



Decreased CO₂ availability and inactivation of Rubisco limit photosynthesis in cotton plants under heat and drought stress in the field

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ABSTRACT

Heat and drought stresses are often coincident and constitute major factors limiting global crop yields. A better understanding of plant responses to the combination of these stresses under production environments will facilitate efforts to improve yield and water use efficiencies in a climatically changing world. To evaluate photosynthetic performance under dry-hot conditions, four cotton (*Gossypium barbadense* L.) cultivars, Monseratt Sea Island (MS), Pima 32 (P32), Pima S-6 (S6) and Pima S-7 (S7), were studied under well-watered (WW) and water-limited (WL) conditions at a field site in central Arizona. Differences in canopy temperature and leaf relative water content under WL conditions indicated that, of the four cultivars, MS was the most drought-sensitive and S6 the most drought-tolerant. Net CO₂ assimilation rates (*A*) and stomatal conductances (*g_s*) decreased and leaf temperatures increased in WL compared to WW plants of all cultivars, but MS exhibited the greatest changes. The response of *A* to the intercellular CO₂ concentration (*A*–*C_i*) showed that, along with stomatal closure, non-stomatal factors associated with heat stress also limited *A* under WL conditions, especially in MS. The activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) decreased in WL compared to WW plants, consistent with thermal inhibition of Rubisco activase activity. The extent of Rubisco deactivation could account for the metabolic limitation to photosynthesis in MS. Taken together, these data reveal the complex relationship between water availability and heat stress for field-grown cotton plants in a semi-arid environment. Both diffusive (drought-stress-induced) and biochemical (heat-stress-induced) limitations contributed to decreased photosynthetic performance under dry-hot conditions.

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1. Introduction

Abiotic stress has been long recognized as a major factor limiting plant productivity. Heat and drought stresses are caused by

elevated temperatures and decreased water availability, respectively, that deviate from the optimal condition for plant life (Larcher, 2003). Water availability, either from rainfall or irrigation, is typically the single most important factor determining yield (Boyer, 1982). In certain geographic regions, heat stress also has a significant impact on plant productivity, with major reductions in yield occurring in response to growing-season temperatures that are only one or two degrees warmer than the optimum (Lobell and Field, 2007; Schlenker and Roberts, 2009). Climate change models predict that the frequency and intensity of both drought and heat stresses will increase in the near future (IPCC, 2007). Development of plants with superior heat and drought tolerance for cultivation under deficit irrigation or water-limited conditions will improve water use efficiencies while minimizing yield penalties (Feres et al., 2011).

The number of studies on plant responses to water deficit has increased markedly over the past two decades, but the majority of these studies examined potted plants in growth chambers or greenhouses and used disparate experimental conditions (Lawlor and Tezara, 2009; Pinheiro and Chaves, 2011). Because the response

Abbreviations: *A*, net CO₂ assimilation rate; *A_c*, RuBP-consumption limited *A*; *A_i*, RuBP-regeneration limited *A*; *C_i*, intercellular CO₂ concentration; DAW, days after watering; DW, dry weight; *E*, transpiration rate; *F_v*/*F_m*, maximum efficiency of PSII; FW, fresh weight; *g_m*, mesophyll conductance to CO₂; *g_s*, stomatal conductance to water vapor; IRT, infrared thermometer; *J_{max}*, maximum rate of electron transport; MS, Monseratt Sea Island; P32, Pima 32; PPFD, photosynthetic photon flux density; PSII, photosystem II; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RWC, leaf relative water content; S6, Pima S-6; S7, Pima S-7; SLA, specific leaf area; TW, turgid weight; *V_{cmax}*, maximum rate of RuBP carboxylation; VPD, vapor pressure deficit; WL, water-limited; WW, well-watered; ϕ_{PSII} , operating efficiency of PSII.

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to drought depends on the frequency, intensity and duration of the water deficit treatment, as well as on the genotype, developmental stage, and tolerance/acclimation potential of the plant species, it is difficult to make generalizations and evaluate the relevance of the available data for field-grown plants. Because of these shortcomings, it is more biologically relevant to evaluate plant responses to water deficit under field conditions and in combination with other commonly coincident stresses, particularly heat.

In semi-arid environments, heat stress is closely linked to water availability. When water is readily available to the plant, a large vapor pressure gradient exists between the leaf and the low humidity air and this gradient provides the driving force for leaf cooling via transpiration. Cotton (*Gossypium* spp.), the primary source of natural fiber in the world, is cultivated in semi-arid environments including the southwestern United States. In cotton plants, the magnitude of leaf cooling on very hot days can be exceptional, sometimes reaching 10 °C (Jackson et al., 1981; Upchurch and Mahan, 1988; Burke and Upchurch, 1989; Lu et al., 1994; Radin et al., 1994). Under these conditions, water loss is substantial and persistent, and can eventually lower the relative water content of the plant, even under well-watered conditions. Breeding efforts over the past century have greatly improved the productivity of Pima cotton (*G. barbadense* L.) cultivars for cultivation under irrigated conditions in Arizona. Cultivars released more recently are well-adapted to high air temperatures (Ulloa et al., 2009), but this adaptation is accomplished through a heat avoidance mechanism that uses considerable water for evaporative cooling (Radin et al., 1994). The genetically diverse Pima cotton germplasm provides an ideal biological system for understanding the biochemical basis for heat tolerance, a pertinent goal that will help to develop cultivars with improved yields while optimizing water use in arid environments.

One of the primary responses of plants to water deficit is stomata closure. This response minimizes water loss, but also lowers the intercellular CO₂ concentration (C_i), causing a stomatal or diffusional limitation to photosynthesis (Chaves et al., 2003) because Rubisco, the enzyme that catalyzes CO₂ assimilation in photosynthesis, has a relatively low affinity for CO₂. In addition, reduced transpiration under hot, sunny conditions decreases the capacity for leaf cooling, increasing leaf temperature and, consequently, the incidence of heat stress. Photosynthesis is acutely sensitive to inhibition by moderate heat stress (i.e., <10 °C above the thermal optimum), and this inhibition has been linked to various causes, including the thermal instability of Rubisco's molecular chaperone, Rubisco activase (Feller et al., 1998; Law and Crafts-Brandner, 1999; Crafts-Brandner and Salvucci, 2000; Salvucci and Crafts-Brandner, 2004a,b). Severe drought stress conditions also cause metabolic limitations to photosynthesis because, as water becomes increasingly scarce, leaf water content decreases to levels that adversely affect metabolism (Lawlor and Tezara, 2009). Photosynthesis in semi-arid environments is therefore prone to inhibition by diffusive and metabolic limitations that are caused by the combined effects of drought and heat stresses.

In the present study, the physiological responses of four Pima cotton cultivars were examined in a hot, arid environment under well-watered (WW) and water-limited (WL) conditions. A high-clearance tractor was used for high-throughput canopy temperature measurements in conjunction with leaf gas-exchanges and other lower-throughput physiological methods. The objective of the study was to characterize the photosynthetic responses of the different cultivars to conditions that promote both drought and heat stress in the field. The hypotheses tested herein are: (1) under WL conditions, stomatal closure is accompanied by metabolic limitations to photosynthesis when transpiration is insufficient to cool the leaves, and (2) these metabolic limitations are associated with temperature-induced changes in Rubisco activation.

2. Materials and methods

2.1. Plant material

A set of 25 diverse cotton (*G. barbadense* L.) accessions was assembled to capture a wide range of genetic variability for heat tolerance. The panel includes Pima cotton cultivars released over a period of 90 years (1918–2009) by breeding programs in Arizona, heat-sensitive Sea Island type cultivars from the Caribbean, and a recently released commercial Pima cotton variety. A subset of four accessions was selected for physiological and biochemical studies: Monseratt Sea Island (MS), Pima 32 (P32), Pima S-6 (S6) and Pima S-7 (S7), which were released in ca. 1931, 1949, 1983 and 1992, respectively. In general, modern Pima cotton cultivars are better adapted to high air temperatures and achieve higher yields in irrigated agricultural areas of Arizona relative to earlier released cultivars (Kittock et al., 1988; Cornish et al., 1991; Ulloa et al., 2009).

2.2. Experimental design, cultivation and irrigation regime

The 25 cultivars were grown in the summer of 2010 at the Maricopa Agricultural Center in Maricopa, AZ (33°04'N, 111°58'W). Meteorological data for the experimental site were recorded by the University of Arizona Meteorological Network (AZMET; Brown, 1989) using the Maricopa weather station (<http://ag.arizona.edu/azmet/06.htm/>). The experiment was planted on May 7, 2010, in a 5 × 5 α-lattice with 4 replications for each water treatment, well-watered (WW) and water-limited (WL) (Supplementary Fig. S1). Experimental units consisted of a single cultivar in an 8.53 m plot per replicate, and plots were overplanted and thinned to an average of 61 plants. Plots were separated by a 0.91 m alley and inter-row spacing was 1.02 m. One plot of a conventional cultivar was planted as border on all sides of each 5 × 5 α-lattice. Standard agronomic and pest-control practices for irrigated cotton production in central Arizona were used. The soil type at Maricopa is a Casa Grande sandy loam (fine-loamy, mixed, superactive, hyperthermic Typic Natrargids).

Subsurface drip irrigation scheduling was performed using a daily soil water balance model calculated for the cotton root zone. Soil water balance inputs included estimated daily evapotranspiration, determined from the FAO-56 dual crop coefficient procedures (Allen et al., 1998), metered irrigation depths, and precipitation and meteorological data obtained from the AZMET weather station located about 270 m from the field. Irrigations to the WW plots were applied to refill the root zone water content to field capacity and were supplied at approximately 35% soil water depletion. Starting on July 14th, the WL plots received one-half of the irrigation amounts applied to the WW plots. The water deficit treatment was imposed at first flower to minimize the interaction of flowering time and drought stress. Weekly soil water content measurements to a depth of 1.5 m were made to monitor the actual soil water depletion and adjust the modeled soil water balance when needed. The last irrigation was on October 4th, thus the differential irrigation regimes were constant and consistent from first flower to defoliation.

Physiological and biochemical measurements were taken periodically, generally once-a-week, during weeks 12–15 after planting (late July–August). This timing coincided with transition from the vegetative to reproductive stage. Plants were analyzed 2–3 days after watering (DAW), i.e., when the WW plants were fully hydrated and the WL plants were experiencing water deficit. At the end of the experiment, gas-exchange measurements were also taken 1 DAW on the WL plants (see below).

2.3. High-throughput measurement of canopy temperature

A high-throughput phenotyping system consisting of a high-clearance tractor with front-mounted radiometric infrared thermometers (IRTs, SI-121; Apogee Instruments, Logan, USA) with a narrow field of view (18° half-angle) was used to measure canopy temperature throughout the growing season. Each IRT was individually calibrated with an empirical procedure using quadratic models. The system was deployed in the field at a constant speed of 0.5 m s⁻¹ with minimal disturbance to the plants within trafficked rows due to the high clearance of the tractor and tire shrouds for deflecting plant branches. Four suites of identical IRTs were mounted on a horizontal boom located 0.75 m ahead of the tractor front; thus, four rows were simultaneously monitored during each tractor pass. Measurements were geo-referenced with simultaneous recording of position from the NMEA-GGA (National Marine Electronics Association, Severna Park, USA) string output by a GPS-RTK receiver with an antenna located in the center of the horizontal boom. Data acquisition code was written to record the output of all IRTs at a frequency of once per second using a CR3000 data logger (Campbell Scientific, Logan, USA). Canopy temperature measurements were performed on four occasions throughout the growing season (corresponding to four consecutive weeks), at three different times of the day: 7:00–8:00 MST (CT1), 10:00–11:00 MST (CT2) and 13:00–14:00 MST (CT3). To reduce the influence of plot edge effects, only measurements collected from the internal 5.49 m of each plot were used to calculate canopy temperature on a plot basis for the four-replicated WW and WL plots of each cultivar (total of 32 plots).

2.4. Leaf relative water content

Samples were collected in the morning (9:00–10:30 MST) for four consecutive weeks of the experiment (weeks 12–15). Each sample consisted of two 2 cm² leaf disks excised from a young fully expanded leaf with a visible cuticle. Two samples were taken, each from a different plant, from each of the four-replicated WW and WL plots of each cultivar (total of 32 plots). The fresh, turgid and dry weights (FW, TW, DW) were determined and used to calculate the leaf relative water content [RWC (%) = (FW – DW)/(TW – DW)] and the specific leaf area [SLA (m² kg⁻¹) = leaf area/DW]. The level of dehydration increased with the number of DAW in a consistent manner over the four weeks of analysis (data not shown). Since plants were usually irrigated on day 4, sampling and analyses were conducted 3 DAW to obtain a consistent level of dehydration throughout the experiment.

2.5. Gas-exchange and fluorescence measurements

Gas-exchange and fluorescence parameters were measured with a LI-COR 6400 portable photosynthesis system equipped with a 6400-40 chlorophyll fluorometer (LI-COR Biosciences, Lincoln, USA). The analyzers were calibrated prior to the season and checked periodically for leaks. Measurements were conducted between 10:30 and 14:00 MST, using a reference CO₂ concentration of 380 μmol mol⁻¹, a photosynthetic photon flux density (PPFD) of 1800 μmol m⁻² s⁻¹ (10% blue light) and a block temperature of 32 °C. The irradiance matched the incident irradiance at the top of the canopy. Leaf temperatures of plants under WW conditions were similar to the block temperature, whereas the leaf temperatures of plants under WL conditions exceeded this temperature, both before and during the measurement. Once steady-state photosynthesis was achieved (generally within 2–4 min) the infrared gas analyzers were matched and values recorded. Gas-exchange and fluorescence parameters were calculated by the LI-COR OPEN software (<ftp://ftp.licor.com/perm/env/LI-6400/Manual/>

[Using_the_LI-6400XT-v6.2.pdf](#)). Calculation of the operating (ϕ_{PSII}) and the maximum (Fv'/Fm') efficiency of photosystem II used the formulas: $\phi_{PSII} = (Fm' - Fs)/Fm'$ and $Fv'/Fm' = (Fm' - Fo')/Fm'$. When measuring light-adapted leaves, Fs is the steady-state fluorescence, Fm' the maximal fluorescence at a saturating light pulse and Fo' the minimal fluorescence when leaves are momentarily darkened.

Measurements were conducted on fully expanded leaves near the top of the canopy, generally corresponding to the youngest fully expanded leaf with a developed cuticle. Because of constraints associated with the duration of the analyses and the supply of electrical power at the field site, measurements for each cultivar were taken from several plants within one single WW or WL plot (total of 8 plots). A limited number of measurements were occasionally taken from plants in the other plot replicates that verified the accuracy of differences observed among cultivars and between treatments (data not presented). Measurements on plants under WW and WL conditions were taken on three occasions during the growing season (weeks 12–15). On week 15, measurements of the plants in the WL plots were also taken on the day immediately following irrigation (i.e., under WL-irrigated conditions). Hence, comparisons between plants under WW, WL and WL-irrigated conditions, used values solely from measurements taken on week 15, to avoid confounding effects of plant age.

The response of net CO₂ assimilation to the intercellular CO₂ concentration (i.e., A–C_i curve) was also measured during week 15, by changing the concentration of the reference CO₂ as described previously (Long and Bernacchi, 2003). The reference CO₂ of the infra-red gas analyzer was changed in increments from 380 to 75 and then from 350 to 1500 μmol mol⁻¹. Modeling of the experimental data used the A/C_i Response Curve Fitting 10.0 utility available at <http://landflux.org/Tools.php>, which uses the curve fitting parameters of Ethier and Livingston (2004).

2.6. Rubisco activation states

Samples were collected at noon (12:00–12:30 MST), in parallel with the gas-exchange measurements, on three occasions during the growing season (weeks 12–15). Each sample consisted of two 0.5 cm² leaf disks, excised from a young fully expanded leaf with a visible cuticle. Several samples were taken, each from a different plant, from the same WW and WL plots used for gas-exchange and fluorescence analyses (total of 8 plots). Disks were plunged into liquid N₂ within 5 s of sampling and maintained at –80 °C until extraction. Leaf extracts were prepared by grinding the frozen disks at 4 °C in a Ten-Broeck glass homogenizer containing 0.5 mL of CO₂-free extraction medium composed of 100 mM Tricine–NaOH pH 8.0, 1 mM EDTA, 5% polyvinylpyrrolidone (PVP-40), 5% polyethylene glycol 3350 (PEG₃₃₅₀), 5 mM dithiothreitol (DTT), 1 mM phenylmethanesulphonyl fluoride (PMSF) and 10 μM leupeptin. Rubisco activity was measured by incorporation of ¹⁴CO₂ into acid-stable products at 30 °C (Salvucci, 1992) in aliquots that were assayed immediately upon extraction (initial activity) or after incubation for 3 min in assays containing all the components except RuBP to allow full carbamylation of the enzyme (total activity). For each sample, assays were conducted in duplicate. Initial and total activities were used to calculate Rubisco activation state, i.e., (initial/total activity × 100) = % activation.

2.7. Statistical analyses

Statistical analyses were carried out using the PROC MIXED procedure in SAS/STAT® 9.2 (SAS Institute Inc., Cary, USA). Statistical significance of trait variation was tested by analyses of variance (ANOVA) with cultivar, water regime, and their interaction as fixed factors. Sampling date and its interaction with cultivar and water

regime were included as random effects in the fitted model for traits measured periodically during weeks 12–15. Replicate plot, nested within date by water-regime, was included as a random effect for traits measured in the four-replicated WW and WL plots. Likelihood ratio tests were conducted to remove non-significant random terms ($\alpha = 0.05$) from the final model. The repeated statement was used in PROC MIXED to model the correlation structure of repeated measurements of a response variable (trait) on the same experimental unit (plot) over a period of time. Data transformations (square, log_e, inverse, or inverse squared) and unequal variance models were applied as necessary to ensure that the assumptions of the analyses were not violated. The Tukey–Kramer method was used for post hoc multiple pairwise comparisons between least square means (LSM) of the levels for each fixed effect. Values presented in the manuscript are means \pm standard error of the mean (SEM) of all measurements taken for each cultivar by water regime (n as indicated).

3. Results

3.1. Meteorological conditions at the experimental site

Air temperatures during early vegetative (June) through late reproductive (August) phase generally exceeded 35 °C and often 40 °C during the daily peak (Fig. 1). Daily low temperatures generally exceeded 20 °C and often 25 °C, particularly in July and August. No measurable precipitation was recorded from planting until July 21 and precipitation was minimal over the season (<60 mm) except for one event in late August (28 mm). Monthly averages of temperature, relative humidity and vapor pressure deficit (VPD) for June, July and August during the day hours (6:00–18:00 Mountain Standard Time; MST) were 36.3, 36.3 and 35.0 °C; 28.4, 38.4 and 33.8% and 4.6, 4.6 and 4.0 kPa, respectively. Diurnal conditions of temperature, humidity and irradiance were monitored for the specific days when measurements were taken (Supplementary Fig. S2).

3.2. Canopy temperatures

The canopy temperature of four cotton Pima cultivars, Monseratt Sea Island (MS), Pima 32 (P32), Pima S-6 (S6) and Pima S-7 (S7), was measured in the early morning (CT1), late morning (CT2) and early afternoon (CT3). There was a significant effect of cultivar on CT2 and CT3 and a significant effect of water regime on CT1 and CT3 (Table 1). In general, temperatures were higher in water-limited (WL) compared to well-watered (WW) plants and in MS compared to the other three cultivars (Fig. 2). Air temperatures increased as the day progressed and the canopy temperatures of the four cultivars also increased, most markedly under WL conditions. Under WW conditions, the canopy temperatures of the four cultivars were less than the air temperatures, particularly in the early morning and early afternoon. Of the four cultivars, only MS had a canopy temperature that occasionally matched the air temperature under WW conditions (Supplementary Fig. S3). In contrast, under WL conditions the canopy temperatures of all four cultivars generally approached or exceeded the air temperatures, especially by late morning and early afternoon (Fig. 2). The cultivar MS had the highest canopy temperatures, especially under WL conditions, frequently exceeding the air temperatures by early afternoon and sometimes by late morning.

3.3. Leaf relative water content and structural properties

The leaf relative water content (RWC) was significantly different among cultivars and water regimes and there was a strong cultivar by water regime interaction (Table 1). WW plants of all four cultivars had very similar RWC (82.5–83.7%; Fig. 3). Conversely, under

WL conditions, all cultivars had lower RWC compared to the WW plants and the two earlier released cultivars, MS and P32, had lower RWC than the more modern cultivars, S6 and S7. The average difference of RWC between WW and WL plants was $5.6 \pm 0.6\%$ for MS, $5.5 \pm 0.3\%$ for P32, $2.4 \pm 0.4\%$ for S6, and $2.5 \pm 0.2\%$ for S7, suggesting that S6 and S7 have a greater capacity to maintain a higher RWC under water deficit conditions compared to MS and P32. The specific leaf area (SLA) was significantly different between cultivars and water regimes, but the effect of water regime was the same across cultivars (i.e., no significant interaction; Table 1). Plants of P32, S6 and S7 had lower SLA than MS and there was a decrease in SLA for all four cultivars when plants were grown under WL compared to WW conditions (Fig. 3). The decrease in SLA was caused by a progressive increase in the leaf dry matter content throughout the growing season, which was greater in plants grown under WL conditions (data not shown).

For all four cultivars, leaf wilting was observed as the day progressed, especially for plants grown under WL conditions. The onset of leaf wilting occurred earliest for MS, generally by late morning, and allowed the plants to reduce the heat load by limiting the amount of incident solar radiation (Fig. 4). Measurements of RWC conducted on samples harvested in the afternoon showed that leaf dehydration through the day occurred in all four cultivars under both WW and WL conditions (data not shown). The lowest afternoon values were observed for plants under WL conditions, but the difference in RWC between WW and WL plants of each cultivar was only 0.5–2.4%, which was much less than the differences measured earlier in the day (see above). Greater differences earlier in the day were related to a greater extent of nighttime rehydration in plants under WW compared to WL conditions. Thus, the small, but significant difference in RWC in the morning served as a more reliable indicator of drought stress than a late day RWC.

3.4. Gas-exchange and chlorophyll fluorescence

The net CO₂ assimilation (A) and transpiration rate (E) were significantly different among cultivars; A , stomatal conductance to water vapor (g_s), intercellular CO₂ concentration (C_i) and leaf temperature (T_{leaf}) were significantly different among water regimes; and there was a significant cultivar by water regime interaction for all the above traits (Table 1). Under WW conditions, A was similar among cultivars, with an average seasonal rate for the four cultivars of $35.1 \pm 0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 5). Conversely, A was lower and more variable under WL conditions: the lowest seasonal average rate was $17.9 \pm 1.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ for MS, and the highest was $31.3 \pm 0.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ for S6. Together with canopy temperatures and RWC, these results indicated that MS was the most drought-sensitive and S6 the most drought-tolerant of the four cultivars. Similar to the response of A to water availability, differences in g_s , E , C_i and T_{leaf} among cultivars became evident under WL conditions. For instance, g_s was similar for the four cultivars under WW conditions, with an overall average of $0.71 \pm 0.01 \text{ mol m}^{-2} \text{s}^{-1}$, and decreased under WL conditions, more markedly for MS.

The photosynthetic properties of the drought-sensitive and -tolerant cultivars, MS and S6, respectively, were compared under WW, WL and WL-irrigated conditions (Table 2). Plants under WL-irrigated conditions were analyzed on the day after irrigation of the WL plots. These plants had fully rehydrated and showed no visible signs of drought stress. All traits were significantly different between water regimes and in most cases there was a strong cultivar by water regime interaction (Table 1). The data show that the decreased A and g_s in WL MS plants were accompanied by a lower C_i , reduced E and higher T_{leaf} (Table 2). In contrast, the drought-tolerant cultivar S6 was much less affected by the WL conditions, with much smaller decreases in A and g_s . The transpiration rate was almost unaffected in WL S6 plants and, as a result, the T_{leaf} increased

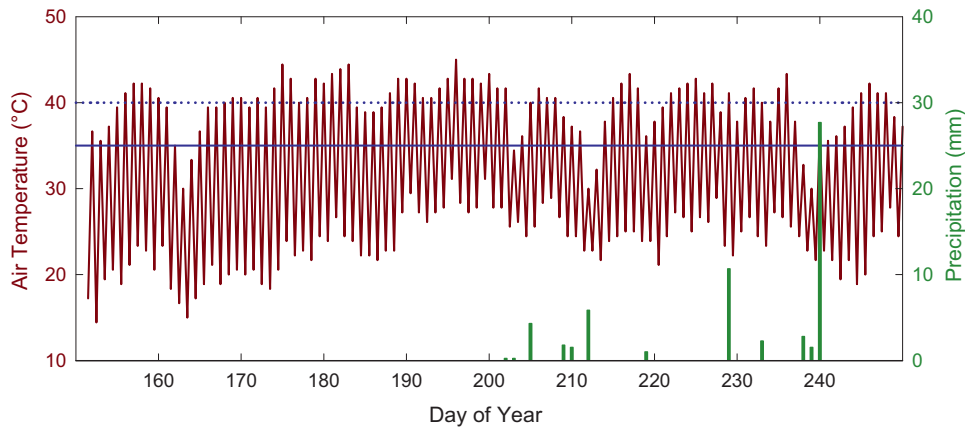


Fig. 1. Meteorological data for the 2010 cotton growing season in Maricopa, AZ. Air temperatures (lines) and precipitation (bars) levels were obtained from the University of Arizona meteorological station (<http://ag.arizona.edu/azmet/06.htm>). Horizontal lines at 35 and 40 °C are for reference.

Table 1

Analysis of variance (ANOVA) results for physiological and biochemical traits. Non-normal data were transformed to meet ANOVA assumptions. Degrees of freedom (df) were calculated via the Satterthwaite approximation. *F*-statistics (*F*) and *P*-values (*P*) are given for the fixed effects of cultivar, water regime, and their interaction. *P*-values representing statistical significance ($\alpha = 0.05$) are highlighted in bold.

Data	Trait	Transformation	Cultivar			Water regime			Cultivar × water regime		
			df num/den	<i>F</i>	<i>P</i>	df num/den	<i>F</i>	<i>P</i>	df num/den	<i>F</i>	<i>P</i>
Fig. 2	CT1	–	0.33	3.3	0.0689	0.2	8.5	0.0303	0.04	1.1	0.3454
	CT2	–	0.5	9.0	0.0116	0.25	7.3	0.0573	0.04	1.2	0.3323
	CT3	Log	0.07	20.0	<0.0001	0.33	22.4	0.0173	0.07	1.9	0.1399
Fig. 3	RWC	Square	0.33	7.0	0.0095	0.04	51.3	<0.0001	0.02	13.8	<0.0001
	SLA	Log	0.33	4.8	0.0286	0.04	40.3	<0.0001	0.02	0.6	0.5960
Fig. 5	<i>A</i>	Square	0.01	40.7	<0.0001	0.25	26.3	0.0065	0.01	10.9	<0.0001
	<i>g_s</i>	–	0.43	2.7	0.1264	0.25	33.5	0.0042	0.02	30.8	<0.0001
	<i>C_i</i>	Square	0.6	1.2	0.4063	0.01	501.3	<0.0001	0.02	32.5	<0.0001
	<i>E</i>	–	0.27	4.1	0.0354	0.5	12.9	0.0766	0.27	23.0	<0.0001
	<i>T_{leaf}</i>	–	0.5	0.9	0.4840	0.5	18.4	0.0481	0.02	42.0	<0.0001
Fig. 7	R-Act	Square	0.04	5.3	0.0024	0.02	54.4	<0.0001	0.05	6.4	0.0007
Table 2	<i>A</i>	–	0.04	54.5	<0.0001	0.09	84.0	<0.0001	0.09	20.3	<0.0001
	<i>g_s</i>	–	0.07	8.8	0.0099	0.14	195.2	<0.0001	0.14	16.8	0.0002
	<i>C_i</i>	Square	0.05	8.4	0.0086	0.18	159.2	<0.0001	0.18	24.3	<0.0001
	<i>E</i>	–	0.05	20.4	0.0002	0.2	61.7	<0.0001	0.2	32.6	<0.0001
	<i>T_{leaf}</i>	Log	0.11	4.1	0.0717	0.29	285.4	<0.0001	0.29	34.3	0.0003
	ϕ_{PSII}	–	0.03	2.0	0.1679	0.07	10.3	0.0004	0.07	1.7	0.2104
	<i>F_v</i> / <i>F_m</i> '	–	0.03	3.0	0.0940	0.07	30.0	<0.0001	0.07	3.5	0.0432
	<i>T_{leaf}</i>	Log	0.08	0.5	0.4839	0.17	78.9	<0.0001	0.17	16.4	0.0004
	<i>C_i</i>	–	0.33	0.1	0.7785	1	68.3	0.0074	1	20.4	0.0306
	<i>C_{trans}</i>	Inverse	0.08	7.3	0.0193	0.17	18.0	0.0002	0.17	3.6	0.0613
Table 3	<i>V_{cmax}</i>	Inverse-squared	0.08	5.0	0.0449	0.17	33.4	<0.0001	0.17	6.1	0.0151
	<i>J_{max}</i>	Log	0.08	6.3	0.0272	0.17	20.1	<0.0001	0.17	1.4	0.2843
	<i>g_m</i>	Log	0.08	0.6	0.4600	0.17	4.3	0.0384	0.17	3.6	0.0587

A, net CO₂ assimilation rate; *C_i*, intercellular CO₂ concentration; *C_{trans}* (*C_i*, *A_c*–*A_j*), CO₂ concentration at which RuBP-regeneration and RuBP-consumption co-limit photosynthesis; CT1, canopy temperature in the early morning; CT2, canopy temperature in the late morning; CT3, canopy temperature in the early afternoon; *E*, transpiration rate; *F_v*/*F_m*' , maximum efficiency of PSII; *g_m*, mesophyll conductance to CO₂; *g_s*, stomatal conductance to water vapor; *J_{max}*, maximum electron transport rate; R-Act, Rubisco activation state; RWC, leaf relative water content; SLA, specific leaf area; *T_{leaf}*, leaf temperature; *V_{cmax}*, maximum RuBP carboxylation rate; ϕ_{PSII} , operating efficiency of PSII.

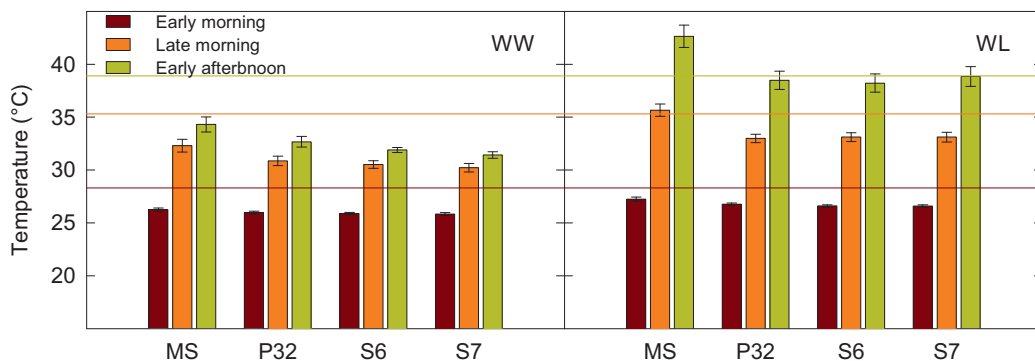


Fig. 2. Canopy temperature of the cotton cultivars Monseratt Sea Island (MS), Pima 32 (P32), Pima S-6 (S6) and Pima S-7 (S7), under well-watered (WW) and water-limited (WL) conditions at 7:00–8:00 MST (CT1), 10:00–11:00 MST (CT2) and 13:00–14:00 MST (CT3). Measurements were taken on four-replicated WW and WL plots, 3 DAW, for four consecutive weeks. Values are means ± SEM (*n* = 12–16). The corresponding average air temperatures at the measurement times are indicated by the horizontal lines (lower line = early morning, middle line = late morning, upper line = early afternoon).

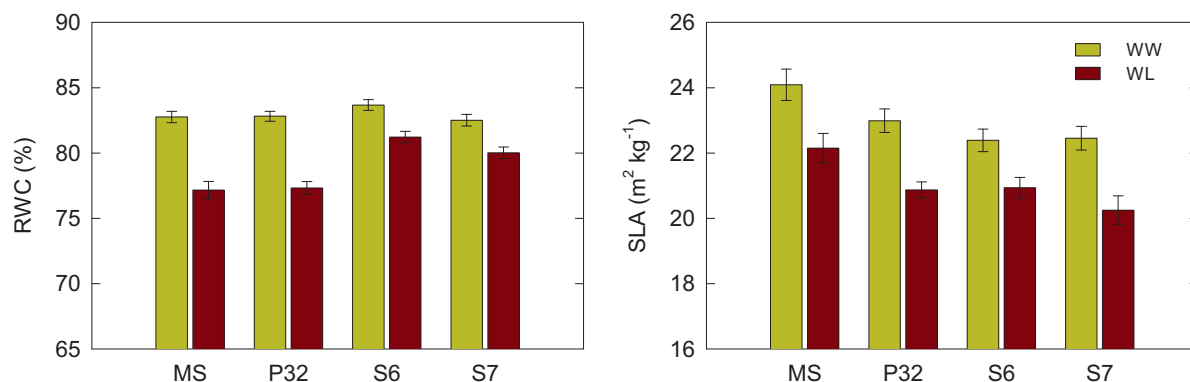


Fig. 3. Leaf relative water content (RWC) and specific leaf area (SLA) of the cotton cultivars Monseratt Sea Island (MS), Pima 32 (P32), Pima S-6 (S6) and Pima S-7 (S7), under well-watered (WW) and water-limited (WL) conditions. Two samples were taken from each of four-replicated WW and WL plots, 3 DAW, for four consecutive weeks. Values are means \pm SEM ($n = 31$ –32).

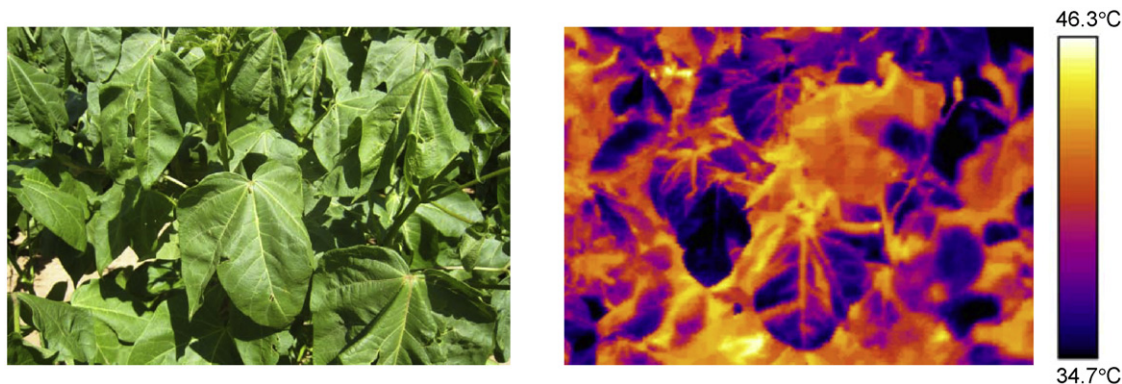


Fig. 4. Photograph (left panel) and forward looking infrared (FLIR) image (right panel) of a water-limited Monseratt Sea Island cotton plant. Both images were collected in the early afternoon, 3 DAW, on week 15 after planting. The color scale to the right shows the variation in leaf temperature produced by leaf wilting and subsequent shading. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

less in S6 than in MS WL plants. Both the operating (ϕ_{PSII}) and the maximum (F_v/F_m') efficiency of photosystem II, determined by chlorophyll fluorescence, were lower under WL conditions for both cultivars.

MS plants under WL-irrigated conditions had slightly lower g_s compared to WW plants, but there were no significant differences in A and ϕ_{PSII} between these plants ($P > 0.05$; Tukey–Kramer). A similar phenomenon of photosynthesis recovery was observed for the cultivar S6; there were no significant differences between WL-irrigated and WW plants for any of the traits in Table 2 for this cultivar ($P > 0.05$; Tukey–Kramer). These results indicate that the photosynthetic properties of plants grown under WL conditions were similar to those of the WW plants once the WL plants were re-watered.

3.5. Response of net CO_2 assimilation to the intercellular CO_2 concentration

The parallel response of CO_2 assimilation and stomatal conductance (Fig. 5) suggested that diffusional (i.e., stomatal) factors that restrict CO_2 availability to the site of carboxylation contributed significantly to the inhibition of photosynthesis under drought conditions. To determine if non-stomatal factors also contributed, the response of net CO_2 assimilation to the intercellular CO_2 concentration ($A-C_i$) was determined in the drought-sensitive and -tolerant cultivars, MS and S6, respectively, under WW, WL and WL-irrigated conditions (Fig. 6). Analysis of the response showed that CO_2 assimilation in both cultivars was limited by RuBP consumption, i.e., Rubisco activity, at atmospheric CO_2 concentrations under WW

Table 2
Comparison of the gas-exchange and fluorescence characteristics of the cotton cultivars Monseratt Sea Island (MS) and Pima S-6 (S6), under well-watered (WW), water-limited (WL) and water-limited but irrigated (WL-irrigated) conditions. Measurements were taken on week 15 of the experiment. Conditions for infra-red gas analysis were reference $CO_2 = 380 \mu\text{mol mol}^{-1}$, PPFD = $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ and block temperature = 32°C . Values are means \pm SEM ($n = 3$ –10). A , net CO_2 assimilation rate; g_s , stomatal conductance to water vapor; C_i , intercellular CO_2 concentration; E , transpiration rate; T_{leaf} , leaf temperature; ϕ_{PSII} (or F_q'/F_m'), operating efficiency of PSII; F_v/F_m' , maximum efficiency of PSII.

Cultivar	Treatment	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	C_i ($\mu\text{mol mol}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	T_{leaf} ($^\circ\text{C}$)	ϕ_{PSII} (ratio)	F_v/F_m' (ratio)
MS	WW	36.3 ± 1.0	0.86 ± 0.02	283 ± 2	12.7 ± 0.1	34.3 ± 0.1	0.25 ± 0.01	0.46 ± 0.01
	WL	12.3 ± 1.2	0.10 ± 0.01	156 ± 6	4.4 ± 0.4	40.7 ± 0.3	0.22 ± 0.00	0.40 ± 0.01
	WL-irrigated	35.8 ± 0.5	0.68 ± 0.03	264 ± 5	11.7 ± 0.3	31.6 ± 0.4	0.28 ± 0.00	0.50 ± 0.01
S6	WW	38.7 ± 0.9	0.77 ± 0.02	269 ± 2	11.5 ± 0.2	34.2 ± 0.1	0.27 ± 0.01	0.49 ± 0.01
	WL	29.6 ± 1.9	0.39 ± 0.05	218 ± 8	10.3 ± 0.7	37.3 ± 0.3	0.25 ± 0.01	0.44 ± 0.01
	WL-irrigated	37.2 ± 0.8	0.70 ± 0.04	264 ± 3	11.2 ± 0.1	33.2 ± 0.3	0.27 ± 0.01	0.48 ± 0.01

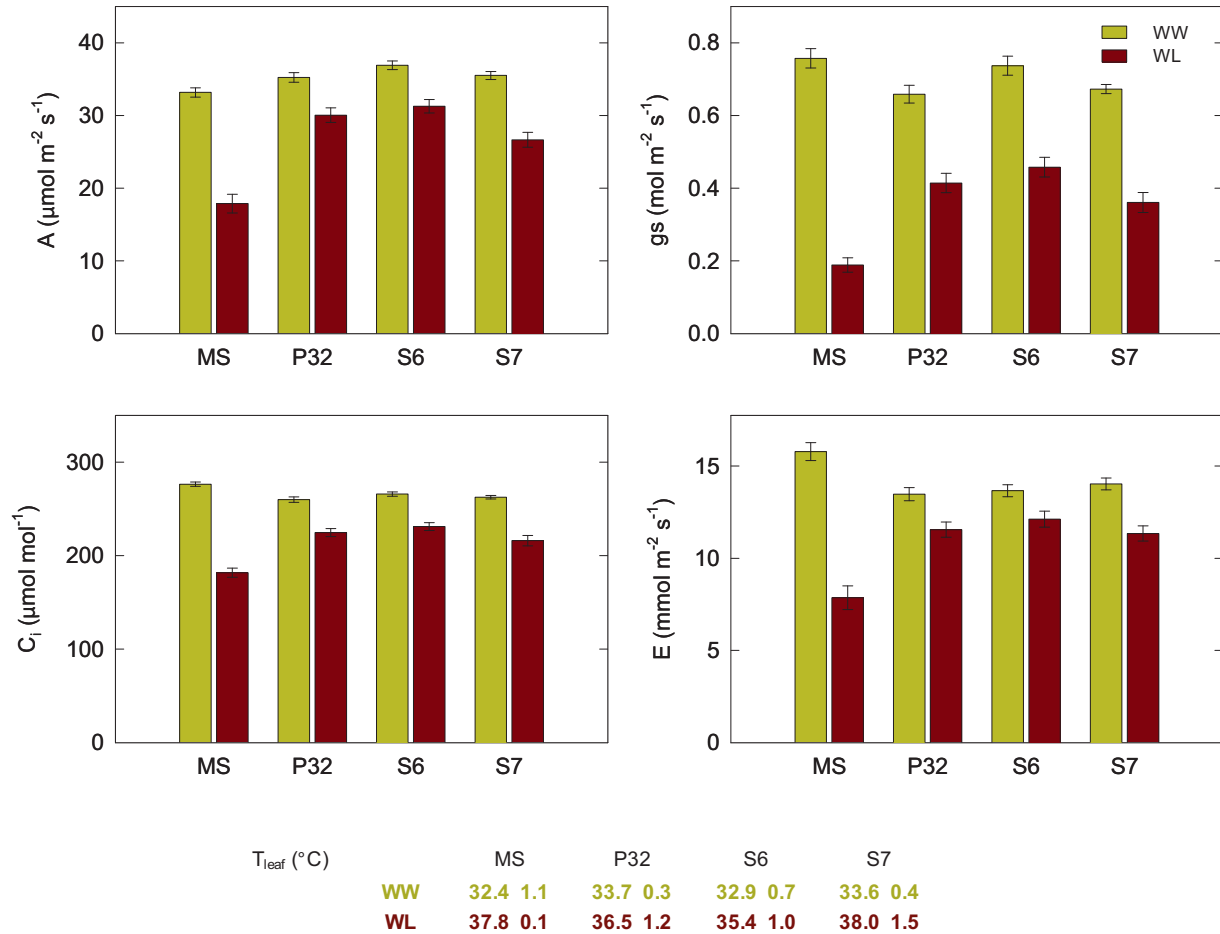


Fig. 5. Net CO_2 assimilation rate (A), stomatal conductance to water vapor (g_s), intercellular CO_2 concentration (C_i), transpiration rate (E) and leaf temperature (T_{leaf}) in the cotton cultivars Monseratt Sea Island (MS), Pima 32 (P32), Pima S-6 (S6) and Pima S-7 (S7), under well-watered (WW) and water-limited (WL) conditions. Measurements were taken from several plants of one WW or WL plot, 3 DAW, on several occasions during weeks 12–15. Conditions for infra-red gas analysis were: reference $\text{CO}_2 = 380 \mu\text{mol mol}^{-1}$, PPFD = $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ and block temperature = 32°C . Values are means \pm SEM ($n = 23\text{--}33$).

and WL conditions for both MS and S6 (see also Table 3). RuBP-regeneration, i.e., electron transport activity, was a limitation or a co-limitation in WL-irrigated plants of both cultivars and in WW plants of MS only. The maximum RuBP carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) estimated by modeling the $A\text{--}C_i$ response curves were higher in WL compared to WW and WL-irrigated plants (significant water regime effect; Table 1), probably as a result of the higher leaf temperatures (Table 3). The predicted mesophyll conductance (g_m) was also higher in WL compared to WW plants, but g_m predicted for WL-irrigated plants was not different from WW or WL plants ($P > 0.05$; Tukey–Kramer).

To quantify the extent of the non-stomatal limitations, the $A\text{--}C_i$ response was used to compare the net CO_2 assimilation

rates of plants under WL and WL-irrigated conditions assuming the same C_i (Table 4). For the drought-tolerant cultivar S6, the initial response of $A\text{--}C_i$ was very similar under both WL and WL-irrigated conditions, whereas for the cultivar MS, A at each C_i was lower in WL compared to WL-irrigated plants (Supplementary Fig. S4). For the cultivar S6, A measured in WL plants was only slightly lower (92%) than A at the corresponding C_i for WL-irrigated plants. In contrast, for the cultivar MS, A in WL plants was less than 70% of A at the corresponding C_i in WL-irrigated plants. These results indicate that, in addition to stomatal closure, non-stomatal factors also contributed significantly to the inhibition of net photosynthesis under WL conditions, particularly in MS.

Table 3

Photosynthetic parameters estimated from modeling of $A\text{--}C_i$ response curves for Monseratt Sea Island (MS) and Pima S-6 (S6) under well-watered (WW), water-limited (WL) and water-limited but irrigated (WL-irrigated) conditions (see Fig. 6). Values are means \pm SEM ($n = 3$). T_{leaf} , leaf temperature (measured); C_i , intercellular CO_2 concentration at atmospheric CO_2 levels (measured); C_{trans} (C_i at A_j), CO_2 concentration at which RuBP-regeneration and RuBP-consumption co-limit photosynthesis; V_{cmax} , maximum RuBP carboxylation rate; J_{max} , maximum electron transport rate; g_m , mesophyll conductance to CO_2 . When $C_i < C_{\text{trans}}$, A is A_c -limited and, when $C_i > C_{\text{trans}}$, A is A_j -limited.

Cultivar	Treatment	$T_{\text{leaf}} (^{\circ}\text{C})$	$C_i (\mu\text{mol mol}^{-1})$	$C_{\text{trans}} (\mu\text{mol mol}^{-1})$	$V_{\text{cmax}} (\mu\text{mol m}^{-2} \text{s}^{-1})$	$J_{\text{max}} (\mu\text{mol m}^{-2} \text{s}^{-1})$	$g_m (\mu\text{mol m}^{-2} \text{s}^{-1})$
MS	WW	32.6 ± 0.2	281 ± 1	281 ± 24	171 ± 1	249 ± 12	0.20 ± 0.00
	WL	38.7 ± 0.7	196 ± 5	412 ± 117	301 ± 41	356 ± 34	0.27 ± 0.03
	WL-irrigated	31.6 ± 0.4	264 ± 5	175 ± 7	179 ± 3	226 ± 3	0.25 ± 0.01
S6	WW	33.5 ± 0.5	244 ± 18	315 ± 17	215 ± 15	315 ± 21	0.23 ± 0.01
	WL	35.6 ± 0.6	240 ± 3	367 ± 11	259 ± 18	361 ± 20	0.25 ± 0.02
	WL-irrigated	33.2 ± 0.3	264 ± 3	261 ± 25	190 ± 4	259 ± 14	0.21 ± 0.00

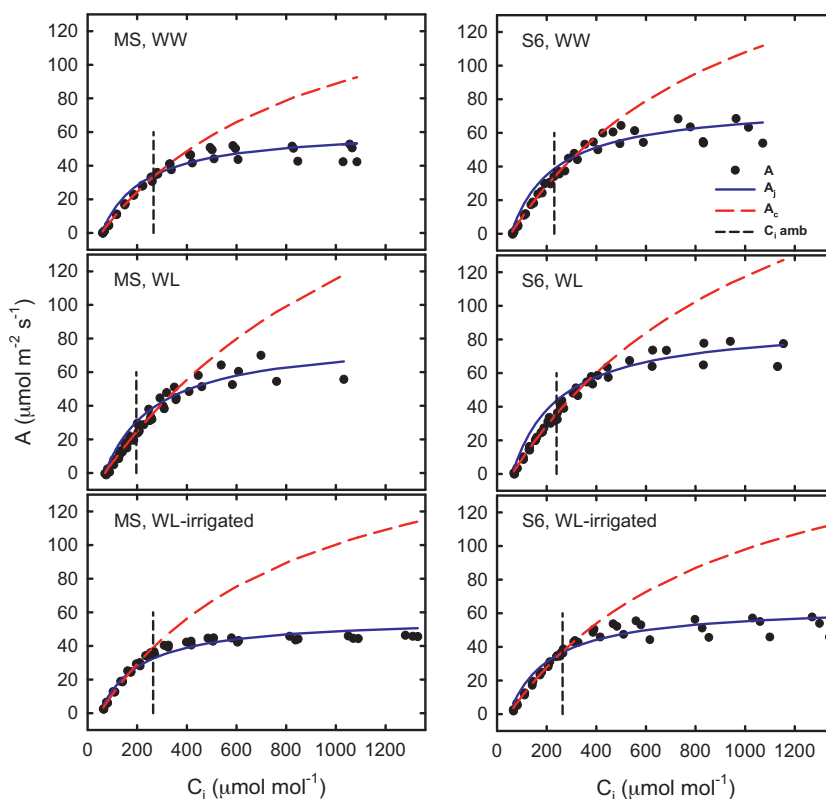


Fig. 6. Response of the net CO_2 assimilation rate (A) to the intercellular CO_2 concentration (C_i) in the cotton cultivars Monseratt Sea Island (MS) and Pima S-6 (S6), under well-watered (WW), water-limited (WL) and water-limited but irrigated (WL-irrigated) conditions. Measurements were taken from three plants of one WW or WL plot, 1 DAW (WL-irrigated) or 3 DAW (WW and WL), on week 15 of the experiment. Conditions for infra-red gas analysis were: reference $\text{CO}_2 = 75\text{--}1500 \mu\text{mol mol}^{-1}$, PPFD = $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ and block temperature = 32°C . Each symbol represents one measurement. The modeled RuBP-regeneration (A_j , solid blue line) and RuBP-consumption (A_c , dashed red line) limitations of photosynthesis are shown. The C_i at atmospheric CO_2 levels is marked by the vertical dashed line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 4
Photosynthetic parameters of Monseratt Sea Island (MS) and Pima S-6 (S6) plants under WL conditions and the corresponding A for plants under WL-irrigated conditions. Values of intercellular CO_2 concentration at atmospheric CO_2 levels (C_i), leaf temperature (T_{leaf}) and net CO_2 assimilation (A) are means \pm SEM of measurements taken throughout the season ($n = 29\text{--}33$). $A_{\text{WL-1}}$ is the A for WL-irrigated plants at the same C_i , determined from three $A\text{--}C_i$ response curves ($n = 3$).

Cultivar	C_i ($\mu\text{mol mol}^{-1}$)	T_{leaf} ($^\circ\text{C}$)	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	$A_{\text{WL-1}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
MS	182 ± 5	38.6 ± 0.4	17.9 ± 1.3	26.0 ± 1.7
S6	231 ± 4	35.4 ± 0.3	31.3 ± 0.9	34.1 ± 1.0

To separate the effects of heat and drought stress, the $A\text{--}C_i$ response was measured for WL-irrigated plants at a leaf temperature that was artificially increased to the temperature observed in the WL plants. The results showed that the $A\text{--}C_i$ response was identical for plants under WL or WL-irrigated conditions when both were measured at the same elevated leaf temperature (data not shown). These observations indicate that the higher leaf temperatures under WL conditions were responsible for the differences in A between WL and WL-irrigated plants at a given C_i . Thus, moderate heat stress accompanied the reduced availability of water in WL MS plants and caused a metabolic limitation to photosynthesis.

3.6. Rubisco activation state

The activation state of Rubisco was high and very similar for the four cultivars under WW conditions, with an average of $86.3 \pm 0.8\%$ (Fig. 7). Under WL conditions, the activation state decreased, with significant differences between WW and WL plants in all but the P32 cultivar ($P > 0.05$; Tukey–Kramer). The greatest decrease in Rubisco activation occurred for MS, followed by S7 and then P32 and S6. Thus, the response of the Rubisco activation state to WL

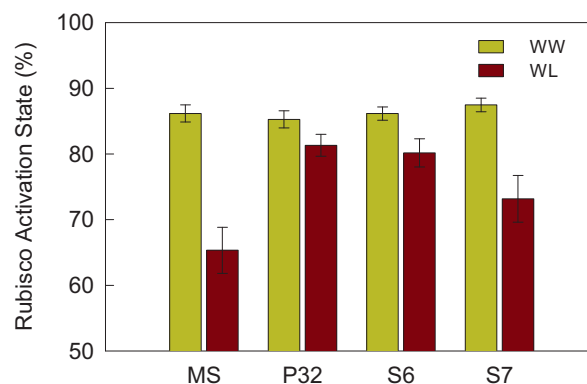


Fig. 7. Rubisco activation state in the cotton cultivars Monseratt Sea Island (MS), Pima 32 (P32), Pima S-6 (S6) and Pima S-7 (S7), under well-watered (WW) and water-limited (WL) conditions. Samples were taken from several plants of one WW or WL plot, 3 DAW, on three occasions during weeks 12–15. Frozen samples were assayed for initial and total Rubisco activity and the activation state was calculated as initial/total activity and expressed as a percentage. Values are means \pm SEM ($n = 9\text{--}16$).

conditions followed the same pattern as that observed for CO₂ assimilation. In MS, Rubisco activation was reduced to $65.3 \pm 3.3\%$ under WL conditions, whereas the total activity of the enzyme was not affected (data not shown). A reduction in Rubisco activation state of this magnitude can readily account for the metabolic limitations to cotton photosynthesis observed in MS plants under WL conditions (see Table 4).

4. Discussion

Climatic and anthropogenic scenarios suggest that maintaining photosynthetic productivity will require genotypes with greater tolerance to decreased water availability and elevated air temperatures. Hence, a better understanding of plant physiological responses to the combined effects of drought and heat stresses in production environments is pertinent. In the present study, four Pima cotton (*G. barbadense* L.) cultivars were grown in the field in a hot, arid environment and supplied with sufficient (WW) and limiting (WL) irrigation. Using gas-exchange and other physiological measurements it was possible to show that reduced water availability in dry-hot conditions caused both drought and heat stress, reducing photosynthesis through diffusional and metabolic limitations.

Despite very high air temperatures, cotton plants maintained high stomatal conductances and transpiration rates when growing under WW conditions, effectively avoiding heat stress through evaporative cooling (Upchurch and Mahan, 1988; Burke and Upchurch, 1989; Lu et al., 1994; Radin et al., 1994). Pima cotton has been bred for irrigated production in hot environments (Kittock et al., 1988; Ulloa et al., 2009), but the ability of the modern cultivars to withstand high temperatures relies on heat avoidance rather than heat tolerance and is highly dependent on the excessive use of water (Radin et al., 1994).

Plants under WL conditions were subjected to consecutive cycles of water availability and water deficit, reminiscent of the situation experienced by plants under both rain-fed and irrigated conditions. Leaf dehydration, stomatal closure and photosynthetic inhibition occurred in WL plants experiencing water deficit. Decreased stomatal conductance and lower transpiration rates reduced the capacity for evaporative cooling. As a result, plants under WL conditions exhibited higher leaf and canopy temperatures than WW plants, reaching temperatures that caused metabolic inhibition of photosynthesis (see below). On the hottest days, the canopy temperature of the most drought-sensitive cultivar was high enough to cause significant heat stress in cotton (Law and Crafts-Brandner, 1999), even under WW conditions. However, for the other cultivars, heat stress was strictly linked to limited water availability, occurring only under WL conditions. Elevated canopy temperatures were therefore an informative indicator of both heat and drought stresses.

Heat tolerance involves the maintenance of a high photosynthetic capacity under conditions of elevated temperature. Since leaf temperature is closely related to stomatal conductance and transpiration rate, the maintenance of net CO₂ assimilation rates (*A*) at higher canopy temperatures can provide a reliable indicator of greater heat tolerance and higher water-use efficiency (Cottee et al., 2010). All four cotton cultivars examined in the present study showed increased leaf/canopy temperatures, and decreased leaf relative water contents, photosynthetic rates and stomatal conductance under WL conditions compared to WW conditions. However, the most drought-sensitive cultivar, MS, was markedly more affected than the other three cultivars. This cultivar exhibited a lower capacity for evaporative cooling (higher canopy temperature) and a greater specific leaf area (SLA) compared to the other cultivars, even under WW conditions. Both increased stomatal

conductances and decreased leaf area contribute to lower leaf/canopy temperatures (Cornish et al., 1991; Lu et al., 1994; Radin et al., 1994) and lower SLA has been associated with plant adaptation to arid conditions (Mitchell et al., 2008; Carmo-Silva et al., 2009).

Under drought stress, photosynthetic CO₂ assimilation can be limited by stomatal closure, decreased mesophyll conductance, and biochemical (i.e., metabolic) constraints (e.g., Pinheiro and Chaves, 2011). In general, diffusional limitations (including stomatal and mesophyll conductance) prevail under mild to moderate stress conditions and metabolic factors only become prominent as the severity of drought increases. Photosynthetic limitations under the combination of drought and heat stresses in the field are less well understood. The large decrease in stomatal conductance that occurred under WL conditions indicated that stomatal closure was a major factor limiting cotton photosynthesis since *C*_i decreased to levels that limited carboxylation by Rubisco. The magnitude of the limitation imposed on photosynthesis by mesophyll conductance to CO₂ (gm) is controversial, but data suggests that gm tends to decrease with drought and increase with elevated temperature, at least up to the optimum temperature (Bernacchi et al., 2002; Galmés et al., 2007; Flexas et al., 2008; Niinemets et al., 2009; Scafaro et al., 2011). In the cotton cultivars MS and S6, gm estimated from the *A*–*C*_i response curves was higher in WL compared to WW plants, suggesting that mesophyll resistance to CO₂ diffusion to the carboxylation site did not limit photosynthesis under dry-hot conditions. On the other hand, the photosynthetic rate of MS plants under WL conditions was lower than the rate measured for the WL-irrigated plants at a given *C*_i, indicating that metabolic factors associated with heat stress added to the limitation imposed by stomatal closure. Modeling of the *A*–*C*_i response curves indicated that photosynthesis was Rubisco-limited under WL conditions and when temperature was elevated for WL-irrigated plants.

Drought and heat stress conditions cause a large number of changes in the chloroplast environment, including alterations in the redox status and the energy balance, that may affect photosynthetic metabolism (Allakhverdiev et al., 2008; Lawlor and Tezara, 2009; Sharkey and Zhang, 2010). In addition, many studies have linked the inhibition of *A* at elevated temperatures to a decrease in Rubisco activation, caused by the exceptional thermal lability of Rubisco activase (Feller et al., 1998; Crafts-Brandner and Salvucci, 2000; Salvucci and Crafts-Brandner, 2004a,b). In a recent study, Signarbieux and Feller (2011) have also implicated Rubisco activity in the inhibition of *A* under drought stress in the field. Severe water deficit decreases the CO₂ concentrations in the chloroplast (*C*_c) to levels sufficiently low (i.e., less than 100 μmol mol⁻¹) to promote decarbamylation and consequently inactivation of Rubisco (Sage et al., 2002). Galmés et al. (2011) suggested that species adapted to lower *C*_c – either as a result of different Rubisco properties (i.e., lower *K*_{act}[CO₂]) or the presence of a more efficient or more abundant Rubisco activase – would have a greater capacity to maintain Rubisco in an active state under drought stress.

In the present study, a decrease in *C*_i occurred in WL plants, but the magnitude of this decrease was not sufficiently large to cause Rubisco decarbamylation (Sage et al., 2002). Instead, analyses of the *A*/*C*_i response curves suggested that metabolic inhibition of *A* was associated with elevated leaf temperatures. The response of Rubisco activase to temperature measured in rapidly prepared leaf extracts showed that Rubisco activase activity in cotton was optimal at 25–35 °C and was inhibited by 50% at 39 °C (Carmo-Silva and Salvucci, 2011). Thus, under WW conditions, canopy temperatures were optimal for activase activity in cotton, consistent with the high *A* and Rubisco activation observed under these conditions. In contrast, leaf temperatures under WL conditions were sufficiently high to inhibit the activity of activase, especially in the early afternoon, when canopy temperatures often exceeded 38 °C. In WL plants,

Rubisco deactivation was correlated with inhibition of *A* in all of the four cultivars and both Rubisco deactivation and *A* inhibition were correlated with the decrease in *g_s* and the concomitant increase in leaf temperature that occurred under dry conditions. These findings indicate that the capacity to maintain Rubisco in an active form represents a major metabolic factor influencing photosynthetic productivity under field conditions in dry-hot environments.

Under severe water deficit, leaf wilting provided a mechanism to conserve water while avoiding heat stress. By limiting the incident light and heat load, leaf wilting protects the leaves of plants under drought from irreversible damage of the photosynthetic apparatus by heat and photoinhibition (Zhang et al., 2010). That *A* was similar in WL-irrigated and WW plants demonstrated that CO₂ assimilation was not irreversibly affected by the WL conditions. However, the temporal limitations to photosynthesis under WL conditions affected the shoot biomass production and yield of the drought-sensitive MS cultivar (Carmo-Silva et al., unpublished data). In contrast, some of the more recently released cultivars showed less of a difference in yield between WW and WL growth conditions (Gore, unpublished data). These cultivars are either more tolerant to heat stress, more efficient in accessing soil water, or a combination of the two.

The results obtained with the four different cotton cultivars confirm that *g_s* is an informative indicator of genotypic differences in response to growth under WL conditions (Munns et al., 2010) in a dry-hot environment. Differences in *g_s* translated into differences in canopy temperature among cultivars. Canopy temperature was easily measured using tractor-mounted infrared thermometers, providing a fast and reliable, high-throughput, phenotyping tool to screen for genotypes that are better adapted to heat and drought conditions. The non-destructive technique allowed continuous monitoring of plant responses throughout the experiment (Berger et al., 2010). The combination of high-throughput techniques with physiological and biochemical analyses facilitated a more thorough characterization of the plant stress tolerance mechanisms and provided insights into the complex relationship between water availability and heat stress for field-grown plants in a semi-arid environment.

5. Conclusions

This study provides new insights into the physiological and biochemical responses of plants to drought and heat stresses imposed under field conditions in a semi-arid environment. The results demonstrate the interactive effects of heat and drought stresses. Most cotton cultivars avoided heat stress when adequate water was available for evaporative cooling. Under water deficit conditions, stomatal closure imposed a diffusive limitation on photosynthesis by decreasing the availability of CO₂ for assimilation by Rubisco. In addition, the reduced capacity of evaporative cooling raised the leaf/canopy temperatures, imposing a metabolic limitation on photosynthesis through the inactivation of Rubisco. Thus, in hot, arid environments, both diffusive (drought-induced) and biochemical (heat-induced) limitations act in concert to inhibit photosynthesis when water supply is limiting.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.envexpbot.2012.04.001>.

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