CELLULAR ADJUSTMENTS OF COTTON DURING DEVELOPMENTAL STAGES OF ANTHESIS

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Abstract

High temperature stress is amongst the greatest threats to yield; however, it is near impossible to control. This unpredictability facilitates the need for an understanding of the cellular processes that may facilitate flower shed during the critical time period of anthesis. Growth chamber analysis was conducted over two years at the University of Arkansas in Fayetteville. Ovaries from flowers the day before flowering, during flowering, and the day after flowering were analyzed along with the subtending leaf for both antioxidant stress and carbohydrate changes. Leaves of the fourth main stem from the top node were analyzed for both electron transport rate changes and membrane leakage changes over the course of five days during the stress. Membrane leakage indicated a significant increase in the damage caused by the heat, but acclimated to the control by day four. Electron transport rates all remained lower than the control, but experienced the greatest difference on day two when membrane leakage was also greatest. Protein concentration differed among the leaves and ovaries of both stressed and the control. Peroxidase levels indicated a greater change in the leaf concentrations than in the leaf. Whereas, glutathione reductase differed significantly for both the leaf and the ovary during all stages of development. Carbohydrate analysis indicated no differences amongst the trends in the leaves, but the ovaries indicated differences in fructose, sucrose, and starch concentrations during development.

Introduction

Under any form of biotic or abiotic stress, a plant must make minor cellular adjustments to maintain homogeneity within the cell. These cellular changes are compounded over the length of tissue affected that then may distinguish it from other plants unaffected by the particular stress. Many stresses can have external physical characteristics. Nitrogen stress, for example, can be characterized by thinner leaves that display a general yellowing. This abiotic stress complicates the ability of the cell to maintain uniformity across the cellular processes, and thus leads to a reduction in CO2 assimilation rates. (Longstreth and Nobel, 1980). Water deficits in cotton results in stunted growth due to reduced cell and leaf expansion, reduced stem elongation, and a reduced leaf area index (Turner et al., 1986). These minor changes in the individual plant is again compounded across the entire field leading to a change in the distribution of bolls on the plants, favoring less secondary and tertiary boll development, leading to reduced yields (Pettigrew, 2004).

In many fields across the southern United States, stresses both biotic and abiotic can be amended. Temperature stress, in particular high temperature, can be the most uncontrollable factor that growers have to endure. When water deficits are taken into account, temperature stress is viewed as the most limiting factor associated with diminished crop yields (Crafts-Brandner and Salvucci, 2004). Under high temperature stress, cotton plants exhibit a range of negative effects including, but not limited to, a reduction in carbohydrate manufacture (Loka and Oosterhuis, 2010) , an increase of reactive oxygen species (Snider et al., 2008), and an increase of molecular protein chaperones such as heat shock proteins (HSPs) (Fender and O'Connell, 1989).

Methods

70 pots of cotton (Gossypium hirsutum L.) cultivar ST5288 B2RF was grown in a large walk-in growth chamber at the Altheimer laboratory at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR. Growth chambers were setup with a day/night temperature of 30/20^{o}C, a relative humidity of 70%, and 14 hour photoperiods of 500 µmol m^{-2} s^{-1} of photosynthetically active radiation. Plants were grown in nutrient deficient potting mix in 1.5L pots. Plants were watered daily using ½ strength Hoagland's solution. The experiment was arranged in a completely randomized design of one factor: high temperature.

Plants were separated and randomized into two chambers set to identical temperatures, humidity, light intensity, and photoperiods once squaring was established. Plants were monitored daily for the beginning of flowering. Once flowering had commenced, temperatures were increased in one chamber to 38/24^{o}C. Measurements were taken of

membrane leakage and fluorescence daily between 1200-1400 hrs. Collections included the subtending leaf and its candle, the subtending leaf and its companion flower, and the subtending leaf and one day post flower. Membrane leakage was collected daily on the fourth main-stem leaf of ten random plants in each chamber. Fluorescence data was collected of ten random plants in each chamber. Fluorescence readings were taken three times on different points of each leaf and averaged to give an average value of electron transport rate for each leaf. Collections were immediately bagged and placed in an ultra-deep freezer (-80^{o}C) for later analysis. Ten leaves and their companion ovaries were each assigned to either antioxidant, carbohydrate, or protein analysis.

Membrane Leakage

Three leaf discs measuring 1 cm in diameter each were collected from the fourth main-stem leaf of ten plants per day. Each disc was placed in 10 ml of double deionized water and gently swirled to remove dust and contaminates. The discs were then transferred to another 10 ml of double deionized water in another tube. Tubes were capped and placed in a dark chamber at room temperature for 24 hours. Conductivity measurements were made prior the tubes being placed in an autoclave for 20 minutes at 150^{o}C to completely disrupt the membrane. Conductivity was measured again and recorded. Calculations were made of the relative change in conductivity for each tube. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

Protein

Leaves and ovaries were processed according to Anderson et al. (1992) with the following modifications. 0.5 grams of leaf tissue and the entire ovary tissue were each ground in liquid nitrogen and placed in a tube containing 0.1 grams PVPP. Ice-cold extraction solution did not contain the PVP or the polyclar-AT. A 10x addition of the ice-cold extraction solution was placed into each tube filled with ground leaf tissue or ovary, i.e. 0.5 grams of leaf would have 5 ml of solution added. The extraction mixture was centrifuged at 20,000g for 15 minutes and the supernatant volume collected and recorded. Samples were stored at -80^{o}C until ready for analysis. Total protein concentration in the solution was determined by the Bio-Rad's protein determination kit (based on Bradford method) according to the manufacturer's instructions. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

Antioxidants

Antioxidants were analyzed from the same supernatant collected for the above protein analysis. Peroxidase was performed using an extraction solution of 50 mM Sodium Acetate buffer (pH 7.0), 25 mM guaiacol, and 25 mM H_2O_2 at room temperature in the dark. H_2O_2 was added after the guaiacol had been completely dissolved in the buffer solution. 25 µl of tissue solution and 975 µl of reaction mix was added together in 1.5 ml cuvette tubes and the absorbance was recorded at 470 nm for 3 minutes. Measurements were recorded as units per gram of fresh weight.

Glutathione reductase was measured using a modified procedure from Schaedle and Bassham (1977). 10 µl of sample extract and 190 µl of reactant solution were transferred to a 96 well microplate reader and analyzed using a Thermo Multiskan Ascent for 1 minute at 340 nm. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

Carbohydrates

Carbohydrates were analyzed according to the procedure presented by Hendrix and Peelen (1987) with the following modifications. Leaf and ovary tissues were freeze dried for 24 hours and ground. 40 mg of each tissue was collected and used for analysis. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

Results

Membrane Leakage

Membrane leakage displayed a significant difference change from the control during the first few days of heat stress (Figure 1). On the first day of stress permeability values were 43% higher than the control. Maximum permeability was seen on the second day when it was 84% greater than the control. Day three saw improvements in membrane leakages of the heat stressed plants with only a 33% difference between it and the control. By day four, the heat stress had reached levels that were within 5% of the control which was similar to values collected on the fifth day when only 4% difference was observed. Values were congruent with previous research establishing that after three

days, membrane permeability is not significantly different than a control. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

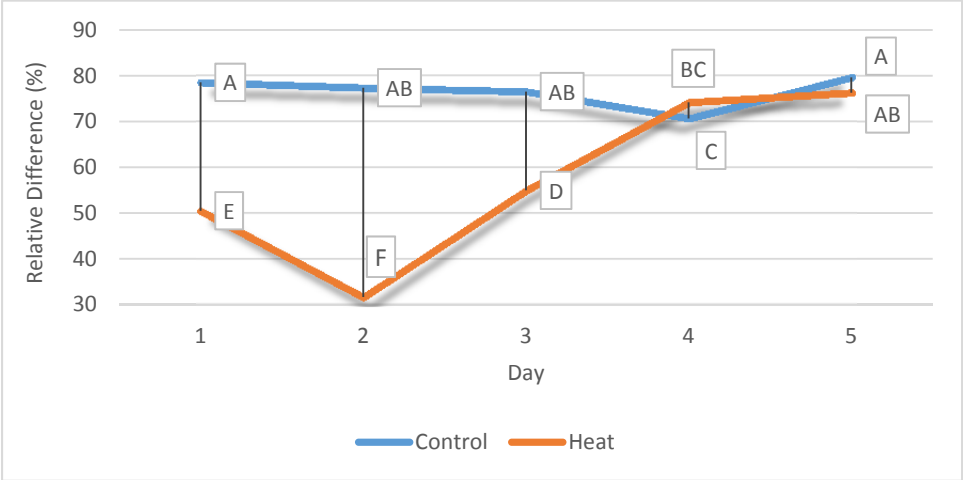

Figure 1: Membrane leakage for each day and stress type. Different letters in each chamber tissue combination represent significant difference at α = 0.05 level.

Electron Transport Rate

Day one experienced a 15% decrease of electron transport rate of stressed plants when compared to the control (Figure 2). Heat stressed plants were consistently below the values of the control with the biggest disparity occurring on day two with a 37% difference coinciding with the largest increase in membrane leakage. Relative differences for the remaining days were 19%, 21% and 16% respectively. General trends followed the control, but with depressed values, falling on the fourth day and remaining stable at day five. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

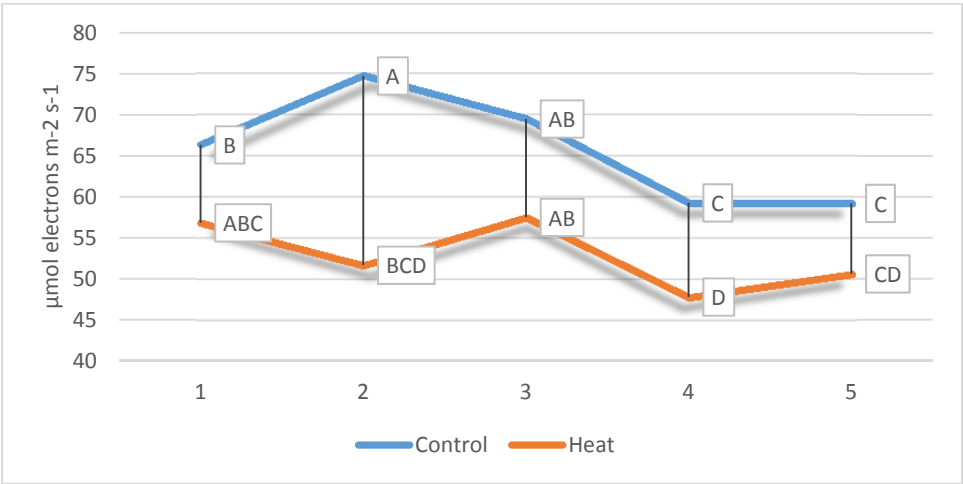

Figure 2: Electron transport rate for each day and stress type. Different letters in each chamber tissue combination represent significant difference at α = 0.05 level.

Protein Concentration

Leaf protein concentration was significantly different over the flowering period (Figure 3). In the control, the leaf concentration followed a positive parabolic trend with the lowest value at flowering. This coincided with a negative parabolic trend for the ovary having the apex during flowering. There appears to be a relationship of a decrease of protein in the leaf at flowering that coincides with an increase in protein concentration at flowering. However, during heat stress, leaf tissue displayed a negative linear trend with the greatest protein concentrations before

flowering. Ovary concentrations leveled off at flowering and were similar at post-flower. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

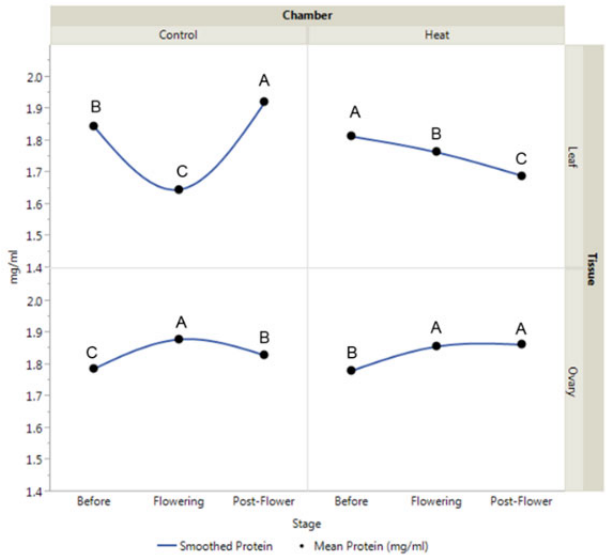

Figure 3: Protein concentration for each development stage separated by chamber treatment and tissue type. Different letters in each chamber tissue combination represent significant difference at $\alpha = 0.05$ level.

Peroxidase

Control leaf levels followed a positive parabolic trend with flowering seeing less activity than either before or after flowering (Figure 4). The ovary saw a rapid rise in activity with each developmental stage significantly greater than the previous. Post-flowering activities were over 150% greater than that of before flowering activity levels. Heat stressed leaves activities had significantly different trends than the control. The lowest levels were found before flowering with peroxidase activities similar both in flowering and post-flowering. Heat stressed activities were lowest before flowering and highest post-flowering, though levels were decreased than in the control. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

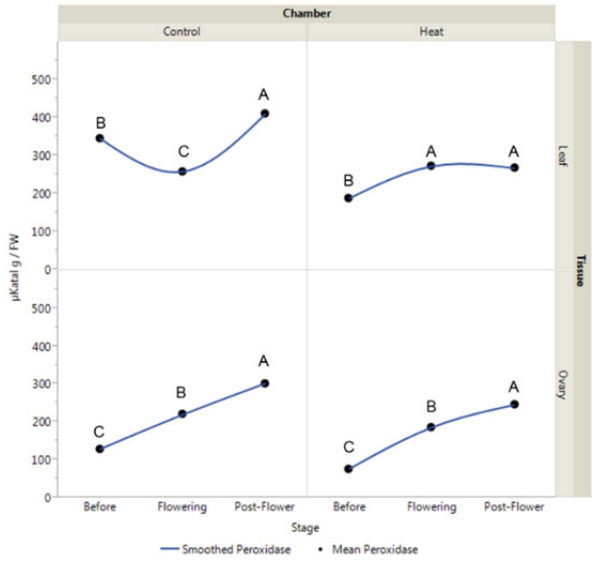

Figure 4: Peroxidase activities for each development stage separated by chamber treatment and tissue type. Different letters in each chamber tissue combination represent significant difference at α = 0.05 level.

Glutathione Reductase

Leaves of the control displayed activity levels that were lowest before flowering, with near a 75% increase at flowering and then a further, though not significant increase post-flowering (Figure 5). Whereas the heat stressed leaf saw increased activities of the enzyme before flowering compared to the control, this dropped significantly at flowering by about 25%. At post-flowering, levels dramatically increased to an almost 100% difference compared to flowering. Control ovaries displayed a negative parabolic trend with flowering exhibiting the highest levels of activity. Heat stressed ovaries had the highest levels before flowering and decreasing activity during flowering and further into post-flowering. Levels at flowering were about 40% less in the heat stressed plants as compared to the control. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

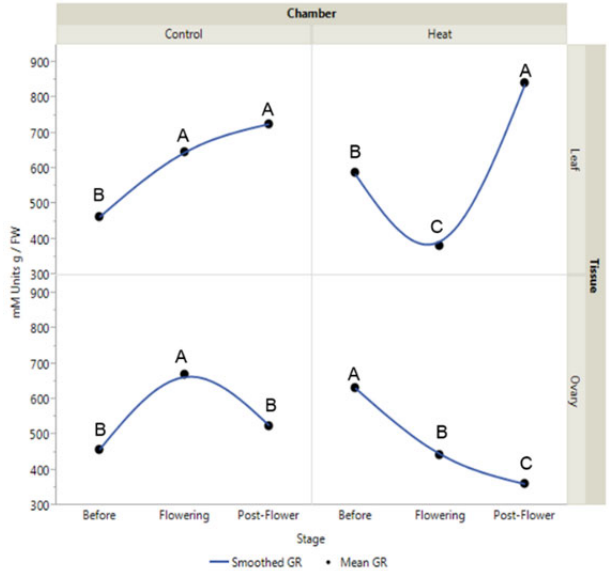

Figure 5: Glutathione reductase activities for each development stage separated by chamber treatment and tissue type. Different letters in each chamber tissue combination represent significant difference at α = 0.05 level.

Sucrose

Sucrose levels for both the control and heat stressed leaves remained level throughout the temperature study (Figure 6). Conversely, ovary concentrations did significantly differ, most particularly before flowering. Levels of sucrose before flowering in the stressed leaves were 18% higher than the levels found in the control. However, levels between the treatments during flowering and post-flowering shared similar levels. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

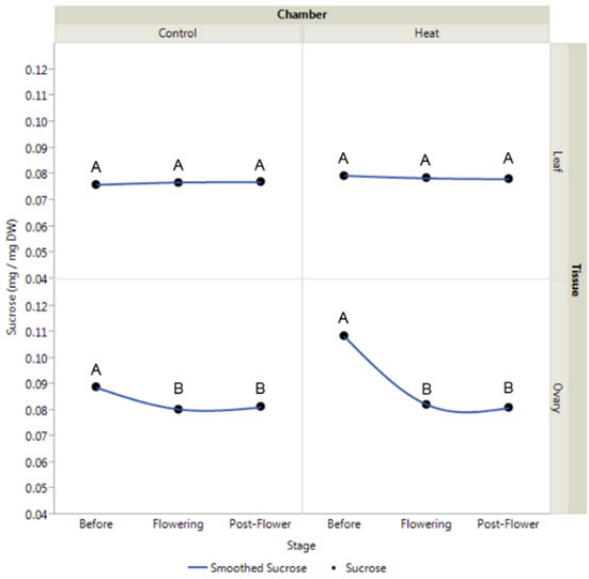

Figure 6: Sucrose concentrations for each development stage separated by chamber treatment and tissue type. Different letters in each chamber tissue combination represent significant difference at $\alpha = 0.05$ level.

Fructose

Levels of fructose in the leaves remained stable at around 0.04 mg / mg dry weight in both the control and of the heat stressed leaves (Figure 7). Similar to sucrose, levels of fructose were significantly higher before flowering in both the control and the heat stressed ovaries. Levels of fructose were higher than in the control, but levels of flowering and post-flower appeared to be similar. However, levels in the heat stressed ovary tissue were about 25% higher than the control. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

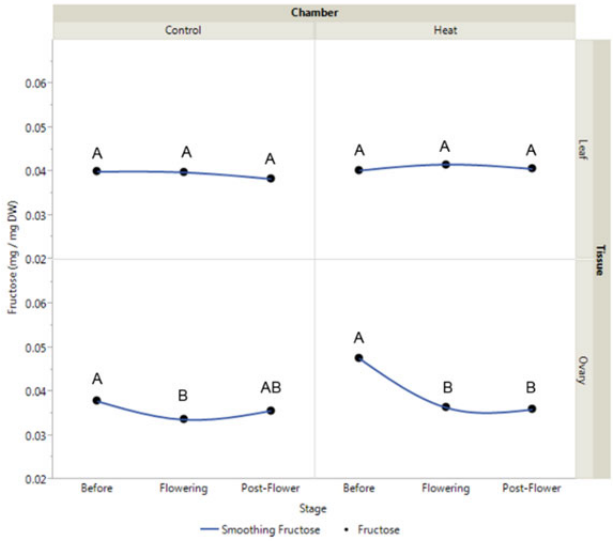

Figure 7: Fructose concentrations for each development stage separated by chamber treatment and tissue type. Different letters in each chamber tissue combination represent significant difference at $\alpha = 0.05$ level.

Glucose

Levels of glucose in the leaves remained steady with flat trends for both the control and heat stressed for all stages of development (Figure 8). Ovary concentration trends did take on slightly different shape characteristics with the heat stressed ovaries having slightly higher levels prior to flowering. The control ovaries did not see any increase in ovary concentrations until post-flowering. The lowest levels were at flowering during heat stress. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

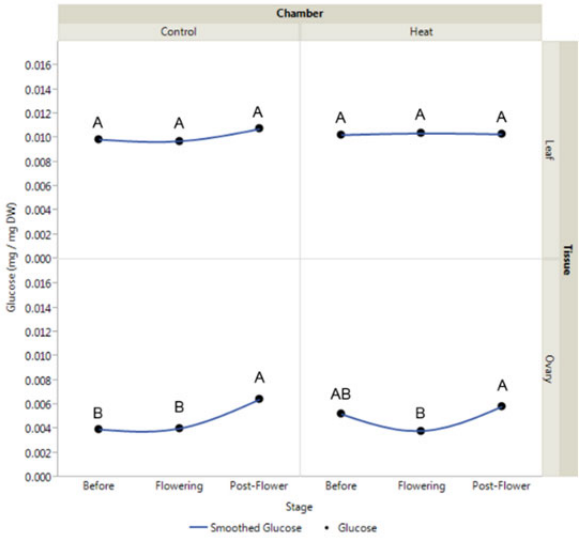

Figure 8: Glucose concentrations for each development stage separated by chamber treatment and tissue type. Different letters in each chamber tissue combination represent significant difference at $\alpha = 0.05$ level.

Starch

Starch levels did display significant differences between the developmental stages. Starch concentrations were least in the control leaves before flowering, increasing at flowering and remaining level post-flower. Heat stressed leaves displayed similar trends with lower concentrations of glucose before flowering and an increase at flowering that is statistically identical to post-flower. All data was statistically run in JMP 10.0 and significance is identified using a t-test set at a probability of 0.05.

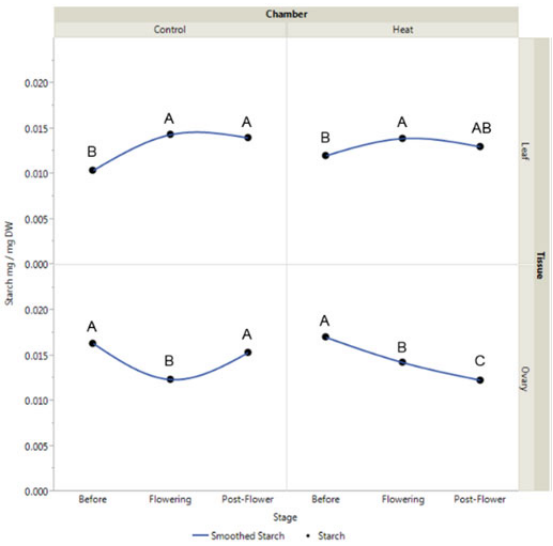

Figure 9: Starch concentrations for each development stage separated by chamber treatment and tissue type. Different letters in each chamber tissue combination represent significant difference at $\alpha = 0.05$ level.

Summary

Membrane leakage presented a very unique example of the speed of which cotton is capable of acclimating itself to a particular stress. After three days, no discernable difference appears to exist between the controls or the heat stressed plants. It does provide a window of opportunity for when the researcher must begin to accumulate data. Too late, and the difference between two treatments may be lost due to this acclimation effect. However, the electron transport rate of the leaves were consistently lower than the control for throughout the duration of the experiment. The decrease may be attributed early on to a combination of many factors, with leakage displaying a large compounding effect on day two. However, it has been reported in several sources (Crafts-Brandner and Salvucci, 2000; Sharkey, 2005) that RUBISCO protein denaturing appears to be the chief limiting factor at high temperatures.

Antioxidants appeared to vary significantly in their roles during the development of the flower and its potential effect on the subtending leaf. Control levels of peroxidase in the leaves experienced a positive parabolic trend with the lowest rates of activity during flowering, but the ovary experienced a continued rise over time. Heat stressed leaves experienced lower values overall, but with similar values during flowering, though not post-flower. Glutathione reductase levels in the control leaves had a rising trend over time, but the ovaries displayed a negative parabolic trend with the greatest activity at flowering. Heat stress levels in the leaves displayed significantly different values over the development period with levels highest post-flower and lowest during flowering. Ovaries experienced a negative trend as the development continued throughout the testing period.

Carbohydrates for all tested leaves did not display any significant differences. Leaves were expected to display some differences, but most research examines water deficit stress and changes in the structural and soluble carbohydrate changes therein. Research on just heat stressed plants in this area is limited for comparison. Sucrose and fructose before flowering concentrations were highest in ovaries the day before flowering, but were similar to concentrations found at flowering and post-flowering in the control. Starch concentrations in the ovary exhibited a change in concentrations compared to the control only post-flower.

The accumulated data indicate that there is significant changes that occur within the developing ovary and leaf during anthesis. Any sudden deviation from the normal levels as indicated in the controls could potentially increase the amount of flower shed that can be experienced in fields under high temperature stress. What has been presented are key areas of changes that occur in both leaves and ovaries that are to be analyzed more in the future as indications of potential crop yield losses.

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