideal temperature environments; however, obsolete cultivars were less sensitive to extreme temperatures.

Keywords: *Gossypium hirsutum* L., fluorescence, membrane leakage, photosynthesis

INTRODUCTION

The U.S. cotton industry has faced very difficult times in recent years due to an increased problem with year-to-year yield variability. It is speculated that this increased yield variability is due to modern cultivars being more sensitive to adverse environmental conditions such as high temperatures during the critical first five weeks of flowering and boll development (Oosterhuis 1990). However, information is lacking on the physiological responses of modern versus obsolete cultivars, under high temperature stress conditions, to try and explain why this variability exists from a physiological perspective.

Even though cotton is a tropical plant and is accustomed to growing in hot climates, it does not necessarily grow best in excessively high temperatures. The ideal temperature range for cotton is reported to be from 20 to 30°C, and once temperatures reach about 35°C, growth begins to decrease (Reddy *et al*. 1991; Bibi *et al*. 2008). Burke *et al*. (1988) reported that the ideal temperature range of cotton for optimal metabolic activity (also known as the thermal kinetic window) was 23-32°C with the optimum for photosynthesis at 28°C. Unfortunately, in the Mid-south, average daily maximum temperature in August and some years in July are well above this optimum temperature for photosynthesis. There is a negative correlation between yield and temperature during early boll development (Oosterhuis 1995, 1997, 1999).

High, above-average temperatures during the day can decrease photosynthesis and carbohydrate production (Bibi *et al*. 2008). On the other hand, high night temperatures (above 23°C) are associated with a significant increase in respiration (Arevalo *et al*. 2004). The overall result of high night temperature is that there is not enough carbohydrate produced to satisfy all the plant's needs, resulting in increased boll shedding, malformed bolls, smaller bolls, fewer seeds and ultimately less lint for yield. High night temperatures during boll development in the Mississippi Delta have been implicated in lower yields and increased yield variability (Oosterhuis 1999; Arevalo *et al*. 2004).

According to McArthur *et al*. (1975) the first signs of temperature stress are associated with decreased boll growth. This decrease in boll growth usually takes place at temperatures above 27°C. Temperatures exceeding 32°C are associated with a decrease in photosynthesis (Perry and Krieg 1981). When temperatures surpass 35°C, plants have poor flower survival and fruit production (Perry and Krieg 1981). If temperatures reach 40°C during the fruiting period, boll mass is typically less than 1% of the total plant mass and this appears to be consistent across cultivars (Reddy *et al*. 1992).

Our hypothesis was that modern cultivars are more sensitive to high temperature stress conditions and, therefore, will have reduced physiological functioning necessary for optimal growth, development and yield in cotton. The objective of this study was to evaluate physiological and biochemical responses of modern versus obsolete cotton cultivars to high daytime temperature stress under controlled growth chamber conditions.

MATERIALS AND METHODS

A growth chamber study was designed at the Altheimer Laboratory in northwest Arkansas at Fayetteville in 2004 to evaluate the effect that high daytime temperature stress had on physiological responses of modern and obsolete cotton (*Gossypium hirsutum* L.) cultivars. Three seeds per pot were planted into 2L pots filled with sunshine potting media (Sun Gro Horticulture Distribution Inc.,

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Bellevue, WA) and watered as needed with a half-strength Hoagland's nutrient solution. After emergence, each pot was thinned to a single plant. The study included two factors, cultivar and temperature, arranged in a completely randomized design with four replications. Temperature treatments included maximum daytime temperatures of 30, 34, and 38°C. Nighttime temperatures were held constant at a minimum of 20°C. The second factor was cultivar and consisted of two modern and two obsolete cultivars. The modern cultivars selected for this experiment were 'Suregrow 747' and 'Stoneville 474'. The obsolete cultivars (developed more than 30 years ago) utilized were 'Stoneville 213' and 'Deltapine 16'. Plants were initially grown in two large walk-in growth chambers (Model PGW36, Controlled Environments Ltd., Winnipeg, Canada) until two weeks after first flower (FF2). At this time, half of the plants in each growth chamber were placed in with half the plants from the other growth chamber to limit the possibility that treatments differences were the result of different growing conditions between the two growth chambers. Also, initially one-third of the plants in each chamber were planted three days later so that final physiological measurements were conducted on plants of the same age.

Beginning at FF2, two separate growth chamber were programmed for 30 and 34°C. Three days after plants were exposed to these temperatures, a series of physiological measurements were assessed to characterize physiological responses of contrasting genotypes under high temperature stress. Measurements include leaf photosynthesis, chlorophyll fluorescence, membrane leakage, total soluble protein, and SPAD chlorophyll. Finally the remaining plants were placed in a growth chamber programmed for 38°C, and measurements taken three days after temperature imposition.

Photosynthesis was monitored within 2 h of solar noon using a Li-Cor 6200 Portable Photosynthesis System (Li-Cor Inc., Lincoln, NE). Photosynthesis was measured on the uppermost fully-expanded main-stem leaf located four nodes down from the top of the plant. Chlorophyll fluorescence was measured on the same leaf position, at the same time of day as photosynthesis, using a portable OS1-FL fluorometer.

Leaf chlorophyll content was measured at approximately the same diurnal time as the other physiological measurements. Chlorophyll was determined by clipping a Minolta SPAD meter (Spectrum Technologies Inc., Plainfield, Il) onto the most fully-expanded main-stem leaf from the top of the plant. A Minolta SPAD meter provides a quick assessment of the chlorophyll content measured in SPAD units.

Once photosynthesis and chlorophyll had been measured, the upper-most fully expanded main-stem leaf four nodes from the top of the plant was collected for membrane leakage and protein determination. Two leaf disks were taken from each leaf using a 0.63 cm^2 area leaf disk punch. The remaining leaf material was then stored in a -80°C freezer for subsequent protein extraction and quantification. Membrane leakage determination consisted of placing these leaf disks into trays with individual cells containing 2Ml of de-ionized water per cell. An automatic seed analyzer (Applied Intelligent Systems Inc., Ann Arbor, MI) was used to determine how much cellular contents had leaked out of the leaf into the de-ionized water via a conductivity test. At 48 hours after the leaf disks were placed in de-ionized water, an electrical voltage from the seed analyzer was applied to the solution containing the leaf disk. The resulting electrical current flow depends upon the ion concentration in the solution, which will depend on the exudates from the leaf disk. A relatively high electrical current means that a relatively large number of ions are in the exudates.

Protein analysis was performed using a protocol by Anderson *et al*. (1992). The first step in protein analysis was to extract total protein from the collected leaf tissue. Initially the frozen leaf was ground in liquid nitrogen with a mortar and pestle. The ground tissue was then placed in a 35 Ml centrifuge tube containing 0.5 g insoluble polyvinylpyrrolidone (PVP, Sigma P6755, MW 111.1), one drop of antifoam-A, 4 ml of ice-cold extraction buffer and homogenized with a Polytron homogenizer. The centrifuge tubes were then centrifuged at 12,000 rpm at 4°C for 20 min and the supernatant passed through a PD-10 desalting column for purification. Total soluble leaf protein levels were then analyzed using a BioSpec-1601 enzyme analyzer (Shimadzu Inc., Columbia, MD). Total soluble leaf protein was measured according to procedures described in a technical bulletin sent from Sigma Chemical with the accompanying Bradford Reagent.

All statistical analyses were performed using SAS version 7.0 (SAS Institute Inc., Cary, NC, 1990). Data were analyzed using analysis of variance and least significant difference (LSD) tests according to the general linear model (GLM) procedures of SAS. Treatment means were separated based on P-values at the 95% confidence level.

RESULTS AND DISCUSSION

Leaf photosynthesis and fluorescence

There were no significant differences in leaf photosynthesis between modern and obsolete cotton cultivars at lower ambient temperatures of 30 and 34°C, however, at an elevated temperature of 38°C the obsolete cultivars showed a significant ($P<0.05$) improvement in leaf photosynthesis (**Fig. 1**). Similar results were observed between modern and obsolete cultivars with regard to measured leaf chlorophyll fluorescence (**Fig. 2**). Modern cultivars had numerically lower chlorophyll fluorescence at 30°C; however, the obsolete cotton cultivars had numerically lower chlorophyll fluorescence at 34°C and significantly lower fluorescence at 38°C compared to the modern cultivars (**Fig. 2**). Higher fluorescence under heat stress conditions indicated that plants were not as efficient at utilizing electrons as they move to a higher energy level in the light reactions of photosynthesis. Bibi *et al*. (2004) reported differences in fluorescence values between old and new cotton cultivars and also indicated an increase in fluorescence under high temperature stress. These results suggest that obsolete cultivars might be less sensitive to temperature-stress compared to modern cotton

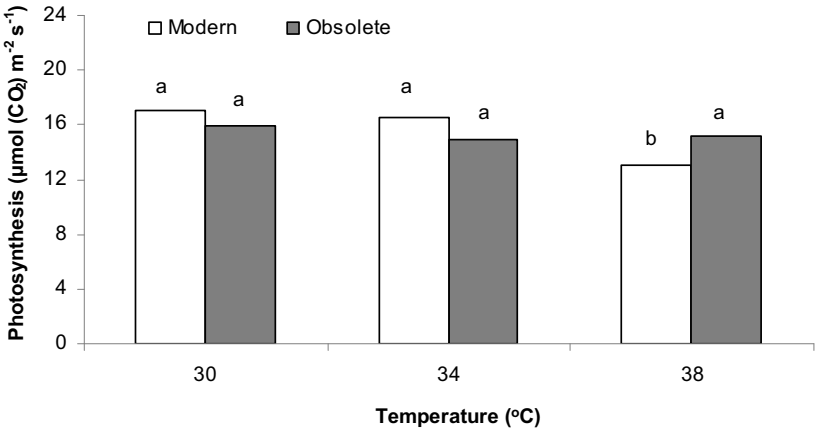

Fig. 1 Effect of high daytime temperature on leaf photosynthesis of modern versus obsolete cotton cultivars. Measurements taken at Fayetteville, AR in 2004 at FF3 under controlled growth chamber conditions. Bars followed by the same letter are not significantly different ($P \leq 0.05$) between paired treatments.

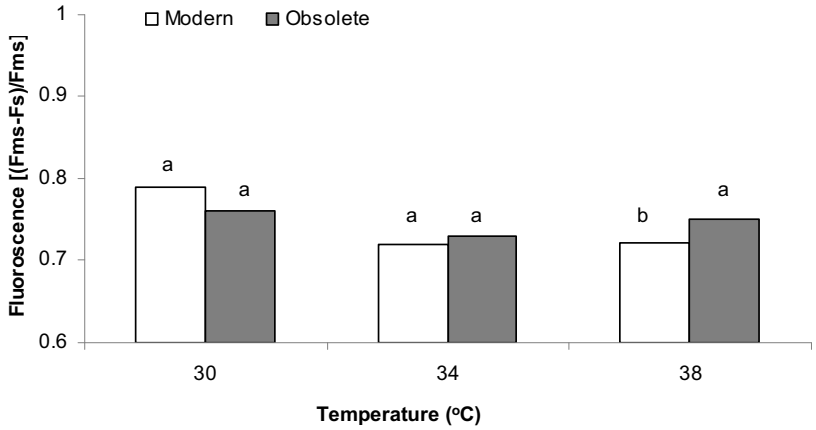

Fig. 2 Effect of high daytime temperature on leaf chlorophyll fluorescence of modern versus obsolete cotton cultivars. Measurements taken at Fayetteville, AR in 2004 at FF3 under controlled growth chamber conditions. Bars followed by the same letter are not significantly different ($P \leq 0.05$) between paired treatments.

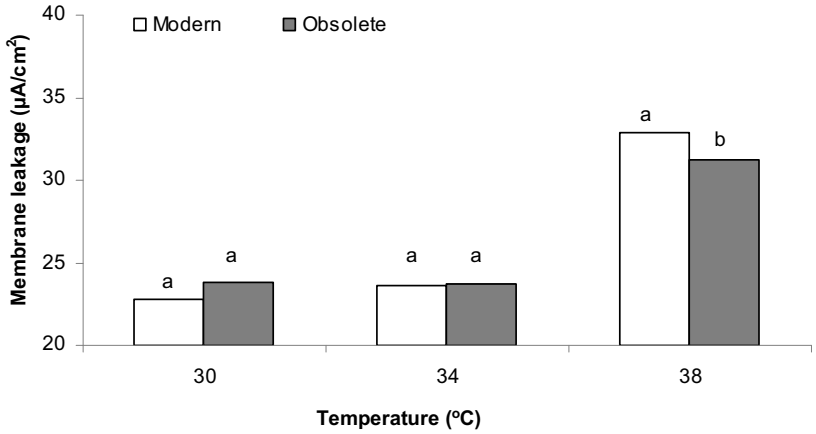

Fig. 3 Effect of high daytime temperature on leaf membrane leakage of modern versus obsolete cotton cultivars. Measurements taken at Fayetteville, AR in 2004 at FF3 under controlled growth chamber conditions. Bars followed by the same letter are not significantly different ($P \leq 0.05$) between paired treatments.

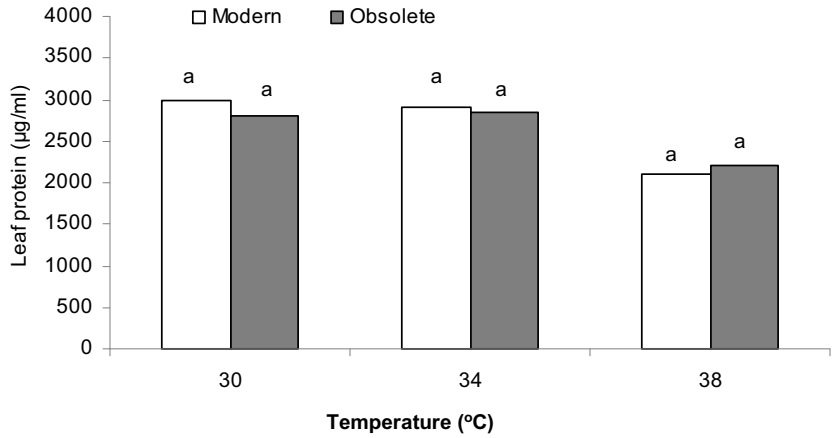

Fig. 4 Effect of high daytime temperature on leaf total soluble protein levels of modern versus obsolete cotton cultivars. Measurements taken at Fayetteville, AR in 2004 at FF3 under controlled growth chamber conditions. Bars followed by the same letter are not significantly different ($P \leq 0.05$) between paired treatments.

cultivars. However, Wells and Meredith (1984) indicated a lack of improved assimilatory activity per leaf area by the modern cultivars.

Membrane leakage

Leaf membrane leakage increased with increased temperature and there was significantly more membrane leakage at 38°C compared to the lower (30 and 34°C) temperatures (**Fig. 3**). There was no significant difference in membrane leakage between old and new cultivars at 30 and 34°C, however at 38°C modern cultivars had significantly ($P < 0.05$) more membrane leakage compared to obsolete cultivars (**Fig. 3**). Bibi *et al*. (2004) indicated that membrane leakage increased with increasing temperature, and there were genotypic differences as well. Increased membrane leakage at elevated temperature by modern cultivars supports the notion that photosynthesis was reduced at elevated temperatures due to a less efficient leaf (**Fig. 1**). Leaf membrane leakage has also been shown to increase with water-deficit stress in cotton (Coker 2004).

Leaf protein

Leaf total soluble protein values indicated no significant differences between modern and obsolete cultivars at all

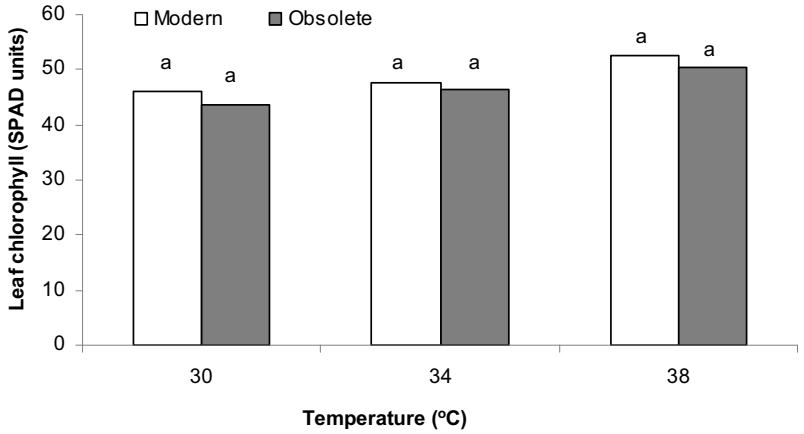

Fig. 5 Effect of high daytime temperature on leaf chlorophyll of modern versus obsolete cotton cultivars. Measurements taken at Fayetteville, AR in 2004 at FF3 under controlled growth chamber conditions. Bars followed by the same letter are not significantly different ($P \leq 0.05$) between paired treatments.

temperatures tested (**Fig. 4**). Leaf protein levels were very similar at 30 and 34°C, however there was a numerical decrease (P<0.08) in leaf protein levels at 38°C (**Fig. 4**). Though not significant, modern cultivars had numerically more leaf soluble protein at 30 and 34°C, but the obsolete cultivars had more leaf protein at 38°C. Proteins tend to denature under extreme temperatures and therefore these higher protein levels further support improved plant functioning under elevated temperatures by the obsolete cultivars. It appears that modern cultivars have improved physiological responses at low temperatures, but are more sensitive to high-temperature stress. Research by Bibi *el al*. (2004) showed no clear trends in protein levels between modern and obsolete cotton cultivars at temperatures up to 31°C. Our research also showed no differences at lower temperatures (30°C and 34°C), but differences at 38°C. Temperatures above 35°C have been shown to be a threshold above which significant physiological differences occur (Bibi *et al*. 2008).

Leaf chlorophyll

There were no significant differences (P<0.05) in leaf chlorophyll between modern and obsolete cultivars across all temperatures evaluated (**Fig. 5**). Furthermore, there were no differences in chlorophyll levels between temperatures when averaged across cultivars. Leaf chlorophyll was the only physiological parameter measured which did not show an advantage by the obsolete cultivars at the highest temperature of 38°C. Modern cultivars had numerically higher leaf chlorophyll values at all temperatures (**Fig. 5**). This suggests that genetics is a bigger determinate in leaf chlorophyll levels than environmental influence.

CONCLUSIONS

Modern cultivars had improved physiological functions at lower maximum daytime temperatures, but obsolete cultivars had improved physiological function sat elevated temperatures of 38^{0}C. Furthermore, overall plant physiological functioning decreased with increasing temperatures. No significant differences in physiological functioning existed between modern and obsolete cultivars at 30 or 34°C; however, at 38°C, obsolete cultivars showed significant improvements (P<0.05) in measured leaf photosynthesis, chlorophyll fluorescence, and membrane integrity of leaves.

Overall, it appears that modern cultivars have improved physiological responses under ideal temperature environments as shown by Reddy *et al*. (1991), whereas obsolete cultivars tend to be less sensitive to extreme temperatures, and therefore exhibit improved physiological functioning under these conditions. Given contrasting environmental conditions which occur from year-to-year, these temperature results help to explain why modern cultivars may have increased variability in terms of yield development. Modern cultivars have great yield potential under favorable environments, but increased variability exist due to reduced yields in years with extreme temperature stress conditions, particularly during flowering and boll development.

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