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Effect of 1-MCP on Gas Exchange and Carbohydrate Concentrations of the Cotton Flower and Subtending Leaf under Water-Deficit Stress

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ABSTRACT

Ethylene is an endogenous plant hormone that increases under adverse environmental conditions, resulting in leaf and fruit abscission and ultimately yield reduction. In cotton, however, the effects of water-deficit stress on ethylene production have been uncertain. In this study it was hypothesized that application of an ethylene inhibitor 1-Methylcyclopropene (1-MCP) would prevent ethylene production and result in alleviation of water-deficit stress consequences on the physiology and metabolism of the cotton flower and subtending leaf. To test this hypothesis, growth chamber experiments were conducted in 2009-2010 with treatments consisting of (C) untreated well-watered control, (C + 1MCP) well-watered plus 1-MCP, (WS) untreated water-stressed control, and (WS + 1MCP) water-stressed plus 1-MCP. The plants were subjected to two consecutive drying cycles during flowering, approximately 8 weeks after planting, and 1-MCP was foliar applied at a rate of 10 g. ai/ha at the beginning of each drying cycle. The results showed that 1-MCP application had no significant effect on gas exchange functions and did not prevent reductions from water stress in leaf photosynthesis, respiration and stomatal conductance. However, application of 1-MCP resulted in a decrease in sucrose content of water-stressed pistils compared to the control indicating that 1-MCP has the potential to interfere in carbohydrate metabolism of reproductive units.

Keywords: Cotton; 1-Methylcyclopropene; Ethylene; Water-Deficit Stress; Carbohydrates; Gas Exchange

1. Introduction

Plant growth and crop yields are greatly affected by limited water supply [1]. Approximately one third of cultivated areas around the world are subjected to inadequate supplies of water [2], and the severity of the problem is expected to increase due to the changing climatic trends [3].

Cotton (*Gossypium hirsutum* L.), a perennial with a complex and indeterminate growth habit that originated in hot and arid areas [4], is considered to be relatively drought tolerant. However, defense mechanisms such as leaf and root osmotic adjustment [5,6] accumulation of compatible osmolytes [7], production of heat shock proteins [8] as well as high water use efficiency [9] are associated with cotton's indeterminate growth habit [10]. As a result, due to its domestication and cultivation as an annual crop, modern cotton cultivars suffer significant yield decreases under conditions of limited water supply [11].

Ethylene is a plant growth hormone involved in numerous physiological functions [12] and its synthesis is continually occurring at low rates under normal conditions. However, the production of ethylene significantly increases under conditions of biotic or abiotic stresses [13-15]. Water-deficit stress and ethylene interaction has been the subject of much debate since contrasting results have been reported for different crops [12,14,16-18].

In cotton, water-deficit stress has been observed to increase ethylene production ultimately resulting in leaf and fruit abscission [19-21]. However, in later studies, Morgan *et al.* [13] reported that ethylene rates of water-stressed intact plants were decreased, and suggested that ethylene production depends on the rate that the stress is imposed, and those findings were confirmed by Bugbee (2011) [22] and Klassen and Bugbee (2003) [18].

Ethylene biosynthesis inhibitors such as silver, silver thiosulfate (STS), and aminoethylvinylglycine (AVG) as well as blockers of ethylene receptors have provided valuable help in ethylene research. 1-Methylcyclopropene (1-MCP) is a gaseous material that acts by binding on ethylene receptors [23] and reducing plant sensitivity to ethylene due to its high affinity to the ethylene recap-

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tors (nearly 10-fold) compared to ethylene [24]. Application of 1-MCP on climacteric fruits has been shown to decrease ethylene production [24-26], respiration [24-27] and chlorophyll degradation [24,27,28]. However, in cotton, application of 1-MCP has produced contrasting results. Kawakami *et al.* (2010) [29] observed that application of 1-MCP on 4-week-old water-stressed cotton plants increased stomatal resistance, water potential and activity of antioxidant enzymes while it decreased membrane leakage compared to the control [29]. On the contrary, da Costa and Cothren (2011) [30] reported that 1-MCP had no effect on gas exchange, chlorophyll content and dry matter partitioning of 16-week-old water-stressed cotton plants while the increase in the number of reproductive nodes that was observed in 1-MCP treated water-stressed plants did not result in higher yield since 1-MCP caused higher fruit abscission [30]. Nevertheless, no further explanation was given on the reason for higher abscission. Recent research in other crops [31-34] has indicated that ethylene increases due to water-deficit stress result in perturbations in the carbohydrate metabolism of reproductive units that consequently result in yield losses. Bearing in mind that the leaf subtending to the fruit is providing the majority of photosynthates to the developing boll in cotton [35], the objective of these studies was to evaluate the possible ameliorating effect of the anti-ethylene plant regulator, 1-MCP on cotton's floral buds and subtending leaves under conditions of limited water supply during reproductive development. It was hypothesized that application of 1-MCP would prevent ethylene action and result in alleviation of water deficit stress effects on the cotton flower and consequently prevent yield loss.

2. Materials and Methods

Growth chamber studies were conducted and repeated at the Altheimer Laboratory, University of Arkansas, during 2009-2010. Cotton (*Gossypium hirsutum* L.,) cultivar ST 5288B2F was planted in 2L pots containing Sunshine potting media mix#1 (SunGro Distribution Inc., Bellevue, WA). Pots were arranged in a growth chamber (Conviron PGW36, Conviron Inc., Winnipeg, Canada) that was equipped with incandescent and fluorescent lamps and set for a 12h photoperiod with a photosynthetic flux density (PPFD) of 800 - 850 µmol/m²s and a relative humidity of 60%. Normal day/night temperatures of 32˚C/24˚C (maximum during the day and minimum during the night) were imposed throughout the duration of the experiments simulating a normal diurnal variation. All pots received half-strength Hoagland's nutrient solution daily to maintain adequate nutrients and water until flowering, approximately eight weeks after planting and induction of water-deficit stress and 1-MCP application treatments, after which plants were watered only with deionized water. The experiments were arranged in a completely randomized design with two factors that consisted of water-deficit stress and 1-MCP application. Application of 1-MCP started at flowering (approximately eight weeks after planting) and the treatments consisted of: Untreated well-watered control (C), Control + 1-MCP (C + 1MCP), Untreated water stressed (WS), and Water Stress + 1-MCP (WS + 1MCP) with fifteen replications for each treatment (**Table 1**). The rate of 1-MCP was 10 g a.i./ha. Control plants received optimum quantities of water during the experiment. Optimum quantity was determined by weighing the plants the day before and after watering to saturation and allowing for excess drainage. A water-stress cycle consisted of withholding water from the pots until stomatal closure, after which the stress was relieved by re-watering with optimum quantity. This process was repeated twice. 1-MCP was applied with a CO_2 backpack sprayer calibrated to deliver 187 l/ha two days after water supply had been discontinued (day 2 and day 8). The adjuvant AF-400 (Rohm Hass, Philadelphia, PA) was used for all 1-MCP applications at 0.375% v/v.

2.1. Stomatal Conductance Measurements

Stomatal conductance measurements (n = 10) were taken daily from the fourth uppermost main-stem leaf from 11:00 a.m. until 1:00 p.m. using a Decagon SC-1 Porometer (Decagon Inc., Pullman, WA). Three measurements on various areas of the leaf were taken and then averaged. The results were expressed as mmol/m²s.

2.2. Photosynthesis and Respiration Measurements

A Li-Cor Model 6200 portable photosynthesis system (LICOR Inc., Lincoln, NE) was used to determine photosynthetic and respiratory rates for the attached, fourth main-stem leaf from the terminal of the plant (n = 10). Photosynthesis measurements were taken at 1:00 p.m. one and four days after spraying. Respiratory rates were taken at 2:00 p.m. one and four days after spraying after turning off the lights in the growth chambers for 15 minutes and additionally covering the plant with a black cloth during the measurement.

2.3. Carbohydrate Content Measurements

White flowers and their subtending leaves were collected the last two days of each drying cycle at noon. Soluble carbohydrate content was measured according to a modification of the Hendrix protocol (1993) [36]. Ten white

Table 1. Rate and timing of treatments applied.

Treatments	Details	Rate	Timing
Control	Untreated well-watered control	-	-
Control + 1MCP	Well-watered control and 1-MCP	10 g a.i./ha	First flower
WS	Untreated water-stressed	-	-
WS + 1MCP	Water-stressed and 1-MCP	10 g a.i./ha	First flower

flowers and their subtending leaves were sampled from each replication-plant and they were oven dried for 3 days at 50˚C and then ground with a mortar and pestle. The ground tissue was extracted 3 times with 80% aqueous ethanol (800 ml ethanol/L) and the samples were centrifuged after each extraction at 5000 rpm and finally the fractions were pooled. Active charcoal was then added to the pooled fractions to remove substances that could interfere with the carbohydrate measurements and the samples were centrifuged again at 3500 rpm. The supernatant was immediately stored at −80˚C for later determination of sucrose and hexose (fructose and glucose) with a MultiScan Ascent Microplate Reader (Thermo Fisher Scientific Inc., Waltham, MA). A glucose HK-assay kit (Sigma Chemical Company, St Louis, MO) was used. A 10µl aliquot of each extract was pipetted into a well of a microtitration plate and the plate was incubated at 50˚C for 40 min to evaporate ethanol. Ten microliters of water were then added to each well along with 100 µl of glucose assay reagent and the plate was incubated again for 15 min at 30˚C. The absorbance was measured at 340 nm using a microplate reader. Subsequently, 0.25 enzyme units of phosphoglucose isomerase was added to the extracts in each well of the plate and the absorbance was again measured at 340 nm after which, 83 enzyme units of invertase were added to the extracts and the microtitration plate was incubated at 30˚C for 60 min. The absorbance was measured at 340 nm.

2.4. Statistical Analysis

A two factor factorial statistical analysis was used to evaluate the results using JMP8 software (SAS Institute, Cary, NC). The factors consisted of experiment, water regime, and 1-MCP application. No interaction was observed between the two separate experiments, so the results were pooled and the means were taken. Analysis of variance and student's t-test were used to analyze statistical significance. The days of the experiment were not considered a factor and a single ANOVA was done for each day to compare differences among treatment combinations.

3. Results

3.1. Stomatal Conductance

Leaf stomatal conductance was significantly lower in water-stressed plants compared to the control (**Figure 1**) in both watering cycles of the study. Stomatal conductance began to decrease 3 days after water supply was stopped, reaching its lowest at 48 mmol/m²s for untreated water-stressed plants and 42 mmol/m²s for 1-MCP treated water-stressed plants in 6 days. Upon rewatering, stomatal conductance measurements returned to same levels as the control. No significant interaction ($P = 0.1043$) was observed between 1-MCP application and water-deficit stress regime in any day of the experiment while a significant effect of the water regime ($P < 0.001$) was observed on days 0, 1, 3, 4, 6, 7, 8, 10 and 11 with water-deficit stress significantly lowering stomatal conductance rates compared to the control. On the other hand, stomatal conductance rates of 1-MCP treated water-stressed plants were similar to the rates of untreated water-stressed leaves and significantly lower compared to the control.

3.2. Photosynthesis and Respiration

Photosynthetic rates for the fourth main-stem leaf from the terminal were significantly decreased under conditions of water-deficit stress compared to the control (**Figure 2**). 1-MCP had no significant effect on preventing reductions in photosynthetic rates under water-limited supply, since photosynthetic rates were decreased by 43% without 1-MCP application, and by 41% after 1-MCP application, compared to the control, respectively.

A similar trend was observed for the respiration rates of the fourth main-stem leaf from the terminal (**Figure 3**). Again water-limited supply caused a significant reducetion in the respiratory rates in the water-stressed leaves compared to the control. Similarly to stomatal conductance and photosynthesis, 1-MCP had no significant ameliorating effect on the respiration rates under water stress, with respiration rates decreasing 35% compared to the control without application of 1-MCP, and 42%

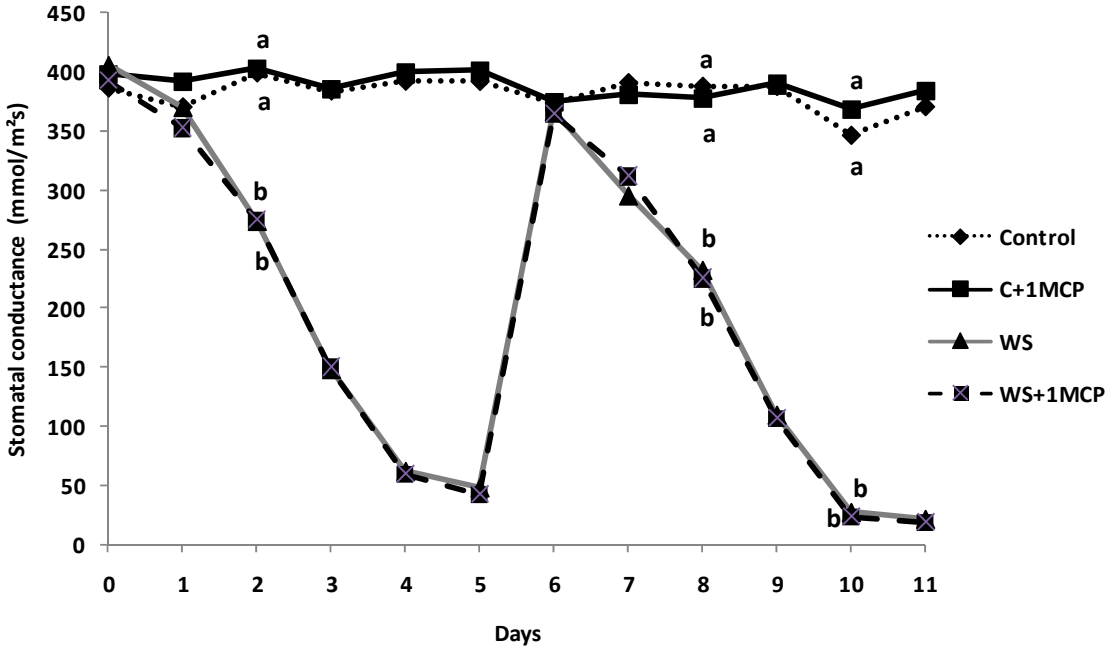

Figure 1. Effect of water-deficit stress and 1-MCP application on leaf stomatal conductance. Plants were imposed on two 5-day drying cycle. Water was withheld on day 0 and reapplied at the end of day 5. 1-MCP was applied on days 2 and 8.

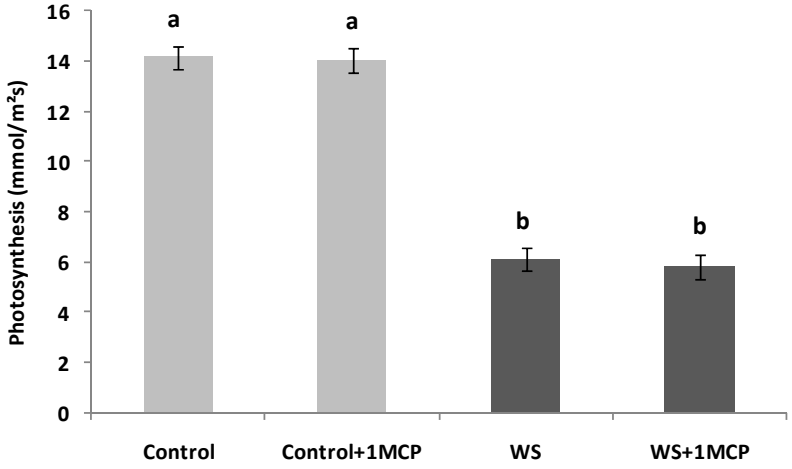

Figure 2. Effect of water-deficit stress and 1-MCP application on leaf photosynthesis 4 days after induction of stress. Bars with the same letter are not significantly different (P = 0.05). Error bars represent ± 1 standard error.

compared to the control after 1-MCP application.

3.3. Total Soluble Carbohydrate Content

Water-deficit stress caused a significant increase in sucrose concentrations of the pistil (**Figure 4**), however, sucrose concentrations of the subtending leaf remained unaffected (**Figure 5**). On the other hand, glucose levels of the pistil remained at the same levels as the control (**Figure 6**), whereas a significant increase was observed in the glucose concentrations of the water-stressed subtending leaves compared to the control (**Figure 7**). Both fructose levels of both the pistil and the subtending leaf remained unaffected under conditions of water-deficit stress and their levels were similar to the control (data not shown).

Application of 1-MCP resulted in a significant decrease in sucrose concentration of the pistils under conditions of water-deficit stress (**Figure 4**). No effect of 1-MCP application was observed in pistil glucose (**Figure 6**) and fructose concentrations (data not shown). Similarly, no significant effects of 1-MCP were observed on glucose, fructose and sucrose levels of water-stressed subtending leaves compared to the control.

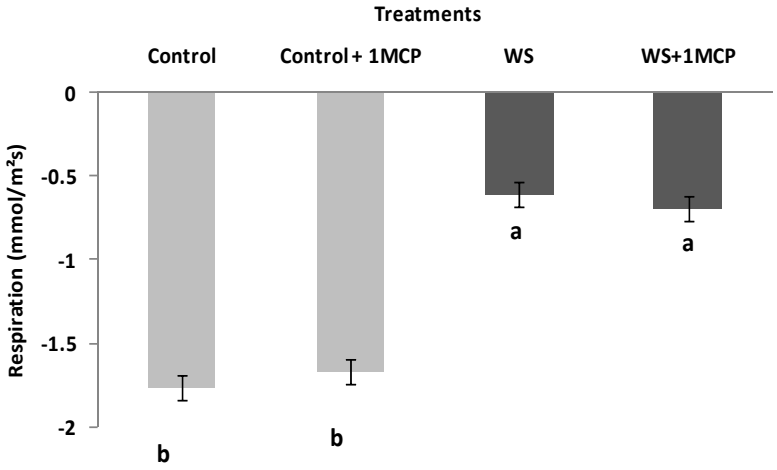

Figure 3. Effect of water-deficit stress and 1-MCP application on leaf respiration 4 days after induction of stress. Bars with the same letter are not significantly different (P = 0.05). Error bars represent ± 1 standard error.

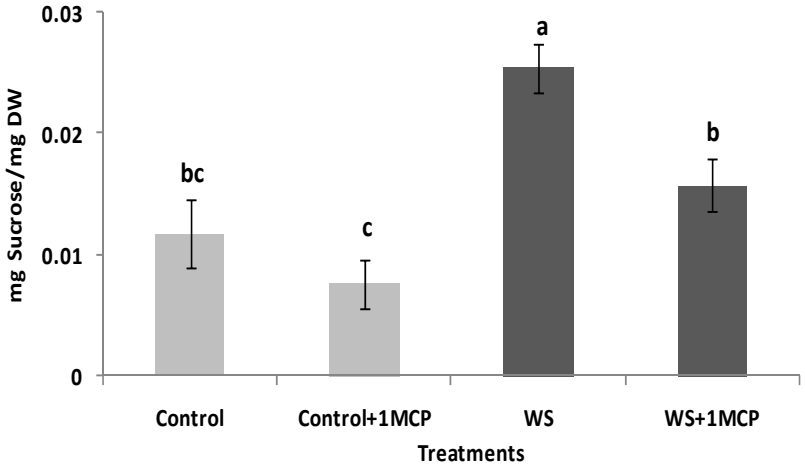

Figure 4. Effect of water-deficit stress and 1-MCP application on pistil sucrose content. Columns with the same letter are not significantly different (P = 0.05). Error bars represent ± 1 standard error.

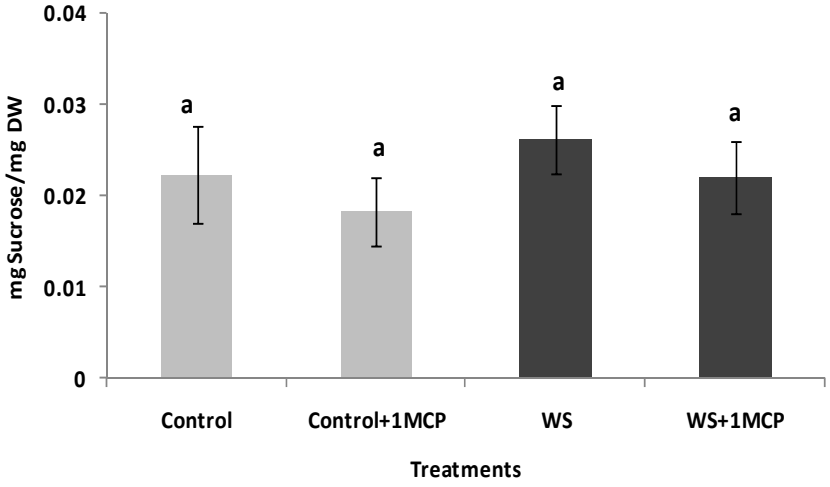

Figure 5. Effect of water-deficit stress and 1-MCP application on leaf sucrose content. Columns with the same letter are not significantly different (P = 0.05). Error bars represent ± 1 standard error.

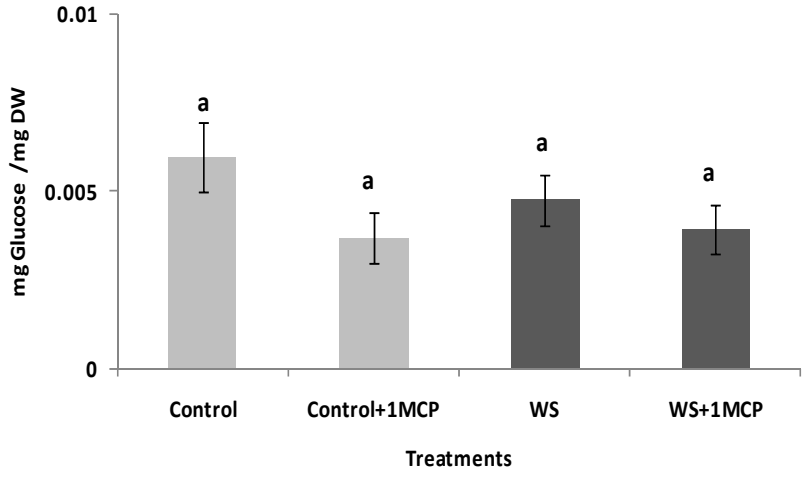

Figure 6. Effect of water-deficit stress and 1-MCP application on pistil glucose content. Columns with the same letter are not significantly different (P = 0.05). Error bars represent ± 1 standard error.

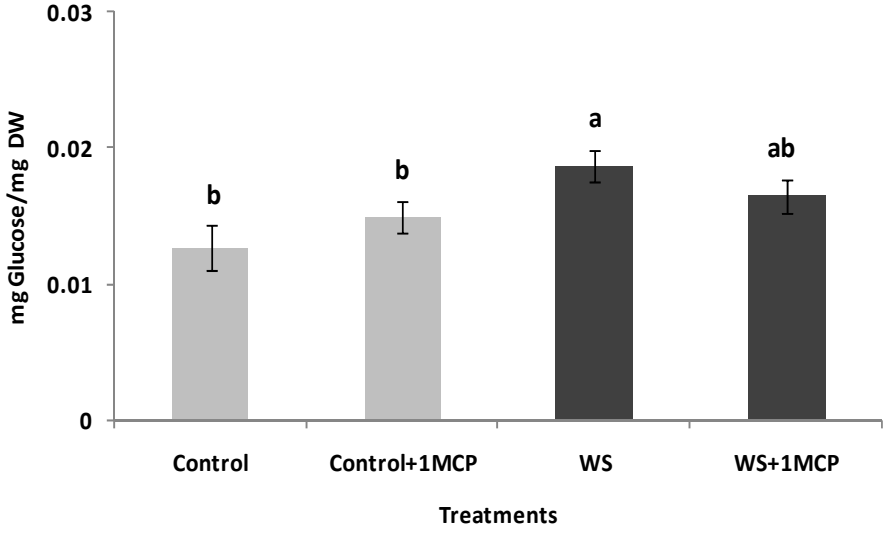

Figure 7. Effect of water-deficit stress and 1-MCP application on leaf glucose content. Columns with the same letter are not significantly different (P = 0.05). Error bars represent ± 1 standard error.

4. Discussion

Stomatal closure and consequently decreased stomatal conductance is a well-documented plant response to limited water conditions [37] in order to prevent excess water loss and in our study significant reductions of leaf stomatal conductance rates were observed in water-stressed plants compared to the control. Similar reductions in leaf stomatal conductance rates under water-deficit stress have been reported in cotton by numerous researchers [24,25,33,34] with the exception of Ackerson *et al.* (1977) [35] who observed that leaf stomatal conductance of field-grown cotton was only slightly affected. However, we speculate that this differential response was due to the differences in light intensity as well as the more gradual imposition of water-deficit stress in the field compared to our potted experiments.

Despite past reports that ethylene production of cotton increases under conditions of limited water supply [13,20,21] inducing stomatal closure and lowering stomatal conductance [38,39], no such responses were observed in our study since application of 1-MCP had no significant effect on stomatal conductance of water-stressed plants. In accordance with our results, Klassen and Bugbee (2003) [18], Bugbee (2011) [22], and da Costa and Cothren (2011) [30] reported that 1-MCP application on potted cotton plants subjected to water deficit during their reproductive stage had no significant effect on leaf stomatal conductance rates and concluded that water-deficit stress had no effect on ethylene evolution rates. The opposite results were reported by Kawa-

kami *et al.* (2010) [29] where it was observed that 1-MCP treated water-stressed plants exhibited higher stomatal resistance compared to untreated water-stressed plants and the authors concluded that ethylene production increases under conditions of limited water supply. However, in their experiment plants were subjected in water stress during their vegetative stage and not during their reproductive stage leading us to speculate that the differential to our study results are due to the differential ethylene production depending on the growth stage [40].

As expected, photosynthetic and respiratory rates of water-stressed plants were significantly compromised compared to well-watered control. Regarding photosynthesis, similar decreases in leaf photosynthesis under conditions of limited water supply have been observed in cotton [25,41,42] as well as several other species [43,44]. Unlike photosynthesis, diverse opinions have been expressed on the effect of water-deficit stress on leaf respiration with several researchers reporting significant decreases [45-47] while Ghashgaie *et al.* (2001) [48] noticed the opposite, and Lawlor and Fock (1977) [49] reported no change. In cotton, contrary to our results, Pallas *et al.* (1967) [50] reported an increase in respiretion under water stress, however we speculate that this is due to the different growth stages the stress was imposed in their studies.While exogenous application of ethylene has been reported to result in decreases in photosynthesis and respiration rates in soybean (*Glycine max* L.) [51], peanut (*Arachis hypogea* L.) [35] wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), pea (*Pisum sativum* L.), beans (*Phaseolus vulgaris* L.), cotton [53] and lettuce (*Lactuca sativa* L.) [53], application of 1-MCP failed to prevent reduction of both photosynthetic and respiratory rates. In accordance with our results Bugbee (2011) [22] and da Costa and Cothren (2011) [30] reported that 1-MCP application had no effect on water-stressed cotton leaf gas exchange functions, leading us to accept their conclusion that water-deficit stress has no effect on leaf ethylene production rates and leaf gas exchange functions in cotton.

Similarly to leaf gas exchanger functions, leaf carbohydrate metabolism was significantly affected from water-deficit stress, while 1-MCP application had no significant effect. In our study, limited water-supply resulted in increases in leaf glucose concentrations whereas, fructose and sucrose concentrations remained similar to the control. Increases in leaf hexose concentrations under conditions of water-deficit stress have also been reported in other crops such as soybean [54], and barley (*Hordeum vulgare*, L.) [55], while in cotton Eaton and Ergle (1948) [56], Timpa *et al.* (1986) [57] and Parida *et al.* (2007) [58] observed that water-stressed leaves contained higher glucose concentrations and similar sucrose levels compared to the control indicating an impairment in photosynthate translocation mechanism. Observations in *Arabidopsis* have suggested that ethylene is implicated in leaf carbohydrate metabolism through modulating translocation of photosynthates [59], and also through regulation of enzymes such as invertase and sucrose synthase [60] and carbohydrate concentrations [61,62]. However, in our study leaf glucose, fructose and sucrose concentrations of 1-MCP treated plants were similar to those of untreated plants under both water-stressed and well-watered conditions leading us to assume that ethylene is not implicated in leaf carbohydrate metabolism of cotton.

Pistil carbohydrate metabolism was expected to be similar to leaf carbohydrate metabolism due to the subtending leaf being the main provider of photosynthate products to the pistil [63]. However, pistil carbohydrate metabolism was significantly affected by both water-deficit stress and 1-MCP application. Water-deficit resulted in significant increases in pistil sucrose concentrations, while pistil glucose and fructose concentrations remained unaffected. The differential responses between leaf and pistil carbohydrate concentrations were attributed to tissue specific regulation of sucrose cleaving enzymes, with invertase being up-regulated in the leaves and down-regulated in the fruiting forms under conditions of water-deficit stress [27,62]. In support of our speculation increases in sucrose accumulation of reproductive units under limited water supply have been previously observed in soybean, wheat, maize and rice (*Oryza sativa* L.) [34,54,64-66] and have been associated with cessation of ovary and anther growth due to inhibition of sucrose cleaving enzymes function. In cotton, Guinn (1976) [21] reported that no significant differences were observed in carbohydrate accumulation of 4-day old bolls that had been subjected to limited water supply. We speculate that the contrasting to our study results are due to differences in water-deficit stress imposition and age of tissue. Nevertheless, Guinn (1976) [21] reported that ethylene levels of water-stressed bolls were significantly higher compared to control.

Ethylene has been observed to modulate sucrose concentrations of reproductive units in sugar beet (*Beta vulgaris* L.) [67], grape berry (*Vitis vinifera* L.) [68] and rice [69]. In support of these observations, reductions in grain filling rate and weight due to increases in ethylene under conditions of water stress have been reported in wheat [70] and rice [34], while Mohapatra *et al.* (2000) [71] and Naik and Mohapatra (2000) [72] observed that application of ethylene inhibitors on rice improved grain filling and enhanced sucrose synthase activity under conditions of water stress. In accordance to the above reports, in our study application of 1-MCP had a pronounced effect on pistil sucrose concentration, signifi-

cantly inhibiting sucrose accumulation under water-stress compared to the untreated water-stressed plants. However, reductions in sucrose concentrations, even though not significant, were observed between the treated well-watered and the untreated well-watered leading us to assume 1-MCP decreased plant sensitivity to ethylene that is produced naturally during cotton flowering [13, 73]. Nevertheless, Morgan *et al.* (1990) [13] reported that flowering cotton plants subjected to water stress showed a decrease in ethylene production rates, while no changes in ethylene production rates were reported by Bugbee (2011) [22] and da Costa and Cothren (2011) [30]. Since ethylene rates were not determined in our study we cannot comment if 1-MCP affected ethylene production rates, however we can conclude that ethylene affects carbohydrate metabolism of cotton reproductive units. We speculate that the differential response of 1-MCP observed in cotton leaves and pistils is attributed either to the lower number of receptors existing in the leaves compared to the reproductive units [74] or to the differential regulation of sucrose cleaving enzymes due to tissue specific response of ethylene under conditions of water-deficit stress [40].

In summary, the results of our study indicated that water-deficit stress during reproductive development resulted in significant decreases in cotton leaf stomatal conductance, photosynthesis and respiration. Application of 1-MCP failed to ameliorate the negative consequences of water-deficit stress on cotton gas exchange functions indicating that either ethylene evolution from the leaves is minimal under conditions of water stress or ethylene evolution is uncoupled from cotton leaf's gas exchange functions. However, the significant decrease in pistil sucrose concentrations indicates that ethylene plays a rather important role in the regulation of carbohydrate metabolism in reproductive tissues. Further research is needed to elucidate the exact site of modulation and factors controlling the interaction between ethylene production under water-deficit stress and cotton carbohydrate accumulation.

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