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Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

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Part 3 – Final Report

(The points below are to be used as a guideline when completing your final report.)

Background

1. Outline the background to the project.

Climate change will affect cotton through rising atmospheric CO_2 levels, higher temperatures, lower humidity (high Vapour Pressure Deficit (VPD)) and reduced water availability. Fortunately predictions for climate change effects are similar to some of the extremes in climate experienced within and across cotton regions; therefore, opportunities exist to harness current understanding of cotton system adaptation to climate variability to plan for projected climate change. Although some research had previously been conducted on the main effects of rising CO_2 and temperature, VPD, and reduced water, there had been virtually no research that had addressed the real-world interaction of rising CO_2, temperature, VPD and reduced water. Research supported by CRDC (PhD project of Katie Broughton: *the integrated effects of projected climate change on cotton growth and physiology*) has shown that while some aspects of cotton growth are improved by elevated CO_2, there are issues emerging on the availability and use of water to generate this growth. In the glasshouse, cotton has shown improved early growth rates in elevated CO_2, which was the result of improvements in both leaf-level photosynthesis and water use efficiency (WUE). However, early cotton growth in elevated CO_2, especially at elevated temperatures, increased total plant water use despite improvement in WUE. In water-limited situations, this suggests that more water may be invested in early vegetative growth (leaves and stems) and therefore, less water may be available for later reproductive growth (bolls and lint). Further research is needed (including field studies) under a wider range of future climatic conditions to validate these initial outcomes, extend them through the full growth cycle, and to begin to explore management options for adaptation. Therefore, the development of the *National Facility for Cotton Climate Change Research* (CSP1402) and the *cotton production in a future climate* sister project (CSP1501) have been crucial aspects of investigating the response and adaptation of field-grown cotton in Australian production systems to projected climate change.

Objectives

2. List the project objectives and the extent to which these have been achieved, with reference to the Milestones and Performance indicators.

1. Post Doctoral Fellow to lead the cotton industry's research into impacts and adaptation to climate change.
   1.1 A post-doctoral fellow (Katie Broughton) was appointed and started 27th January 2015.

2. Quantify the impacts of the interactive effects of future climate change on Australian cotton production at the field scale.
   2.1 Investigate the impact of elevated CO_2 and elevated temperature on cotton growth and productivity: Two seasons of field experiments completed in Narrabri where yield, quality and water use were measured; data analysed and used in refereed journal articles or industry publications.

   2.2 Investigate the impact of elevated CO_2, elevated temperature and water availability on cotton growth and productivity: One season of field experiments completed in Narrabri where yield, quality and water use were measured, data analysed and used in refereed journal articles or industry publications.

   Field-based climate change studies were conducted in Narrabri during the 2014/15, 2015/16 and 2016/17 cotton seasons. For each field season, the experiment has consisted of two ambient [CO_2] chambers (aCO_2: ~400 ppm), two elevated [CO_2] chambers (eCO_2: ~550 ppm) and two control plots without chambers. Soil water content was measured in all three seasons using GLRL capacitance probes. The experiment conducted during 2014/15 was a short-season experiment focusing on early season growth inside the chambers, and plants were harvested at 64 DAP. The 2015/16 and 2016/17 experiments have focused on full season growth and ultimate yield responses to elevated CO_2 and warmer temperatures.

Data from these experiments have been analysed, preliminary results have been reported in CRDC progress reports, CRDC Spotlight magazine (Autumn 2016), presented at conferences, and will potentially be used in refereed journal articles and further industry publications in the future.

Our data show that warmer temperatures may increase stomatal conductance and transpiration, resulting in reduced leaf-level water use efficiency. The two full season experiments showed that cotton grown in warmer temperatures had greater vegetative biomass and leaf area, but large reductions in fruit biomass. Despite elevated [CO_2] increasing photosynthesis and leaf-level water use efficiency, there was no difference in fruit biomass, indicating that elevated [CO_2] does not negate the negative effects of higher temperature on fruit retention. Furthermore, increases in vegetative biomass and leaf area with elevated [CO_2] during the 2016/17 season suggests there is potential for elevated atmospheric [CO_2] to exacerbate the negative effects of warmer temperature.

3. Quantify the impacts of more extreme climate change scenarios of higher average temperatures and more severe weather limitations
   3.1 Investigate the impact of elevated CO_2 with higher temperatures and more severe water limitations on cotton growth and productivity: Two glasshouse experiments completed at UWS where detailed measurements of cotton physiology were taken; results analysed and used in publications that enable a greater understanding of impacts that may result from greater extremes in a future climate.

Two glasshouse experiments to investigate the impact of more extreme climate change scenarios have been completed at UWS:

TransPhys experiment

A glasshouse experiment was conducted to investigate the effects of elevated CO_2 and heatwave events on cotton physiology and growth under well-watered conditions. Cotton was grown at ambient temperature (T_A: 28/17°C, day/night) with two CO_2 treatments (ambient = 400 ppm; and elevated = 640 ppm). Plants were exposed to a heatwave treatment for six days (HW, Day 1: 35/24°C and Days 2-6:40/29°C), whilst maintaining growth CO_2 conditions. Plant growth and physiology were measured and results have been analysed and reported in CRDC progress reports.

Day/night temperature x drought x heatwave experiment

A glasshouse experiment has been conducted at UWS to: (a) determine the impact of warmer average day and night temperatures, and heatwave events, on photosynthesis, growth, development, water use and boll-retention; (b) determine whether higher growth temperatures increase or decrease sensitivity of cotton to heatwave events; and (c) determine the impact of longer term drought on the response of cotton physiology and development to warmer day and night temperatures, and extreme heatwave conditions. There were four temperature treatments: 28/18°C, 28/22°C, 32/22°C and 32/26°C (day/night). Plants were exposed to a heatwave event of 42/26°C (day/night) for a period of five days. Plant growth and physiology were measured. Results have been analysed, reported in CRDC progress reports, and a manuscript for journal publication is in preparation.

These experiments suggest that warmer air temperatures may accelerate growth rates of cotton plants, but not necessarily equate to greater yields. Furthermore, plants grown at warmer air temperatures may be more susceptible to extreme climatic events, specifically water deficits and heatwave conditions. Therefore, adaptive strategies may become increasingly important to mitigate the negative effects of warmer air temperatures, drought and heat stress in cotton production systems.

4. Identify management practices that mitigate the effects of interactive effects of climate change

   4.1 Investigated the results of use of growth hormones (Mepiquat chloride (Pix) and 1-MCP) on mitigating the effects of climate change on cotton growth: Experiment completed at UWS where detailed measurements of cotton physiology were taken; results analysed and used in publications that may provide insight on management practices to optimise cotton productivity in a future climate

   A glasshouse experiment has been conducted at UWS to meet two outcomes: (1) assess the effectiveness of using mepiquat chloride, applied according to current Australian cotton industry standards, to manage vegetative growth of cotton grown in warmer temperatures and at elevated [CO_2] in the glasshouse; and (2) assess the physiological responses of cotton grown in future environmental conditions. Cotton was grown at ambient temperature (T_A: 32/18°C) or elevated temperature (T_E: 36/22°C). For each temperature treatment, plants were grown at either ambient [CO_2] (C_A: 400 ppm) or elevated [CO_2] (C_E: 640 ppm). Growth regulator treatments were applied at flowering and were: (a) a single application of mepiquat chloride at a rate of 22.8 g ai ha^{-1}; (b) an application of mepiquat chloride as described in (a) followed by a second application of mepiquat chloride at a rate of 11.4 g ai ha^{-1} applied 10 days later; or (c) an application of aminoethoxyvinylglycine (AVG) at a rate of 125 g ai ha^{-1}. Plant growth and physiology were measured, results have been analysed and will potentially be used in refereed journal articles and/or industry publications in the future. Our data suggest that vegetative biomass of cotton may be reduced with mepiquat chloride under some environmental conditions, but may not be effective in controlling vegetative growth of plants grown at both high temperature (36/22°C, day/night) and elevated [CO_2] (640 ppm). Therefore, further studies are required to explore management strategies for cotton grown in high temperature, high CO_2 environments.

5. Improve the external parameters of the OZCOT simulation capacity to better capture interactive effects of climate change

   5.1 Undertake a simulation study that uses OZCOT to assess its ability to capture the effects measured in field studies investigating climate change: Detailed report assessing OZCOT's ability to simulate the interactive effects of elevated CO_2, elevated temperature and water availability.

   This objective was not completed during the course of this project cycle, however, activities have begun to plan the approach that will be used to undertake this assessment. The first activity that is currently underway is to document those variables within and external to the model that could be used to capture the response of integrated climate change effects. A documented approach to model assessment and initial model sensitivity analysis will be provided as part of a progress report of the ongoing project: *Improving water use efficiency in a changing climate* (CSP1804).

Additional activities have also been undertaken, such as starting a review/summary of the climate change research that has been conducted within the Australian cotton industry.

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

Definitions and descriptions of terminology

A/Ci response curves: measured using the Licor 6400XT to determine the CO_2 assimilation rate (A; the rate of photosynthesis) as a function of intercellular CO_2 (C_i; the concentration of CO_2 inside the leaf).
ACRI: Australian Cotton Research Institute
A_{sat}: carbon assimilation at saturating light (μmol mol^{-2} s^{-1})
A_{sat}/E: leaf-level water use efficiency (μmol mmol^{-1})
AVG: aminoethoxyvinylglycine; ReTain®
DAP: days after planting
E: transpiration rate (mmol m^{-2} s^{-1})
GLRL: green light red light sensors; capacitance probe used for measuring soil water content
$g_{s\text{-}sat}$: stomatal conductance at saturating light (mol m^{-2} s^{-1})
J_{max}: maximum rate of electron transport (μmol m^{-2} s^{-1}). Derived from the A/Ci response curve to assess electron transport limitation.
MC: mepiquat chloride; Pix®
Temperature response curves: measured using the Licor 6400XT to determine the CO_2 assimilation rate (A) as a function of air temperature
UWS: Western Sydney University
V_{cmax}: maximum carboxylation efficiency (μmol m^{-2} s^{-1}). Derived from the A/Ci response curve to assess the Rubisco limitation.
VGR: vegetative growth rate (cm/node)
VPD_L: leaf vapour pressure deficit (kPa)

Field experiments at the Australian Cotton Research Institute (ACRI), Narrabri

Field based climate change studies were conducted in Narrabri during the 2014/15, 2015/16 and 2016/17 cotton seasons. For each field season, the experiment has consisted of three treatments; (a) warmer temperature and elevated CO_2, (b) warmer temperature and ambient CO_2, and (c) ambient temperature and ambient CO_2. The objective of these experiments were (1) to construct large field chambers that will elevate [CO_2] in the field and evaluate the utility of these chambers for the purpose of field-based climate change studies; (2) identify the impacts of increased atmospheric [CO_2] and warmer temperature on whole canopy physiology of field-grown cotton in high-input production systems; (3) quantify the impact of increased atmospheric [CO_2] and warmer temperature on whole canopy physiology, growth, water use and yield of field-grown cotton in high-input production systems; and (4) assess the effectiveness of using mepiquat chloride, applied according to current Australian cotton industry standards, to manage excessive vegetative growth of cotton grown in warmer temperatures and elevated [CO_2]. A summary of the objectives and treatments of these experiments is shown in Table 1.

Table 1: Summary of objectives and treatments of field-based climate change studies conducted at ACRI, Narrabri over three cotton seasons from 2014 to 2017.

Objectives	**Treatments**
Construct chambers to elevate [CO_2] and temperature in the field and evaluate the utility of these chambers for the purpose of field-based climate change studies.	Elevated [CO_2] and warmer temperature (C_E: 550 ppm + ~2°C) Ambient [CO_2] and warmer temperature (C_A: 400 ppm + ~2°C)

Identify the impacts of increased atmospheric [CO_2] and warmer temperature on whole canopy physiology of field-grown cotton in high-input production systems.	Ambient [CO_2] and ambient temperature (C_C: control)
Quantify the impact of increased atmospheric [CO_2] and warmer temperature on whole canopy physiology, growth, water use and yield of field-grown cotton in high-input production systems.	
Assess the effectiveness of using mepiquat chloride, applied according to current Australian industry standards, to manage excessive vegetative growth of cotton grown in warmer temperatures and elevated [CO_2].	

Four chambers were constructed as part of the National Facility for Cotton Climate Change Research (CSP1402) and the methods developed during these two projects (CSP 1402 and CSP 1501) may be used to benefit other research in investigating responses to altered environmental conditions in field-based studies for cotton and potentially other crops.

Field chamber design and operation

Four chambers were constructed as part of the National Facility for Cotton Climate Change Research (CSP1402). The 4 x 4 x 3 metre chambers were constructed of galvanised steel attached to a 1cm thick transparent plastic segment in the front and the back. The chambers were enclosed with two layers of transparent plastic (F-CLEAN®; inside 80µm and outside 100µm thick; 94% light transparent), which were inflated during the experiments. The chambers are portable, which enables standard crop and field rotations, and a different experimental configuration each cotton season.

Temperature inside the chambers were controlled by a Wadsworth EnviroSTEP controller, which operates an air conditioner unit (Vicot VRPA072A5) to maintain air temperature inside the chambers on average 2-4°C higher than ambient air temperature. Throughout the project, the temperature control systems have been fine-tuned and sensors were re-calibrated to improve day/night performance.

Food grade CO_2 was injected into the chambers using perforated tubing located above the crop canopy, to maintain the desired CO_2 concentration (targets for ambient CO_2 were 400 ppm and for elevated CO_2 were 550 ppm). The concentration of CO_2 and H_2O inside each chamber was monitored at 9 positions within the chamber and the injection of CO_2 was regulated using a Li-Cor 840A CO_2/H_2O Gas Analyser.

Software (LabVIEW 2013, National Instruments) was used to monitor environmental conditions within each chamber on a real-time basis, and was used to set controls for variables such as atmospheric CO_2 and air temperature inside the chambers.

Drip irrigation was set up in the chambers and control plots, and the system was calibrated each year. Following rainfall events, the area inside the chambers were irrigated with the equivalent quantity of water to match precipitation in all of the treatments.

Field-season measurements

Cotton seeds were planted into the field using standard farming practices and chambers were moved into the field after the cotton had emerged. The injection of CO_2 into the chambers commenced between 19 and 22 DAP for each season (Table 2). Commercially available cultivars were grown each season. Sicot 71 BRF was grown in the 2014/15 experiments, and Sicot 74 BRF was grown for the 2015/16 and 2016/17 experiments (Table 2). The 2014/15 experiment was a short season experiment (harvested at 64 DAP) due to late planting, however, both the 2015/16 and 2016/17 season experiments were conducted over the full cotton season (Table 2).

Table 2: Details of field experiments conducted using the National Facility for Cotton Climate Change Research.

Cotton Season	Date Sown	Variety	CO_2 injection in chambers	Date Harvested
14/15	4/2/15	Sicot 71 BRF	26/2/15 (22 DAP)	9/4/15 (64 DAP)
15/16	25/11/15	Sicot 74 BRF	14/12/15 (19 DAP)	18/5/16 (175 DAP)
16/17	27/10/16	Sicot 74 BRF	17/11/16 (21 DAP)	24/4/17 (179 DAP)

Plants were monitored weekly for growth and development (i.e. heights and nodes). Plant physiology was measured using a Licor 6400 XT at first square, first flower and first open boll (or around the time that each of these developmental stages were expected to occur). Physiological measurements included mechanistic response curves (*A/*Ci and temperature) to determine Rubisco enzyme and electron transport limitations (see definitions and descriptions above). Soil water content was measured using a neutron probe and green light red light (GLRL) capacitance sensors (Odyssey Dataflow Systems, Christchurch, NZ). Biomass samples were collected at final harvest.

Mepiquat chloride (Pix) application (2016/17 season)

Vegetative growth rate (VGR) of plants were monitored throughout the season. Height and the number of nodes were measured weekly. VGR was calculated as:

$$VGR\ \left(\frac{cm}{node}\right) = \frac{This\ week's\ height\ (cm) - Last\ week's\ height\ (cm)}{This\ week's\ node\ number - \ Last\ week's\ node\ number}$$

Application of MC was considered when VGR exceeded 5.5 cm/node. During the 2016/17 season, mepiquat chloride was applied at 60 DAP (chambers only), 75 DAP (all plots) and 83 DAP (elevated [CO_2] chambers only).

Glasshouse experiments at Western Sydney University, Richmond

Glasshouse experiments were conducted utilising the naturally-lit, [CO_2] and temperature controlled glasshouses at the Hawkesbury Institute for the Environment at Western Sydney University (UWS), Richmond. Specific details for each set of experiments are detailed below.

- ***Experiment: Day/Night Temperature x Soil water deficit x Heatwave***

A number of questions were addressed in conducting a single, very large glasshouse experiment at UWS to investigate the effects of warmer temperatures, drought stress and heatwave events on cotton growth and physiology. The objectives of this large experiment were to (1) determine the impact of warmer night-time/day-time temperatures on cotton growth, photosynthesis and respiration; (2) determine whether warmer night-time/day-time temperatures increase or decrease the sensitivity of cotton to heatwave events; and (3) determine the impact of long term drought on the response of cotton physiology and development to warmer temperatures and extreme heatwaves.

Cotton (*Gossypium hirsutum* L. cv., Sicot 71BRF [Bollgard II® Roundup Ready Flex®], CSIRO, Australia) was grown at four temperature treatments (28/18°C, 28/22°C, 32/22°C, 32/26°C; day/night). Well-watered plants were maintained at 100% field capacity, and water-stressed plants were maintained at 50% field capacity. Five plants in each temperature treatment were exposed to a heatwave event of 40/26°C for a duration of five days at the beginning of boll development, before being returned to growth temperature. The objectives and treatments of this experiment are summarised in Table 3.

Table 3: Summary of objectives and treatments of the day/night temperature x water x heatwave experiment conducted using the controlled environment glasshouse at UWS, Richmond.

Objectives	**Temperature treatments (day/night)**	**Water treatments**	**Heatwave treatment**
Determine the impact of warmer night-time and day-time temperatures on cotton growth, photosynthesis and respiration.	28/18°C 28/22°C 32/22°C 32/26°C	100% field capacity 50% field capacity	40/26°C for 5 days; applied at the beginning of boll development
Determine whether warmer night-time and day-time temperatures increase of decrease the sensitivity of cotton to heatwave events.			
Determine the impacts of long term drought on the response of cotton physiology and development to warmer temperatures and extreme heatwaves.			

Leaf gas exchange measurements

Gas exchange was measured at four stages: baseline at the eight-leaf stage, drought measurement at squaring heatwave measurement on the fifth day of the heatwave treatment, and a recovery measurement on the second and seventh day of the recovery period. Photosynthesis (A_{sat}) and stomatal conductance ($g_{\text{s-sat}}$) were measured at a photosynthetic photon flux density of 1200 µmol m^{-2} s^{-1}, growth atmospheric CO$_2$ concentration of 400 ppm and mid-day growth temperature of 28 or 32°C.

Plant growth measurements

Once the seedlings were established, height, and the number of leaves, squares and bolls were measured were taken once per week. At the end of the experiment, each plant was harvested and partitioned into vegetative (leaves, stem, and branches) and reproductive (bolls) sections. Harvested samples were oven-dried at 40°C for a minimum of seven days, and weighed.

- ***Experiment: TransPhys***

The objective of this experiment was to determine the effects of warmer temperatures, elevated [CO$_2$] and heatwave events on the growth and physiology of well-watered cotton.

Plant materials and growing conditions

Cotton plants (*Gossypium hirsutum L.* cv, 71BRF [Bollgard II® Roundup Ready Flex®], CSIRO Australia) were grown in a naturally-lit, [CO$_2$] and temperature controlled glasshouse at Western Sydney University, Richmond, Australia. Seeds were sown on the 8th of April 2016 into nine L pots containing composted pine bark. Upon emergence, plants were thinned to one plant per pot for a total of 40 experimental plants. Plants were watered approximately every 3rd day.

Glasshouse bays were set to simulate ambient temperature (T_A: 28/17°C, mid-day/night) with two CO$_2$ treatments: (1) ambient CO$_2$ (C_A: 400 ppm) and (2) elevated CO$_2$ (C_E: 640 ppm). CO$_2$ gas (Food grade, AirLiquide, Australia) was injected into the glasshouse bays from pressurised cylinders through solenoid valves connected to a CO$_2$ controller (Lambda T, ADC BioScientific Ltd., Hoddesdon, Hertz, UK). [CO$_2$] was continually monitored by logging the voltage output of the CO_2 monitors/controllers using a data logger (DL2e, Delta-T Devices Ltd, Cambridge, UK). At 67 DAP (14th June 2016), 10 plants from each CO_2 treatment were exposed to a heatwave treatment for 6 days (HW; Day 1: 35/24°C and Days 2-6: 40/29°C, mid-day/night), whilst maintaining growth CO_2 conditions. After the heatwave treatment, the glasshouse bays were returned to ambient temperature (28/17°C, mid-day/night). The objectives and treatments of this experiment are summarised in Table 4.

Table 4: Summary of objectives and treatments of the day/night temperature x CO_2 x heatwave experiment conducted using the controlled environment glasshouse at UWS, Richmond.

Objectives	**Temperature treatment (day/night)**	**CO_2 treatments**	**Heatwave treatment**
Determine the effects of warmer temperatures, elevated [CO_2] and heatwave events on the growth and physiology of well-watered cotton.	28/17°C	400 ppm (C_A) 640 ppm (C_E)	Day 1:35/24°C and Days 2-6: 40/29°C

Leaf gas exchange measurements

The response of photosynthesis (A) to intercellular [CO_2] (C_i) (A/C_i) was measured to calculate maximum Rubisco activity (V_{cmax}), and maximum rate of electron transport used in the regeneration of RuBP (J_{max}). A/C_i curves were obtained using an automatic programme with the portable open gas exchange system (LI-6400XT, LI-COR, Lincoln, USA) on three consecutive days 20th – 22nd June 2016 (73-75 DAP). Measurements were taken on recently expanded leaves at saturating light (photosynthetic photon flux density 1800 µmol m^{-2} s^{-1}) with the cuvette temperature set to 28°C. Leaf vapour pressure deficit (VPD_L) in the leaf cuvette was maintained within the range 1.0-3.3 kPa using the Licor 6400XT desiccant scrub function. Leaves were allowed to equilibrate before measurements were taken. Net photosynthesis (A_{sat}), stomatal conductance ($g_{s\text{-}sat}$) and transpiration (E) at saturating light (photosynthetic photon flux density 1800 µmol m^{-2} s^{-1}) and mid-day growth temperature and [CO_2] (400 or 640 ppm) were derived from the A/C_i data. $A_{sat}/g_{s\text{-}sat}$ and A_{sat}/E were calculated to describe leaf-level water use efficiency.

R version 3.1.3 was used to fit curves to A/C_i data and generate V_{cmax} and J_{max} coefficients. GenStat version 16 was used to analyse the coefficients by REML. Data were assessed at a P=0.05 level of significance.

Plant growth measurements

At the end of the experiment (30th June 2016; 83 DAP), final height, number of nodes and fruit retention was recorded. Each plant was harvested and partitioned into vegetative (leaf, stem) and reproductive (squares and bolls) organs. Leaf area was measured using a portable leaf area meter (LI-3100A, LI-COR, Lincoln, USA). Harvested samples were oven-dried at 80°C for a minimum of 48 h, and weighed.

The effects of elevated CO_2 and temperature on plant biomass and growth characteristics were analysed by REML using GenStat version 16. Data were assessed at a P=0.05 level of significance.

- ***Experiment: Pix rate and AVG***

The objectives of this experiment were to: (1) assess the effectiveness of using mepiquat chloride, applied according to current Australian cotton industry standards, to manage vegetative growth of cotton grown in warmer temperatures and elevated [CO_2] in glasshouse conditions; and (2) assess the physiological responses of cotton grown in future environmental conditions to the application of mepiquat chloride.

Plant materials and growing conditions

Cotton plants (*Gossypium hirsutum L.* cv, Sicot 746B3F, CSIRO Australia) were grown in a naturally-lit, [CO_2] and temperature controlled glasshouse at Western Sydney University, Richmond, Australia. Seeds were sown into nine L pots containing composted pine bark. Two glasshouse bays were set to simulate ambient temperature (T_A: 32/18°C, mid-day/night) and two bays were set for an elevated temperature treatment (T_E: 36/22°C, day/night). Planting was staggered to coincide developmental stages of plants grown in the two temperature treatments. Cotton grown at T_A was planted on the 1st February 2018 and cotton grown at T_E was planted on the 15th February 2018. For each of the temperature treatments, there were two CO_2 treatments: ambient CO_2 (C_A: 400 ppm) and elevated CO_2 (C_E: 640 ppm). CO_2 gas (Food grade, AirLiquide, Australia) was injected into the glasshouse bays from pressurised cylinders through solenoid valves connected to a CO_2 controller (Lambda T, ADC BioScientific Ltd., Hoddesdon, Hertz, UK). [CO_2] was continually monitored by logging the voltage output of the CO_2 monitors/controllers using a data logger (DL2e, Delta-T Devices Ltd, Cambridge, UK).

Chemical treatments

The treatment structure of the growth regulator experiment is shown in Figure 1. On the 3rd April 2018 (61 DAP for plants grown at T_A and 47 DAP for plants grown at T_E), at anticipated first flower, plants were sprayed with mepiquat chloride at a rate of 600 ml ha^{-1} or 125 g ai ha^{-1} of AVG. A second mepiquat chloride application was administered on the 13th April 2018 (71 DAP for plants grown at T_A and 57 DAP for plants grown at T_E) for the high-rate MC treatment (Table 5).

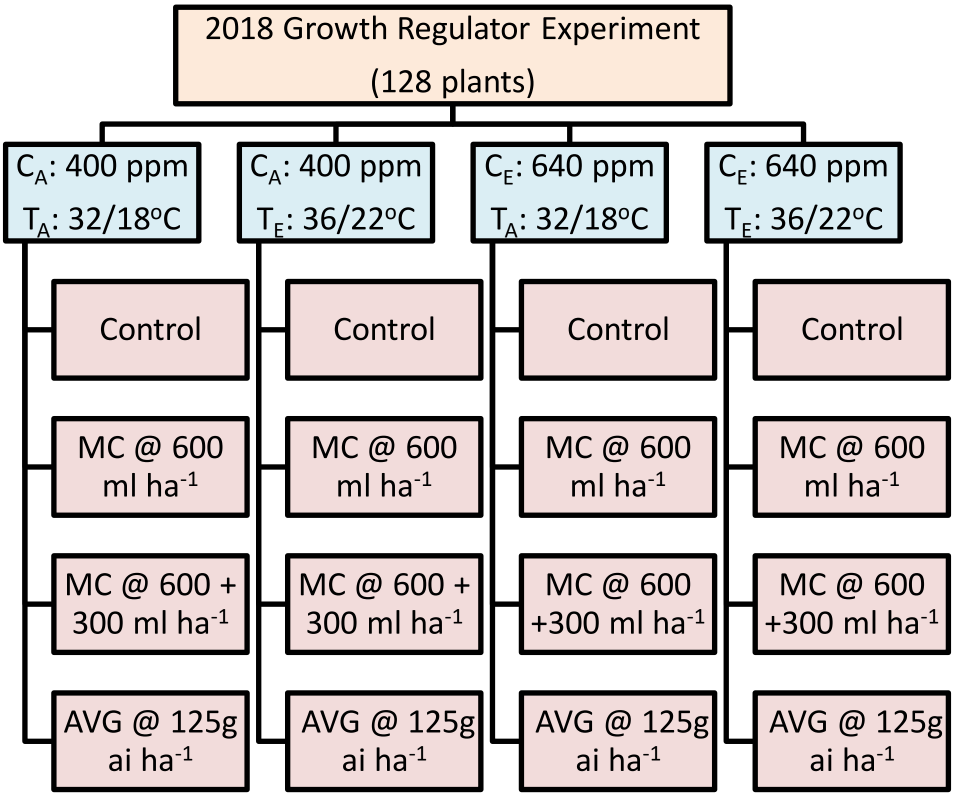

Figure 1: Treatment structure for the 2018 growth regulator experiment at UWS, Richmond.

Table 5: Timing of chemical applications for the 2018 growth regulator experiment at UWS, Richmond.

Chemical Application	First application (at flowering)	Second application (10 days post 1st application)
Control	×	×
Pix 600	✓	×
Pix 600 + 300	✓	✓
AVG	✓	×

Growth and leaf gas exchange measurements

The response of photosynthesis (*A*) to intercellular [CO_2] (C_i) (A/C_i) was measured to calculate maximum Rubisco activity (V_{cmax}), and maximum rate of electron transport used in the regeneration of RuBP (J_{max}). A/C_i curves were obtained using an automatic programme with the portable open gas exchange system (LI-6400XT, LI-COR, Lincoln, USA) on three consecutive days 18th – 20th April 2018 (76-78 DAP for T_A and 62-64 DAP for T_E). Measurements were taken on recently expanded leaves at saturating light (photosynthetic photon flux density 1800 µmol m^{-2} s^{-1}) with the cuvette temperature set to the mid-day growth temperature (T_A: 32°C; and T_E: 36°C). Leaves were allowed to equilibrate before measurements were taken.

R version 3.1.3 was used to fit curves to A/C_i data and generate V_{cmax} and J_{max} coefficients. GenStat version 16 was used to analyse the coefficients by REML. Data were assessed at a P=0.05 level of significance.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

Quantify the impacts of the interactive effects of future climate change on Australian cotton production at the field scale (Objective 2).

Field experiments have been conducted over three consecutive cotton seasons (2014/15, 2015/16 and 2016/17) to investigate the impact of elevated CO_2 and warmer temperature on growth and productivity of cotton. The outcomes of these experiments are summarised in Table 6. These experiments indicate that elevated atmospheric [CO_2] and warmer temperatures increased early season vegetative growth and alter leaf-level physiology. Similarly, the full season experiments showed that cotton grown at warmer temperatures had greater vegetative biomass, but large reductions in fruit biomass resulting in decreased plant water use efficiency. This indicates that greater vegetative biomass does not necessarily equate to increases in yield. Our data also show that elevated [CO_2] does not negate the negative effects of warmer growth temperatures on fruit retention, and furthermore, may in fact exacerbate the negative effects of warmer temperatures. Detailed results are shown below.

Table 6: Summary of results and outcomes for field experiments conducted at the Australian Cotton Research Institute, Narrabri.

Key outcomes: Quantify the impacts of the interactive effects of future climate change on Australian cotton production at the field scale	
Growth and biomass	Cotton grown in warmer temperatures were consistently taller and had a greater number of nodes than plants grown in the control conditions. In comparison, cotton grown at elevated [CO_2] did not have consistent differences in height and the number of nodes across all three seasons. The effects of the different environmental treatments on plant biomass varied over the seasons. Elevated [CO_2] and warmer temperatures increased early-season vegetative biomass and leaf area during the 2014/15 cotton season, although there were no significant effects on biomass and leaf area with warmer temperatures when compared to the control plots. However, across the two full season experiments, cotton grown in warmer temperatures had greater vegetative biomass and leaf area, but large reductions in fruit biomass. When comparing the chamber treatments, there was no difference in fruit biomass with elevated [CO_2], indicating that elevated CO_2 does not negate the

	negative effects of higher temperature on fruit retention. Furthermore, increases in vegetative biomass and leaf area with elevated CO_2 during the 2016/17 season suggests that there is the potential for elevated [CO_2] to exacerbate the negative effects of warmer temperatures.
Leaf physiology	Warmer temperatures increased stomatal conductance and transpiration, and reduced leaf-level water use efficiency of cotton in two of the three field seasons. Elevated [CO_2] consistently increased photosynthesis and leaf-level water use efficiency. Cotton grown at elevated [CO_2] had lower stomatal conductance in two seasons and reduced transpiration in one season.
Water use	There were some changes in soil water content for some irrigation periods and depths for two of the three cotton seasons. However, across the majority of the depths and irrigation periods, there were no consistent changes in soil water content between any of the temperature and CO_2 treatments.

Chambers were used to elevate atmospheric CO_2 concentration and generate warmer temperature in the field. Mean daily [CO_2] (Figure 2), minimum (Figure 3) and maximum (Figure 4) daily air temperatures, and atmospheric vapour pressure deficit (Figure 5) for the chamber and control treatments during each season are shown.

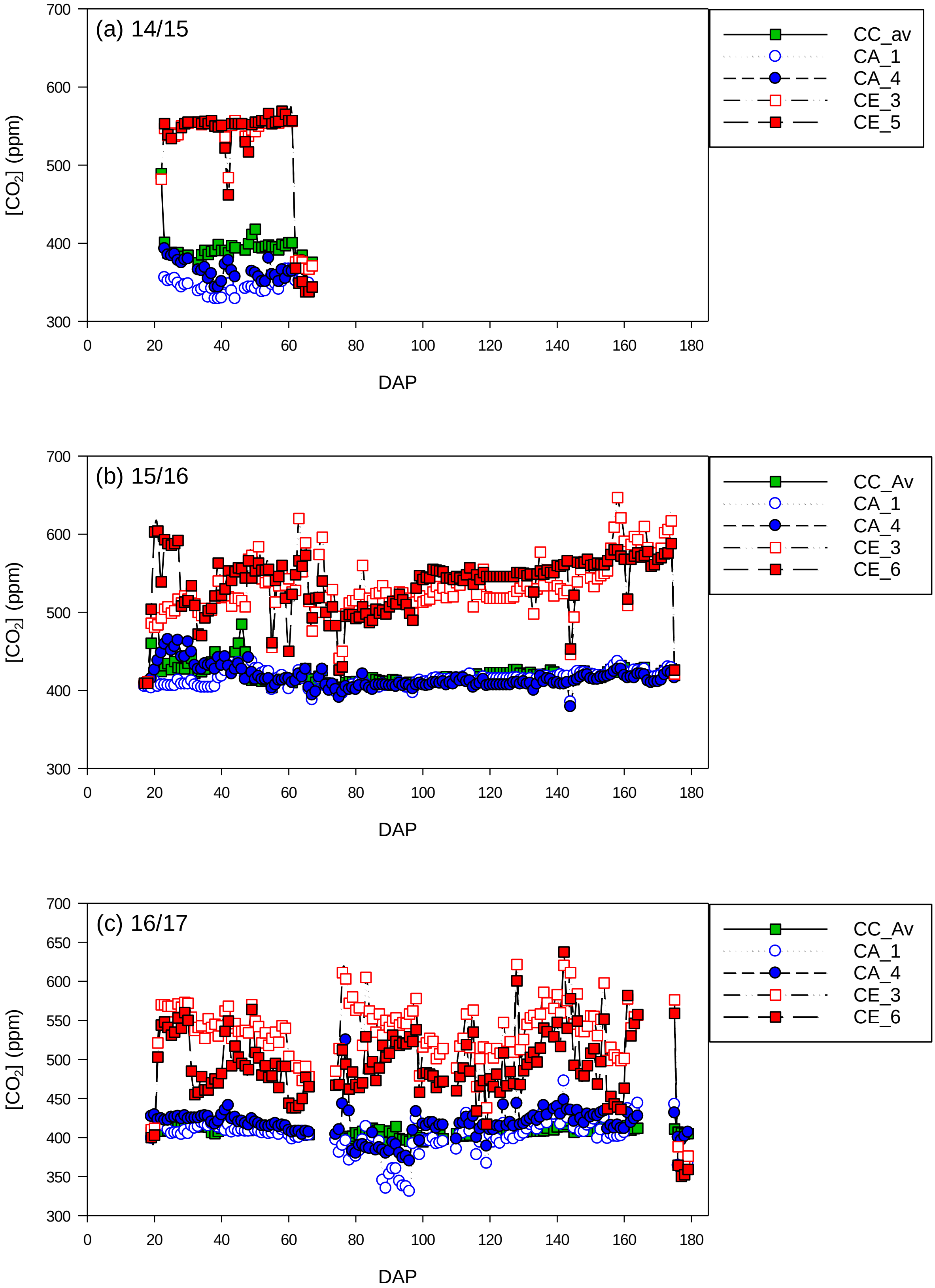

Figure 2: Mean daily [CO$_2$] (ppm) for each of the plots for (a) 2014/15, (b) 2015/16 and (c) 2016/17. C_C represents control plots (green), C_A are ambient CO$_2$ chambers (blue) and C_E are elevated CO$_2$ chambers (red). C_C represents the average of 4 control points measured outside the chamber.

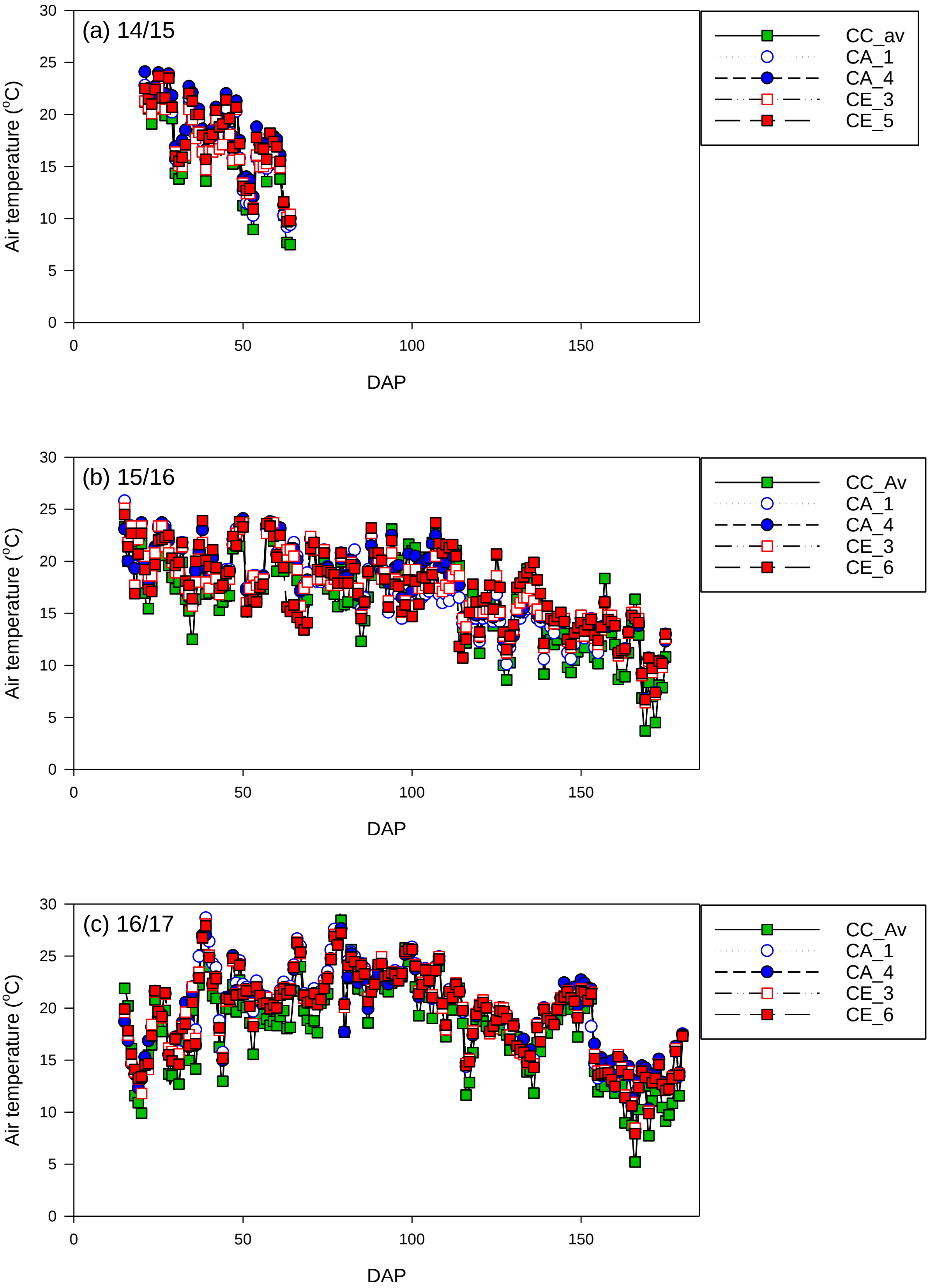

Figure 3: Minimum daily air temperature (°C) for each of the plots for (a) 2014/15, (b) 2015/16 and (c) 2016/17). C_C represents control plots (green), C_A are ambient CO_2 chambers (blue) and C_E are elevated CO_2 chambers (red). C_C represents the average of 2 control plots.

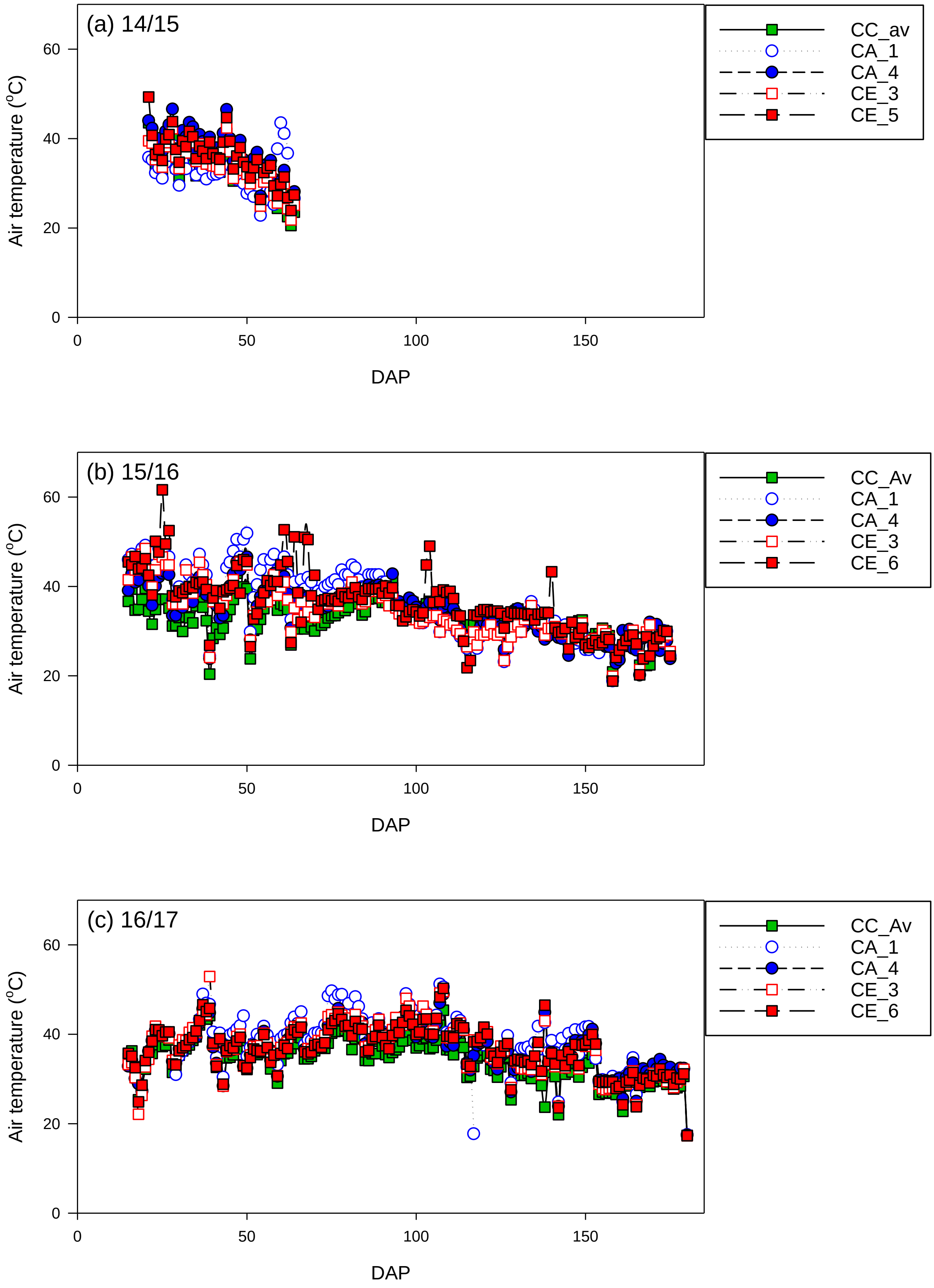

Figure 4: Maximum daily air temperature (°C) for each of the plots for (a) 2014/15, (b) 2015/16 and (c) 2016/17). C_C represents control plots (green), C_A are ambient CO_2 chambers (blue) and C_E are elevated CO_2 chambers (red). C_C represents the average of 2 control plots.

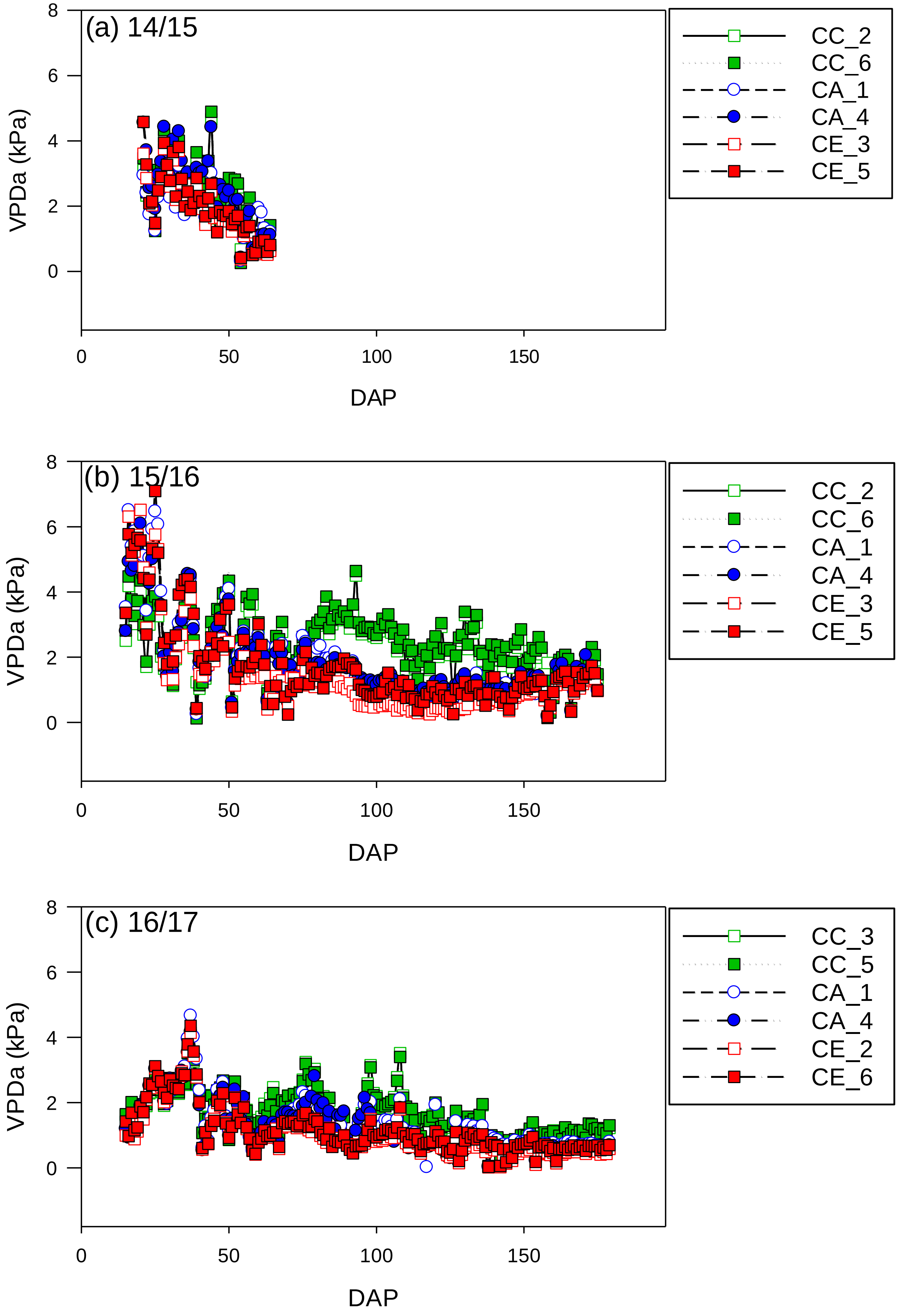

Figure 5: Mean daily atmospheric vapour pressure deficit (VPDa , kPa) for each of the plots for (a) 2014/15, (b) 2015/16 and (c) 2016/17) between the hours of 8am and 6pm. C_C represents control plots (green), C_A are ambient CO_2 chambers (blue) and C_E are elevated CO_2 chambers (red).

Plant growth and development throughout the season

Chamber effect (C_C compared with C_A): plants grown in the ambient [CO_2] chambers (C_A) were consistently taller and had a greater number of nodes than plants in the control plots (C_C) across all three seasons (Table 7; Figure 6). In 2014/15, cotton was 15% taller and had 6% more nodes in the C_A treatment compared with C_C, possibly due to the shorter growing season than the two subsequent seasons. In the 2015/16 season, where cotton growth was not managed using mepiquat chloride, plants grown in the C_A treatment were 48% taller and had 26% more nodes than plants grown at C_C. In the 2016/17 season, plants grown at C_A were 26% taller and had 24% more nodes than plants grown at C_C. This demonstrates a reduction in height compared with plants grown in the 2015/16 season, possibly indicating to a certain extent, that cotton height may be managed using a number of mepiquat chloride applications throughout the season. However, this requires further research.

Effect of elevated [CO_2] (C_A compared with C_E): Cotton grown at elevated [CO_2] (C_E) did not have consistent differences from the ambient [CO_2] (C_A) treatment across all three seasons (Table 7; Figure 6). In the 2014/15 season, plants grown at C_E were 29% taller and had 7% more nodes than plants grown at C_A. During the 2015/16 season, plants grown at C_E had 7% more nodes but were not any taller than cotton grown at C_A. In the 2016/17, there were not any significant differences in either height or the number of nodes between plants grown at C_A or C_E.

Table 7: Effects of [CO_2] and chambers on the height and nodes of cotton over three consecutive growing seasons.

	Effect of the chamber (C_C-C_A)			**Effect of [CO_2] (C_A-C_E)**		
	14/15	**15/16**	**16/17**	**14/15**	**15/16**	**16/17**
Height (cm plant^{-1})	+15%	+48%	+26%	+29%	0	0
Nodes (plant^{-1})	+6%	+26%	+24%	+7%	+7%	0

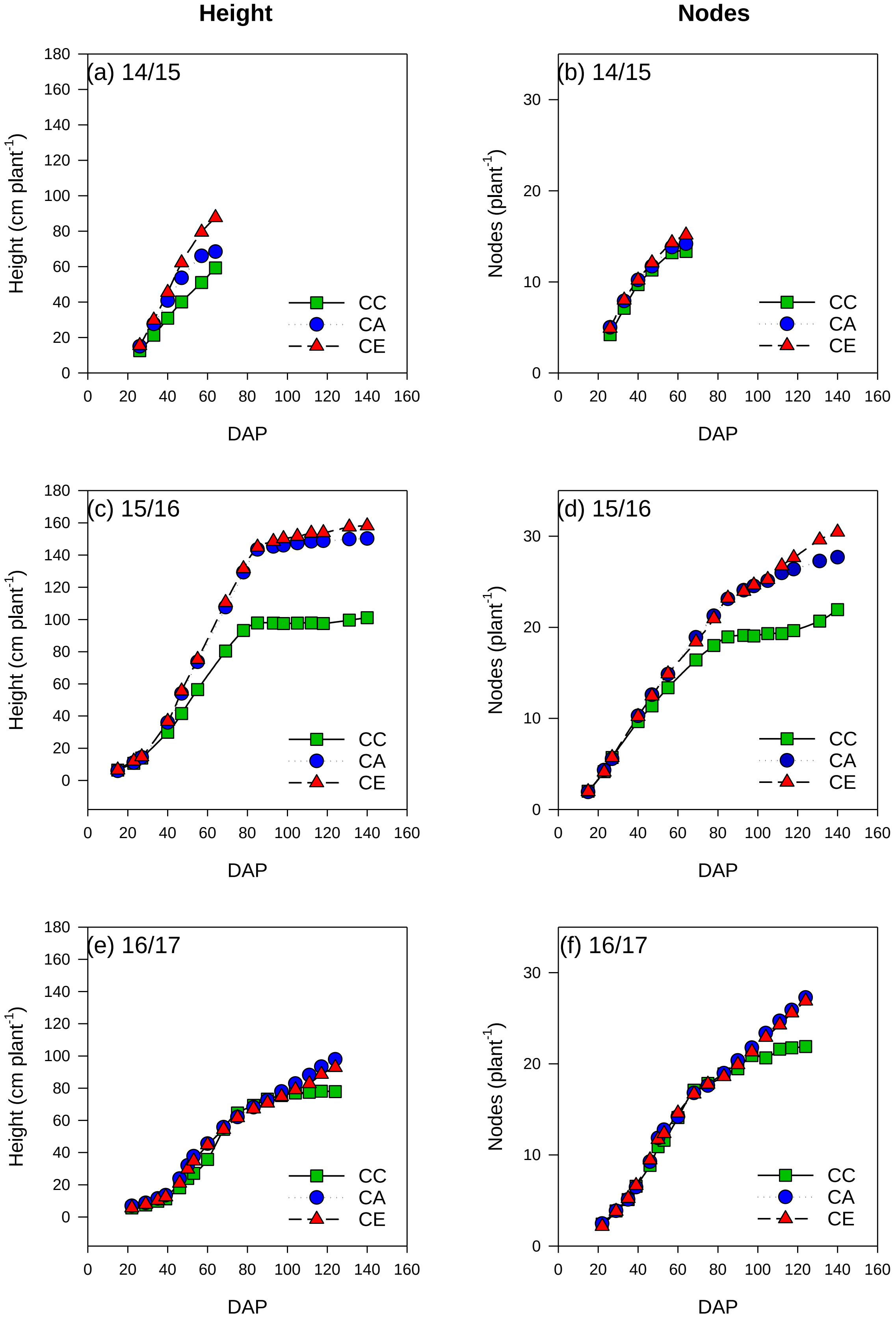

Figure 6: Height (cm plant^{-1}) and nodes (plant^{-1}) of plants grown in control plots (C_C, green), ambient CO_2 (C_A, blue) and elevated CO_2 (C_E, red) chambers over each of the three cotton seasons: 2014/15 (a and b), 2015/16 (c and d) and 2016/17 (e and f).

Physiology

Chamber effect (C_C compared with C_A): The effect of the field chambers on cotton physiology was variable across the three seasons (Table 8), possibly due to different environmental conditions for each season (Table 9; Table 10). During the 14/15 cotton season, the chambers did not have an effect on any of the plant physiological parameters measured, possibly due to a shorter season length. However, over the 15/16 cotton season and the 16/17 cotton season, cotton grown at warmer temperatures inside the chambers had increased stomatal conductance ($g_{s\text{-}sat}$; 50-535%), increased transpiration (E; 15-109%) and reduced the leaf-level water use efficiency (A_{sat}/E; 22-28%) compared with plants grown at ambient temperature (control plots).

Effect of elevated [CO$_2$] (C$_A$ compared with C$_E$): Compared with plants grown at C$_A$, plants grown at C$_E$ had consistently higher photosynthesis (11-34%) and leaf-level water use efficiency (24-30%) across all three seasons (Table 8). For 2015/16, stomatal conductance ($g_{s\text{-}sat}$) was 26% lower and transpiration (E) was reduced by 15% for plants grown at C$_E$ compared with plants grown at C$_A$, but there were no significant differences in the other two years.

Table 8: The effects of field chambers and elevated [CO$_2$] on physiology of cotton measured at squaring over three field seasons in Narrabri. Comparison is at growth [CO$_2$]. Values indicate the percentage increase or decrease.

Physiology	**Effect of the chamber (C$_C$-C$_A$)**			**Effect of [CO$_2$] (C$_A$-C$_E$)**		
	14/15	**15/16**	**16/17**	**14/15**	**15/16**	**16/17**
A_{sat}	0 (P=0.262)	-11% (P=0.001)	+54% (P=0.001)	+34% (P=0.003)	+11% (P=0.007)	+21% (P=0.001)
$g_{s\text{-}sat}$	0 (P=0.172)	+50% (P=0.001)	+535% (P=0.001)	0 (P=0.992)	-26% (P=0.018)	0 (P=0.072)
E	0 (P=0.467)	+15% (P=0.009)	+109% (P=0.001)	0 (P=0.714)	-15% (P=0.032)	0 (P=0.574)
A_{sat}/E	0 (P=0.278)	-22% (P=0.001)	-28% (P=0.001)	+24% (P=0.017)	+30% (P=0.001)	+27% (P=0.008)

Table 9: Timetable of physiology measurements during the three field seasons in Narrabri.

Cotton Season	**Physiology measurement**	**DAP**	**Date**
2014-15	ACi	41	17th March 2015
2015-16	ACi	56 - 58	20th /21st /22nd January 2016
2016-17	ACi	46 – 47	12th /13th December 2016
2016-17	ACi	69 – 70	4th /5th January 2017
2016-17	ACi	118 – 119	22nd /23rd February 2017
2016-17	Temperature	81 – 82	16th /17th January 2017
2016-17	Temperature	97	1st March 2017

Table 10: Summary of environmental conditions at the time of physiology measurements

	2014-15	**2015-16**	**2016-17**
$T_{air(chamber)}$	16-39°C	17-41°C	21-40°C
$VPD_{a(chamber)}$	0-4 kPa	0-6 kPa	0-5 kPa
$T_{block(Licor)}$	28°C	30°C	32°C
$VPD_{L(Licor)}$	1.8 kPa	1.5 kPa	1.2-2.2 kPa

Plant biomass

The effects of the different environmental treatments on plant biomass varied over the seasons (Figure 7, Table 11 and Table 12). It is important to consider the stage of crop development in the interpretation of these results. In the 2014/15 cotton season, plants were harvested at 64 DAP. The results for the 2014/15 season indicate that elevated CO_2 had greater vegetative and total plant biomass compared with cotton grown in the ambient CO_2 (C_A) chambers, although there was no significant difference in fruit biomass (Figure 7, Table 11 and Table 12). Consistent with increased vegetative biomass, leaf area was also greater in the elevated CO_2 (C_E) treatment than in the C_A treatment (Figure 8 and Table 12). For cotton grown in the 2014/15 season and harvested at 64 DAP, there were no significant differences in biomass or leaf area between plants grown in the control and C_A chambers (Table 12).

To examine the effects of warmer temperatures and elevated CO_2 on cotton grown over an entire season, biomass samples of plants grown in the 2015/16 and 2016/17 experiments were harvested at 145 and 139 DAP, respectively. Importantly, although there were no significant differences in total plant biomass between the three treatments (C_C, C_A and C_E), there were differences in the ratio of vegetative to fruit biomass. Consistently across both seasons, cotton grown in the ambient CO_2 chambers had greater vegetative growth and larger leaf area at the end of the season than cotton grown in the control treatments. However, this was coupled with a large (75-84%) reduction in fruit biomass for the plants grown in C_A compared with C_C. For both seasons, there was no difference when comparing the fruit biomass of C_A and C_E grown plants, indicating that elevated CO_2 does not mitigate the negative effects of higher temperatures on fruit retention.

There was variability in biomass comparing plants grown in the ambient and elevated CO_2 chambers, whereby elevated CO_2 increased vegetative biomass (+35%) and leaf area (+28%) only in the 2016/17 season. This suggests that there is the potential that elevated CO_2 may exacerbate the negative effects of warmer temperature. For example, when fruit is shed as a result of high temperature, the plant may be putting resources (i.e. CO_2) into more vegetative growth rather than reproductive growth resulting in a feedback loop of high rates of vegetative growth. Furthermore, we observed “parrot beak” of green bolls grown in the chambers during the 2016/17 season, which demonstrate evidence of high temperature damage to the fruit (Figure 9).

Shedding and total retention of fruit were mapped for cotton grown during the 2016/17 season (Figure 10, Figure 11, Table 13). Although there was no difference in the number of fruiting sites from Nodes 0-10, total retention of plants grown at C_A was reduced by 46% compared with plants grown at C_C, yet increased by 78% for plants grown at C_E compared with C_A. Compared with the control treatment (C_C), cotton grown in the C_A treatment had a greater number of fruiting sites for nodes 11-15, nodes 16-20 and nodes 21+, however, the retention of fruit at each of these nodes were significantly reduced. Compared to cotton grown at C_A, plants grown at C_E had an increased number of fruiting sites for nodes 11-15 (+18%) and nodes 16-20 (+17%) although retention of fruit was not improved by elevated CO_2. Consequently, final yield of cotton grown in the C_A and C_E chamber treatments during the 2016/17 cotton season were much lower than the C_C plot (Figure 12).

Table 11: REML analyses for biomass and leaf area of cotton grown over three field seasons. Values represent P values, where *, **, *** shows significant interactions at $P \leq 0.05$, 0.01 and 0.001 respectively. Refer to Table 12 for *post hoc* analysis.

Cotton season	**14/15**	**15/16**	**16/17**
DAP	**64**	**145**	**139**
Vegetative biomass (g plant^{-1})	0.001***	0.005**	0.001***
Fruit biomass (g plant^{-1})	0.641	0.001***	0.001***
Total biomass (g plant^{-1})	0.001***	0.578	0.147
Leaf area (cm^2 plant^{-1})	0.001***	0.001***	0.001***

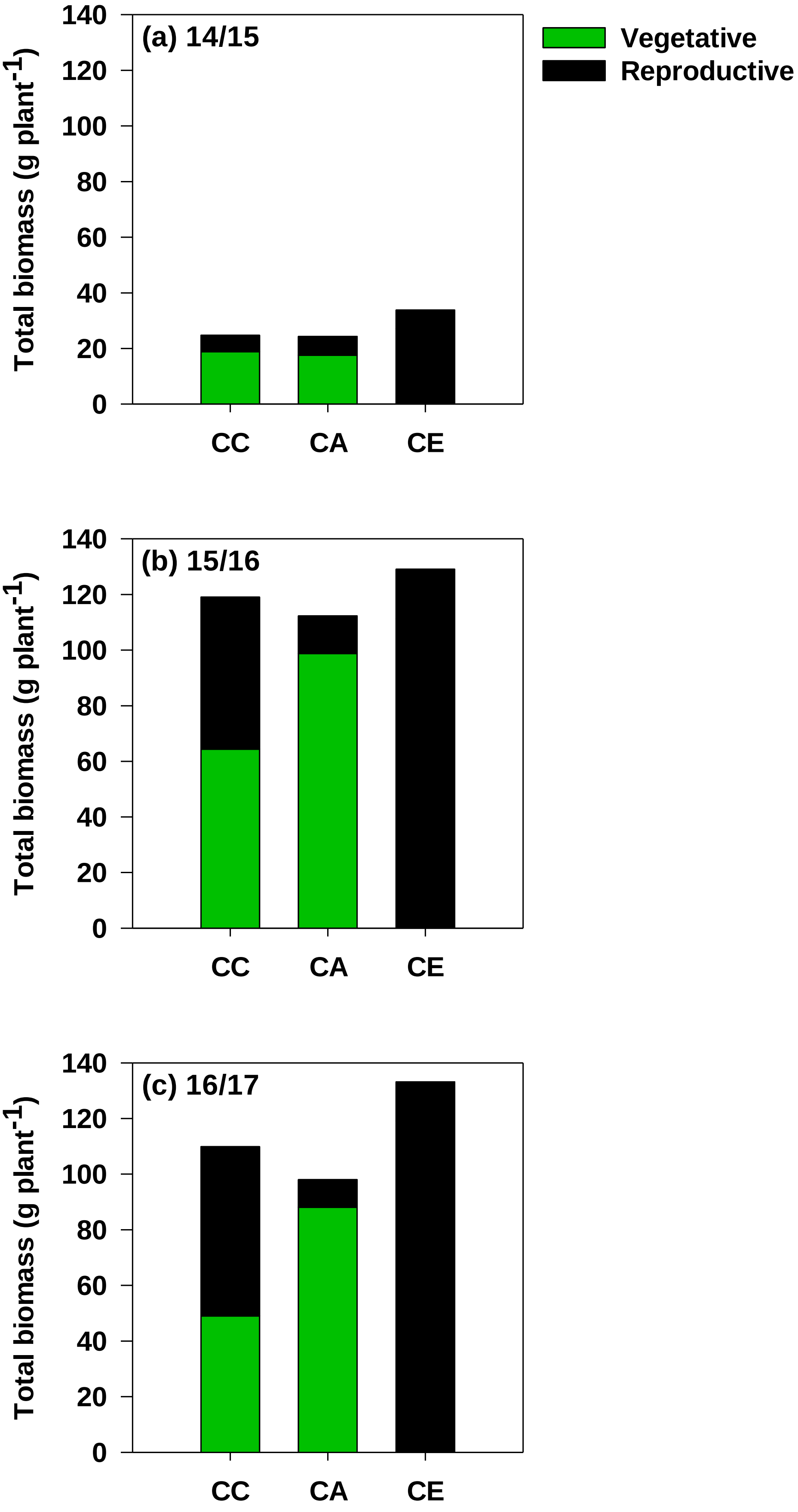

Figure 7: Vegetative (green) and reproductive (black) biomass for cotton grown in control (CC), ambient CO_2 (CA) and elevated CO_2 (CE) during the (a) 2014/15, (b) 2015/16, and (c) 2016/17 cotton seasons.

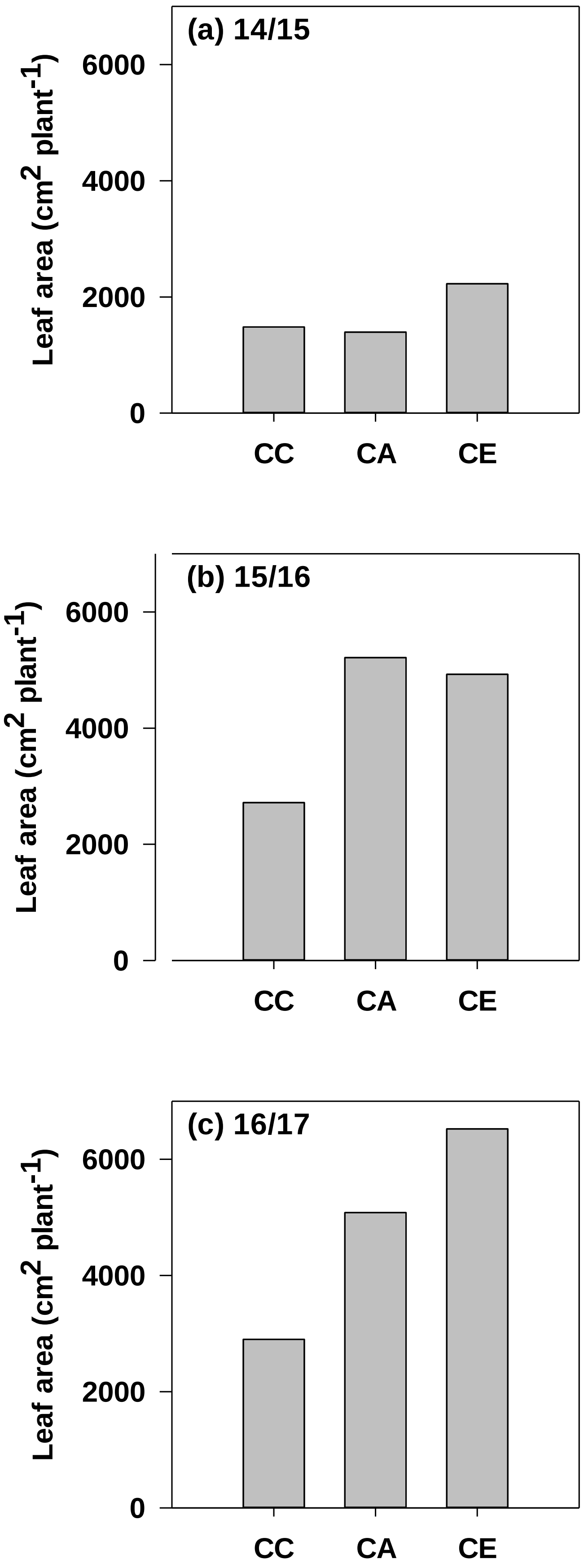

Figure 8: Leaf area of cotton grown at control (CC), ambient CO_2 (CA) and elevated CO_2 (CE) over three consecutive seasons: (a) 2014/15, (b) 2015/16, and (c) 2016/17.

Table 12: The effects of the field chambers and elevated [CO_2] on plant biomass and leaf area of cotton over three field seasons. Values show the percentage increase or decrease.

	Effect of the chamber (C_C-C_A)			**Effect of [CO_2] (C_A-C_E)**		
Cotton season	**14/15**	**15/16**	**16/17**	**14/15**	**15/16**	**16/17**
DAP	**64**	**145**	**139**	**64**	**145**	**139**
Vegetative biomass (g plant^{-1})	0	+53%	+80%	+54%	0	+34%
Fruit biomass (g plant^{-1})	0	-75%	-84%	0	0	0
Total biomass (g plant^{-1})	0	0	0	+51%	0	0
Leaf area (cm^2 plant^{-1})	0	+92%	+75%	+60%	0	+28%

Figure 9: Green bolls from plants grown in the chambers during the 2016/17 cotton season demonstrate evidence for high temperature stress ("parrot beak").

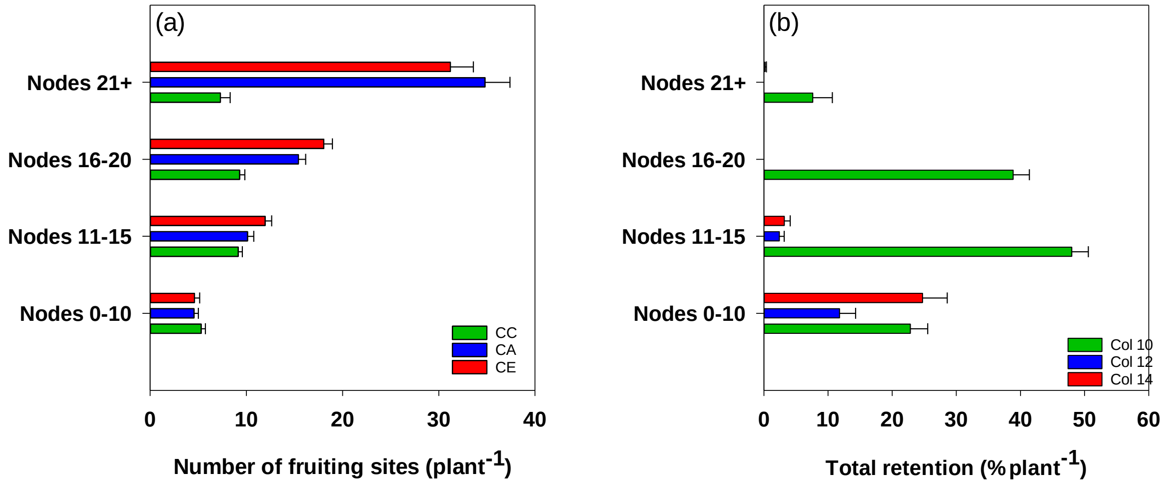

Figure 10: (a) Number of fruiting sites and (b) total retention of cotton plants grown at control (CC), ambient CO_2 (CA) and elevated CO_2 (CE) during the 2016/17 cotton season.

Table 13: ANOVA analysis and percentage difference between plants grown at control (CC), ambient CO_2 (CA) and elevated CO_2 (CE) during the 2016/17 cotton season.

	F pr.	**CC-CA**	**CA-CE**
Number of fruiting sites (plant^{-1})			
Node 0-10	0.471	0	0
Node 11-15	**0.005**	11	18
Node 16-20	**0.001**	66	17
Node 21+	**0.001**	377	0
Total retention (% plant^{-1})			
Node 0-10	**0.045**	-46	78
Node 11-15	**0.001**	-95	0
Node 16-20	**0.001**	-100	0
Node 21+	**0.006**	-100	0

Figure 11: High numbers of fruit loss was observed for plants grown inside the chambers during the 2016/17 cotton season.

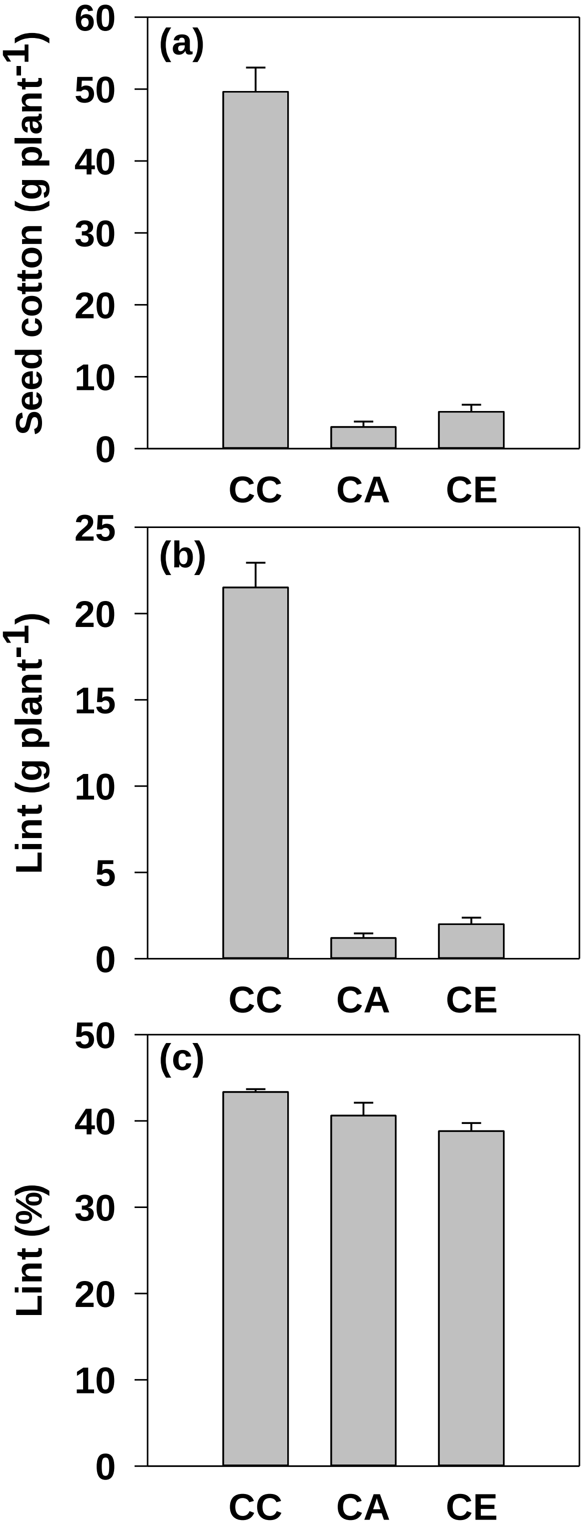

Figure 12: (a) Seed cotton weight, (b) lint weight, and (c) lint percentage of cotton grown at control (CC), ambient CO_2 (CA), and elevated CO_2 (CE) during the 2016/17 cotton season.

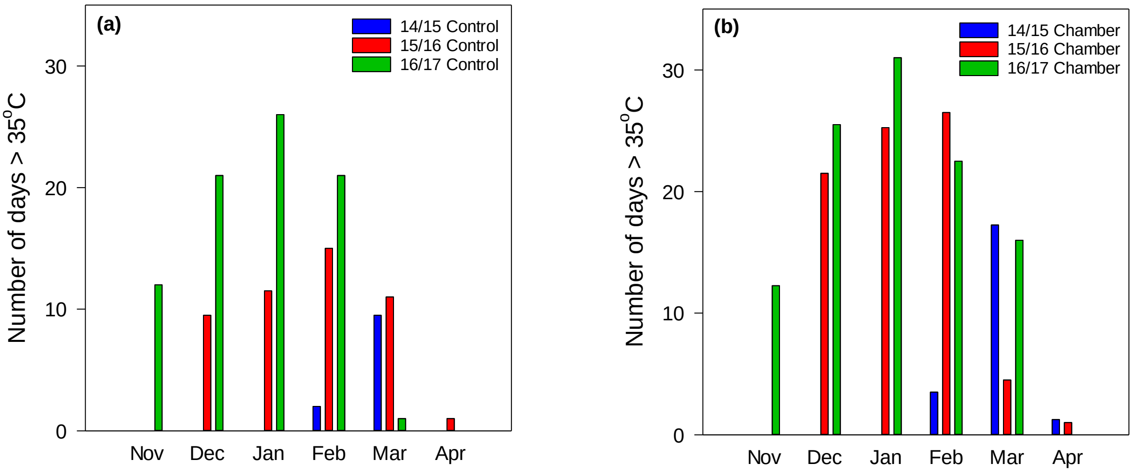

Figure 13: Number of days above 35°C (a) in the control plots and (b) in the climate chambers for three consecutive cotton seasons (14/15, 15/16 and 16/17). Note that experiments started during late February in the 2014/15 cotton season.

The frequency of temperatures reaching above 35°C in the control plots and chambers are shown in Figure 13. High temperatures may have been responsible for the abscission of fruit and consequent excessive vegetative growth.

Plant water use

Each season soil water content was measured to a depth of 90 cm using capacitance probes (GLRL). The change in soil water content between irrigation periods (ΔSWC) was calculated and analysed using ANOVA. For the 2014/15 cotton season, there were no significant differences in ΔSWC (Table 14). There were some significant differences in ΔSWC during each of the 2015/16 and 2016/17 cotton seasons.

Chamber effect (C_C compared with C_A): The change in soil water content (ΔSWC) for some irrigation periods and depths was lower in the C_A plots compared with the control plots. During the 2015/16 season and compared with the control plots, ΔSWC of CA plots was 63% lower at a depth of 90cm for the irrigation period 52-58 DAP, 198% lower at a depth of 30cm and 86% lower at a depth of 90cm for the irrigation period 60-78 DAP, 387% lower at a depth of 10cm for the irrigation period 108-119 DAP, and 725% lower at a depth of 10cm for the irrigation period 120-175 DAP (Table 15). During the 2016/17 season, compared with the control plots, ΔSWC of C_A plots was 61% lower at a depth of 50cm for the irrigation period 64-75 DAP, 75% lower at a depth of 90cm for the irrigation period 93-98 DAP, 74% lower at a depth of 50cm for the irrigation period 100-105 DAP, 62% lower at a depth of 10cm for 107-112 DAP and 75% lower at a depth of 10cm for the irrigation period 114-119 DAP (Table 15). These differences could potentially be due to altered atmospheric vapour pressure deficit (VPDa) inside the chambers, leading to a reduction in transpiration, however, these differences are not seen consistently throughout the soil profile for each irrigation interval.

Effect of elevated [CO_2] (C_A compared with C_E): During the 2015/16 season and compared with C_A, ΔSWC of C_E plots was 160% lower at a depth of 10cm for the irrigation period 60-78 DAP, and 686% lower at a depth of 30cm for the irrigation period 120-175 DAP (Table 15). ΔSWC of the C_E plots was 394% higher at a depth of 20cm for the irrigation period 94-106 DAP. During the 2016/17 cotton season, ΔSWC was lower for C_E compared with C_A on two occasions at a depth of 50cm. For the irrigation period 64-75 DAP ΔSWC of C_E plots was 64% lower than for C_A plots and for the irrigation period 85-91 DAP ΔSWC was 146% lower than for C_A plots (Table 15).

However, across the majority of the depths and irrigation periods, there were no consistent differences in ΔSWC between C_C, C_A and C_E plots for any of the three cotton seasons.

Table 14: ANOVA comparison of SWC between irrigation cycles for each cotton season. Soil water content was measured to a depth of 90cm using GLRL sensors. Values in bold represent a significant difference at P< 0.05 level of significance.

DAP	**10cm**	**30cm**	**50cm**	**70cm**	**90cm**
2014-15 season					
24-49	0.422	0.320	0.882	0.462	0.270
51-58	0.489	0.744	0.996	0.962	0.984
2015-16 season					
42-50	0.134	0.637	0.344	0.135	0.811
52-58	0.161	0.907	0.221	0.172	**0.035**
60-78	**0.005**	**0.012**	0.083	0.220	**0.049**
80-92	0.730	**0.030**	0.165	0.087	**0.015**
94-106	0.421	**0.006**	0.157	0.414	0.566
108-119	**0.048**	0.424	0.798	0.407	0.610
120-175	**0.033**	**0.023**	**0.027**	0.134	0.247
2016-17 season					
18-39	0.361	0.479	0.471	0.511	0.446
41-55	0.041	0.297	0.934	0.835	0.611
57-62	0.432	0.752	0.586	0.599	0.429
64-75	0.561	0.974	**0.032**	0.405	0.179
77-83	0.512	0.103	**0.027**	0.322	0.636
85-91	0.203	**0.046**	**0.014**	**0.021**	0.146
93-98	0.657	0.497	0.430	0.357	**0.045**
100-105	0.211	0.222	**0.023**	0.081	0.118
107-112	**0.025**	0.103	0.120	0.981	0.202
114-119	**0.015**	0.153	0.096	0.564	0.389
121-133	0.201	0.866	0.805	0.367	0.384
134-179	0.270	0.738	0.499	0.402	0.441

Table 15: Percentage difference in soil water content at five depths between irrigation cycles for each cotton season. Soil water content was measured using GLRL sensors. 0 represents no significant difference, values in bold represent significant difference at P<0.05. Data was analysed by ANOVA (see Table 14).

DAP	**10cm**	**30cm**	**50cm**	**70cm**	**90cm**
2015-16 season					
24-49	0	0	0	0	0
51-58	0	0	0	0	0
2015-16 season					
42-50	0	0	0	0	0
52-58	0	0	0	0	**-63% (CA vs CC)**
60-78	**-160% (CE vs CA)**	**-198% (CA vs CC)**	0	0	**-86% (CA vs CC)**
80-92	0	**+170% (CA vs CC)**	0	0	**+311% (CA vs CC)**
94-106	0	**+394% (CE vs CA)**	0	0	0
108-119	**-387%**	0	0	0	0

	(CA vs CC)				
120-175	**-725% (CA vs CC)**	**-686% (CE vs CA)**	**+1428% (CA vs CC)**	0	0
2016-17 season					
18-39	0	0	0	0	0
41-55	0	0	0	0	0
57-62	0	0	0	0	0
64-75	0	0	**-61% (CA vs CC)**	0	0
77-83	0	0	**-64% (CE vs CA)**	0	0
85-91	0	**CC vs CE**	**-146% (CE vs CA)**	**CC vs CE**	0
93-98	0	0	0	0	**-75% (CA vs CC)**
100-105	0	0	**-74% (CA vs CC)**	0	0
107-112	**-62% (CA vs CC)**	0	0	0	0
114-119	**-75% (CA vs CC)**	0	0	0	0
121-133	0	0	0	0	0
134-179	0	0	0	0	0

Quantify the impacts of more extreme climate change scenarios of higher average temperatures and more severe weather limitations (Milestone 3).

Two glasshouse experiments have been completed at UWS where detailed measurements of cotton physiology were taken to investigate the impact of elevated CO_2 with higher temperatures and more severe water limitations on cotton growth and productivity. The outcomes of these two experiments are summarised in Table 16. These experiments suggest that warmer air temperatures may accelerate growth rates of cotton plants, but not necessarily equate to greater yields. Furthermore, plants grown at warmer air temperatures may be more susceptible to extreme climate events, including water deficits and heatwave conditions. Therefore, adaptive strategies may become increasingly important to mitigate warmer air temperatures, drought and heat stress in cotton production systems.

Table 16: Key outcomes of glasshouse experiments conducted to quantify the impacts of more extreme climate change scenarios of higher average temperatures and more severe weather limitations.

	Key outcomes: Quantify the impacts of more extreme climatic scenarios of higher average temperatures and more severe weather limitations	
	TransPhys experiment	**Temperature x water deficit x heatwave experiment**
Growth and biomass	Elevated [CO_2] increased plant height, leaf area and total biomass. The heatwave treatment did not affect biomass of plants harvested 10 days after the heatwave. Significant effects on biomass may be evident in the longer term, but would need to be investigated.	Warmer day and night temperatures (32/22 and 32/26°C) accelerated the growth rate of cotton plants, but high growth rates did not equate to higher yields.
Leaf physiology	Cotton exposed to a six day heatwave treatment (day 1: 35/24°C; days 2-6: 40/29°C) had higher photosynthesis,	Heatwave stress detrimentally affected photosynthesis,

	stomatal conductance and transpiration, resulting in reduced leaf-level water use efficiency.	particularly in plants grown at warmer temperatures.

TransPhys Experiment

Physiological response of cotton to elevated atmospheric [CO_2] and heatwave conditions

There were no significant CO_2 by heatwave treatment interactions for any of the leaf gas exchange measurements (P>0.05, Table 17). In the C_E treatment, A_{sat} was 30% higher than in the C_A treatment (P= 0.001, Table 17, Table 18). In the C_E treatment $A_{sat}/g_{s\text{-}sat}$ was also higher by 66% (P=0.001) and A_{sat}/E by 27% (P= 0.011) than in the C_A treatment. Across both CO_2 treatments, plants grown in the HW treatment had higher A_{sat} by 25%, $g_{s\text{-}sat}$ by 76% and E by 55% than in the T_A (P= 0.001). Thus, the heatwave treatment had 29% lower $A_{sat}/g_{s\text{-}sat}$ (P= 0.001) and 17% lower A_{sat}/E (P= 0.046) than the T_A.

Across both CO_2 treatments, the HW treatment reduced J_{max} by 17% (P= 0.013, Table 17, Table 18) and reduced J_{max}/V_{cmax} by 25% (P= 0.001). C_E increased J_{max}/V_{cmax} by 18% (P= 0.015).

Table 17: REML analysis for leaf gas exchange at growth [CO_2] treatment (i.e. 640ppm compared with 400ppm). Leaf gas exchange was measured over three consecutive days at 28ºC, one day after the heatwave treatment. Values are F probabilities; bold represents a significant difference at P<0.05.

	CO_2 Treatment	**HW Treatment**	**[CO_2] x HW**
Leaf gas exchange			
A_{sat}	**0.001**	**0.001**	0.266
$g_{s\text{-}sat}$	0.565	**0.001**	0.507
E	0.853	**0.001**	0.572
$A_{sat}/g_{s\text{-}sat}$	**0.001**	**0.001**	0.073
A_{sat}/E	**0.011**	**0.046**	0.483
V_{cmax}	0.107	0.271	0.952
J_{max}	0.772	**0.013**	0.119
J_{max}/V_{cmax}	**0.015**	**0.001**	0.508

Table 18: Main treatment effects for leaf gas exchange at growth [CO_2] (i.e. 640ppm compared with 400ppm). Leaf gas exchange was measured over three consecutive days at 28ºC, one day after the heatwave treatment. Values represent the percentage change with CO_2 or temperature treatment.

	C_E compared with C_A (%)	**HW compared with T_A (%)**
Leaf gas exchange		
A_{sat}	30	25
$g_{s\text{-}sat}$	0	76
E	0	55
$A_{sat}/g_{s\text{-}sat}$	66	-29
A_{sat}/E	27	-17
V_{cmax}	0	0
J_{max}	0	-17
J_{max}/V_{cmax}	18	-25

Plant growth

Under the C_A conditions, plants exposed to the heatwave had 14% higher number of nodes than plants grown at T_A (P=0.027, Table 19). However, no significant difference in node number was detected in plants grown under C_E conditions given the heatwave.

At ambient temperature, plants grown at C_E had 88% higher fruit biomass than in C_A, but not for cotton exposed to the heatwave treatment (P=0.036, Table 19). Plants that experienced the heatwave had 37% lower fruit biomass than plants that did not experience the heatwave in C_E conditions, but not under C_A conditions.

Plants grown in C_E had greater height (22%), leaf area (34%), and total biomass (57%) compared with plants grown in C_A (P= 0.001, Table 19, Table 20). Plants grown at C_E also had higher leaf biomass (42%) and stem biomass (79%) than plants grown in C_A (P= 0.001). The heatwave treatment did not have a significant effect on vegetative mass or plant characteristics (P>0.05, Table 19), due to the short period of time between the heatwave and completion of the experiment.

Table 19: REML analysis for vegetative biomass and plant characteristics at growth [CO_2] treatment (i.e. 640ppm compared with 400ppm). Vegetative mass and plant characteristics were measured 10 days post heatwave treatment. Values are F probabilities; bold represents a significant difference at P<0.05.

	CO_2 Treatment	**HW Treatment**	**[CO_2] x HW**
Vegetative mass and plant characteristics			
Leaf biomass (g plant^{-1})	**0.001**	0.091	0.209
Stem biomass (g plant^{-1})	**0.001**	0.566	0.398
Fruit biomass (g plant^{-1})	0.065	0.251	**0.036**
Total aboveground biomass (g plant^{-1})	**0.001**	0.946	0.140
Nodes (plant^{-1})	**0.001**	0.173	**0.027**
Leaf area (cm^2 plant^{-1})	**0.001**	0.145	0.471
Plant height (cm plant^{-1})	**0.001**	0.284	0.425

Table 20: Main treatment effects for vegetative biomass and plant characteristics at growth [CO_2] (i.e. 640ppm compared with 400ppm). Vegetative mass and plant characteristics were measured 10 days post heatwave treatment. Values represent the percentage change with CO_2 or temperature treatment.

	C_E compared with C_A (%)	**HW compared with T_A (%)**
Vegetative mass and plant characteristics		
Leaf biomass (g plant^{-1})	**42**	0
Stem biomass (g plant^{-1})	**79**	0
Fruit biomass (g plant^{-1})	0	0
Total aboveground biomass (g plant^{-1})	**57**	0
Nodes (plant^{-1})	**22**	0
Leaf area (cm^2 plant^{-1})	**34**	0
Plant height (cm plant^{-1})	**22**	0

Summary of findings

The objective of this experiment was to determine the effects of warmer temperatures, elevated [CO_2] and heatwave events on the growth and physiology of well-watered cotton. Elevated CO_2 increased photosynthetic rates, water use efficiency and J_{max}/V_{cmax}, leading to taller plants, greater leaf area and total biomass. Cotton exposed to the six day heatwave event of around 40°C had significantly altered physiology, measured over three consecutive days starting one day after the end of the heatwave treatment. Cotton exposed to the heatwave had higher photosynthesis, stomatal conductance and transpiration, resulting in a reduced water use efficiency than the control plants. After a short frame, the heatwave treatment did not affect biomass of plants harvested 10 days after the heatwave. Significant effects on biomass may be evident in the longer term, but would need to be investigated.

UWS Day/Night Temperature x Drought x Heatwave

Fruit development

Elevated day and night temperature accelerated the development of cotton. Across all temperature treatments, plants at 28/18°C grew the slowest and the plants grown at 32/26°C grew the fastest. Cotton grown at 32/26°C took 59 days to reach the maximum square number, which was 28 days faster than plants grown at 28/18°C (Figure 14). Similarly, plants grown at 32/26°C reached the maximum boll number 80 days after planting, but the plants grown at 28/18°C took 108 days to reach the maximum number of bolls (Figure 14). Nevertheless, the plants grown at cooler day temperatures (28/18 and 28/22°C) developed more squares and bolls than plants grown at warmer day temperatures (32/22 and 32/26°C).

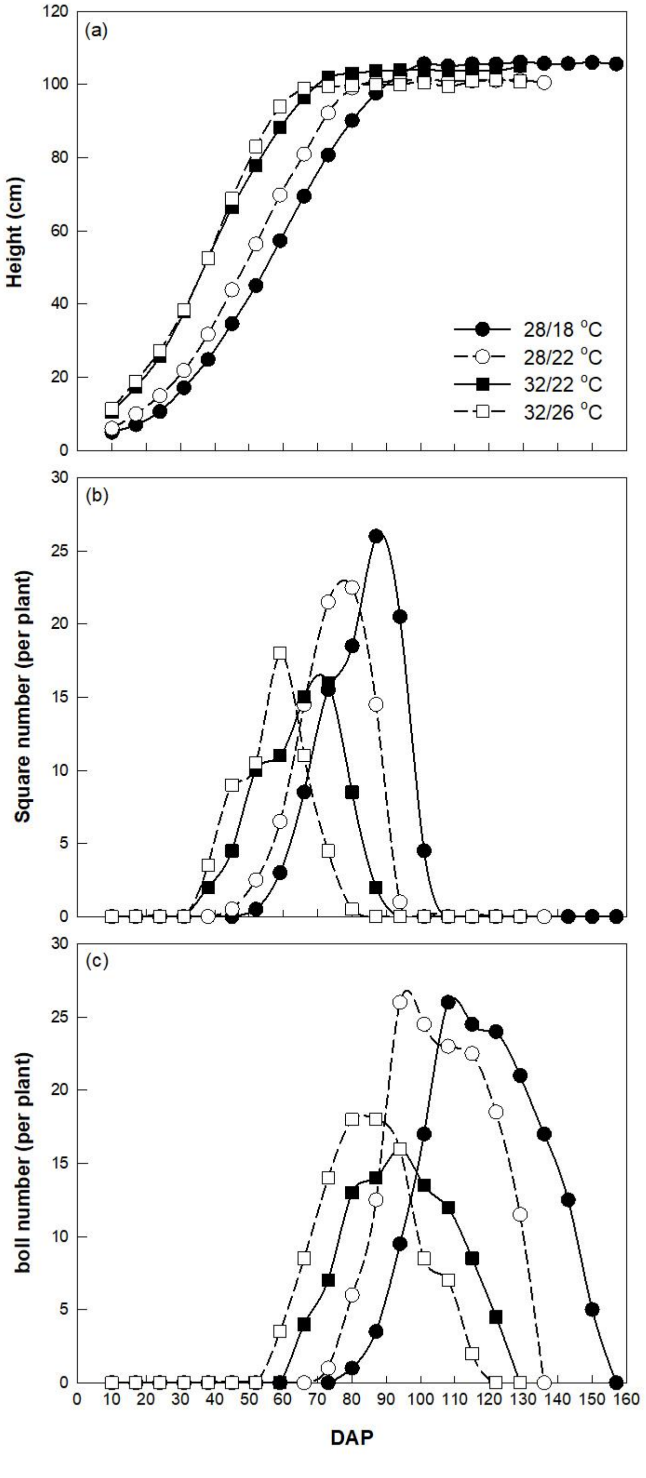

Figure 14: Effect of growth temperature on the development of (a) plant height, (b) number of squares, and (c) the number of green bolls. Solid/open circles are the plants grown at cooler day temperatures (28/18 and 28/22 °C, respectively); Solid/open squares are the plants grown at warmer day temperatures (32/22 and 32/26 °C, respectively). Values represent the mean (n= 20).

Plant growth and biomass

Effect of day/night temperature: Generally, temperature treatment had no significant effects on plant height under well-watered conditions. Compared to the well-watered plants grown at 28/22°C, warmer day-time temperature reduced the above ground biomass by 12.1% in well-watered plants grown at 32/22°C (Table 21, Figure 15). Elevated night temperatures either in cooler or warmer daytime temperature treatments did not affect above ground biomass.

Effect of water treatment: Water-limitation consistently decreased above ground biomass across all temperature treatments, however, varying magnitude of reduction resulted in a significant temperature by water treatment interaction (P= 0.026, Table 21). Water-limitation decreased above ground biomass by 23% at 32/26°C, which is twice as greater as water-limitation reduction at 32/22°C (11%). Compared with well-watered plants in each temperature treatment, the above ground biomass of plants was reduced by 10% for water-limited plants grown at 28/18°C and by 12% for water-limited plants grown at 28/22°C. Averaged across all temperature and heatwave treatments, plant height of water-limited plants was reduced by 6% and above ground biomass was reduced by 14% compared with plants grown in the well-watered treatment (P= 0.001, Table 21).

Effect of heatwave treatment: Compared to the non-heatwave control plants, there was no heatwave effect on either plant height or plant above ground biomass (Table 21, Figure 15).

Table 21: Analysis of variance (ANOVA) showing the effect of growth temperature (28/18, 28/22, 32/22, 32/26 °C), water treatment (50% vs 100% field capacity), heatwave event and their interactions on cotton growth, biomass and cotton yield. Values shown in bold are probability at the $P<0.05$ significance level.

	Main effects			Interactions			
Parameters	Temperature	Water	Heatwave	TxW	TxHW	WxHW	TxWxHW
Growth and biomass							
Height	**0.004**	**0.001**	0.159	0.205	0.815	0.155	**0.021**
Above ground biomass	**0.001**	**0.001**	0.479	**0.026**	0.156	0.677	0.223
Cotton yield							
Seed cotton	**0.001**	**0.001**	**0.001**	0.112	0.083	0.534	0.151
Lint (g)	**0.001**	**0.001**	**0.001**	**0.042**	0.380	0.552	0.217
Lint (%)	**0.001**	0.257	**0.007**	0.902	0.419	0.282	0.261

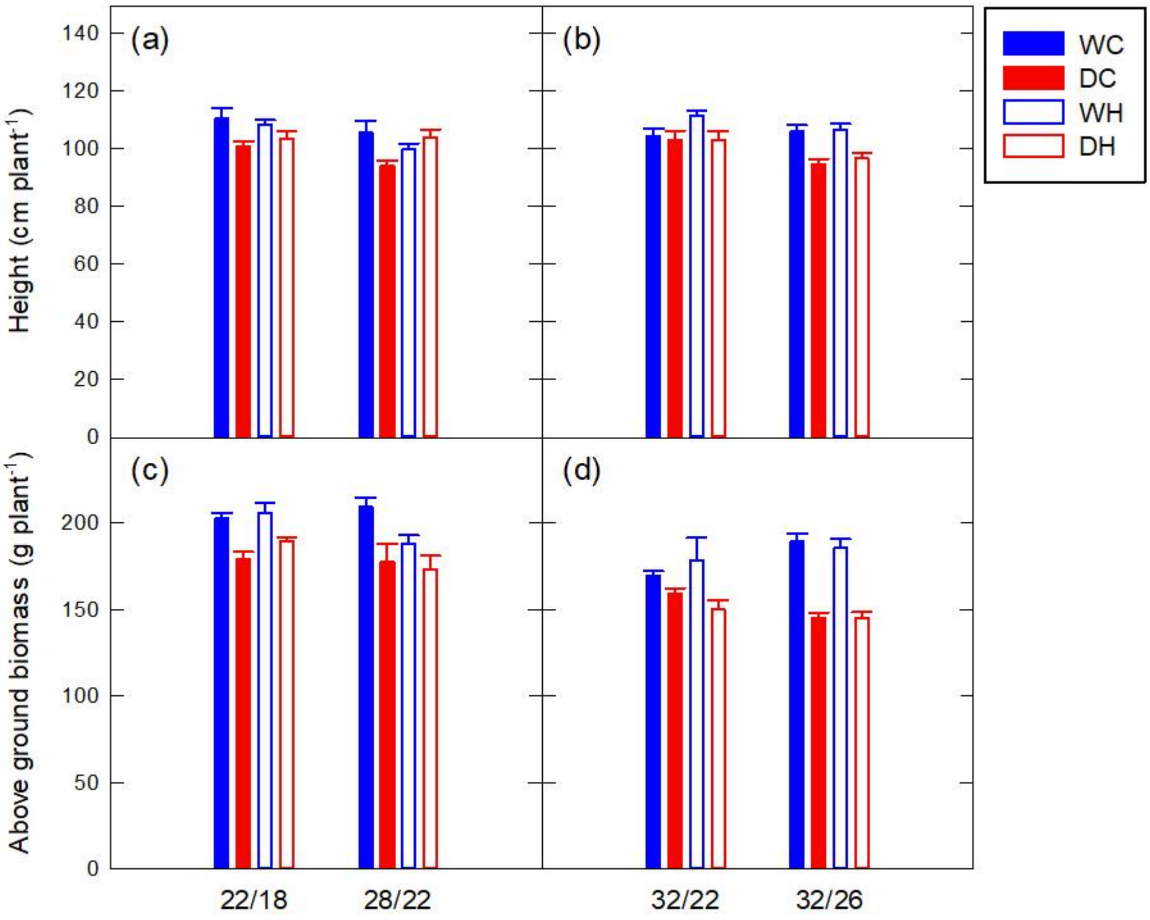

Figure 15: Mean final plant height (a and b) and above ground biomass (c and d) of cotton grown at four temperature treatments. WC is well-watered control, DC is water-limited control, WH is well-watered exposed to the heatwave event, and DH is water-limited exposed to the heatwave event.

Effect of day/night temperature on cotton yield

Well-watered plants grown at 28/22°C had the greatest lint mass (Figure 16; Table 22). In comparison, well-watered cotton grown at cooler night temperatures (22/18°C) had 11% lower lint mass. Well-watered cotton grown at warmer day temperatures (32/22°C) had 21% lower lint mass than well-watered plants grown at 28/22°C.

Plants grown at 28/22°C had the greatest seed cotton mass (Figure 16). In comparison, plants grown at cooler night-time temperatures (28/18°C) had 8% less seed cotton than plants grown at 28/22°C. Compared with cotton grown at 28/22°C, warmer daytime temperatures also reduced seed cotton mass by 21% in the 32/22°C treatment and by 16% in the 32/26°C treatment. There was no difference in seed cotton mass for plants grown at 32/22°C and 32/26°C.

Cotton grown at high day and night temperature (32/36°C) had the lowest lint percentage compared with three other temperature treatments (Figure 16).

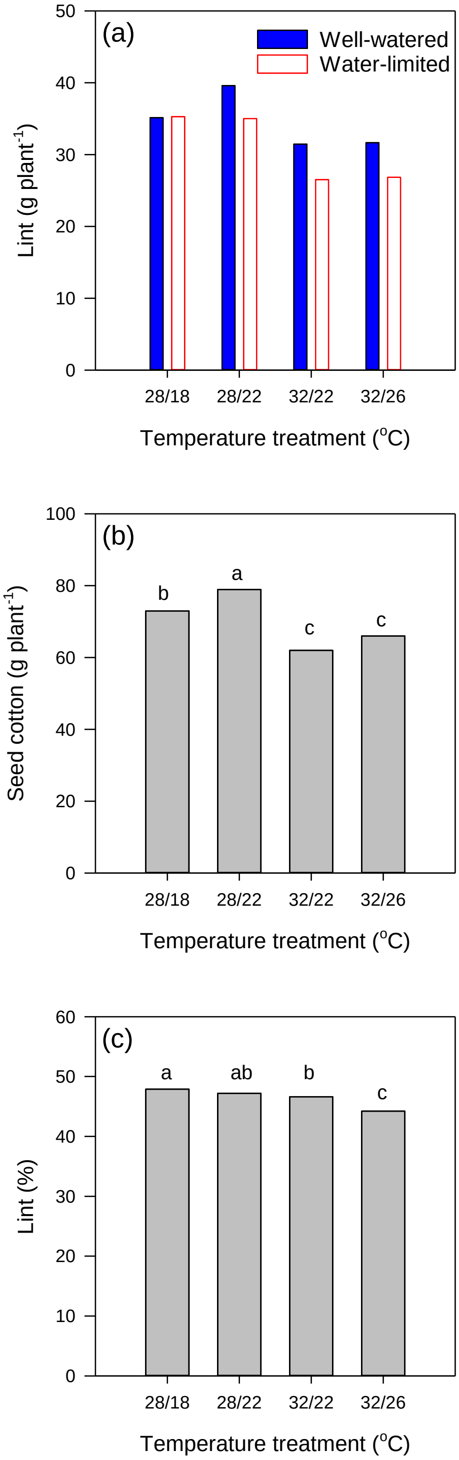

Figure 16: Effect of day/night temperatures on (a) lint mass, (b) seed cotton mass, and (c) lint percentage. Different letters represent significant difference at $P<0.05$.

Effect of water treatment on cotton yield

Averaged across all treatments, cotton grown with limited water had a 13% lower seed cotton mass than well-watered plants. Similarly, cotton grown under water-limited conditions generally had lower lint mass than well-watered cotton in all temperature treatments, with exception to cotton grown at 28/18°C where there was no significant difference in lint mass between well-watered and water-limited cotton (Figure 16; Table 22). However, there was no difference in lint percentage between well-watered and water-limited cotton.

Effect of heatwave treatment on cotton yield
Averaged across all treatments, plants exposed to the heatwave treatment had a 13% reduction in seed cotton mass, a 14% reduction in lint mass and a 2% reduction in lint percentage compared with control plants (Figure 17; Table 22).

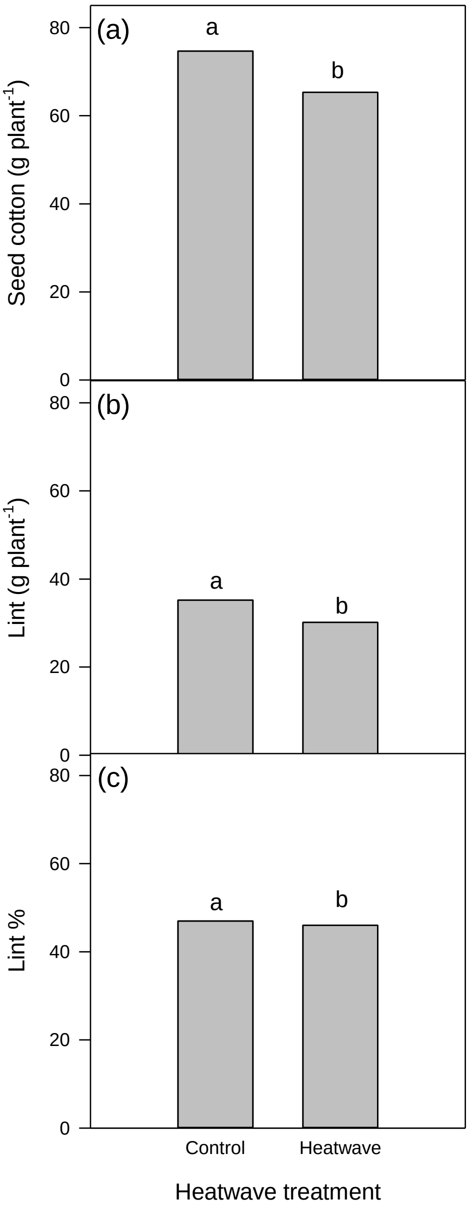

Figure 17: Effects of the heatwave treatment on (a) seed cotton mass, (b) lint mass, and (c) lint percentage

Table 22: REML analysis on yield components of cotton grown in different day/night temperature, water and heatwave conditions. Values in bold represent significance at P< 0.05.

	Temperature	Water	Heatwave	TxW	TxHW	WxHW	TxWxHW
Seed cotton (g plant^{-1})	**0.001**	**0.001**	**0.001**	0.112	0.083	0.534	0.151
Lint (g plant^{-1})	**0.001**	**0.001**	**0.001**	**0.042**	0.380	0.552	0.217
Lint (%)	**0.001**	0.257	**0.007**	0.902	0.419	0.282	0.261

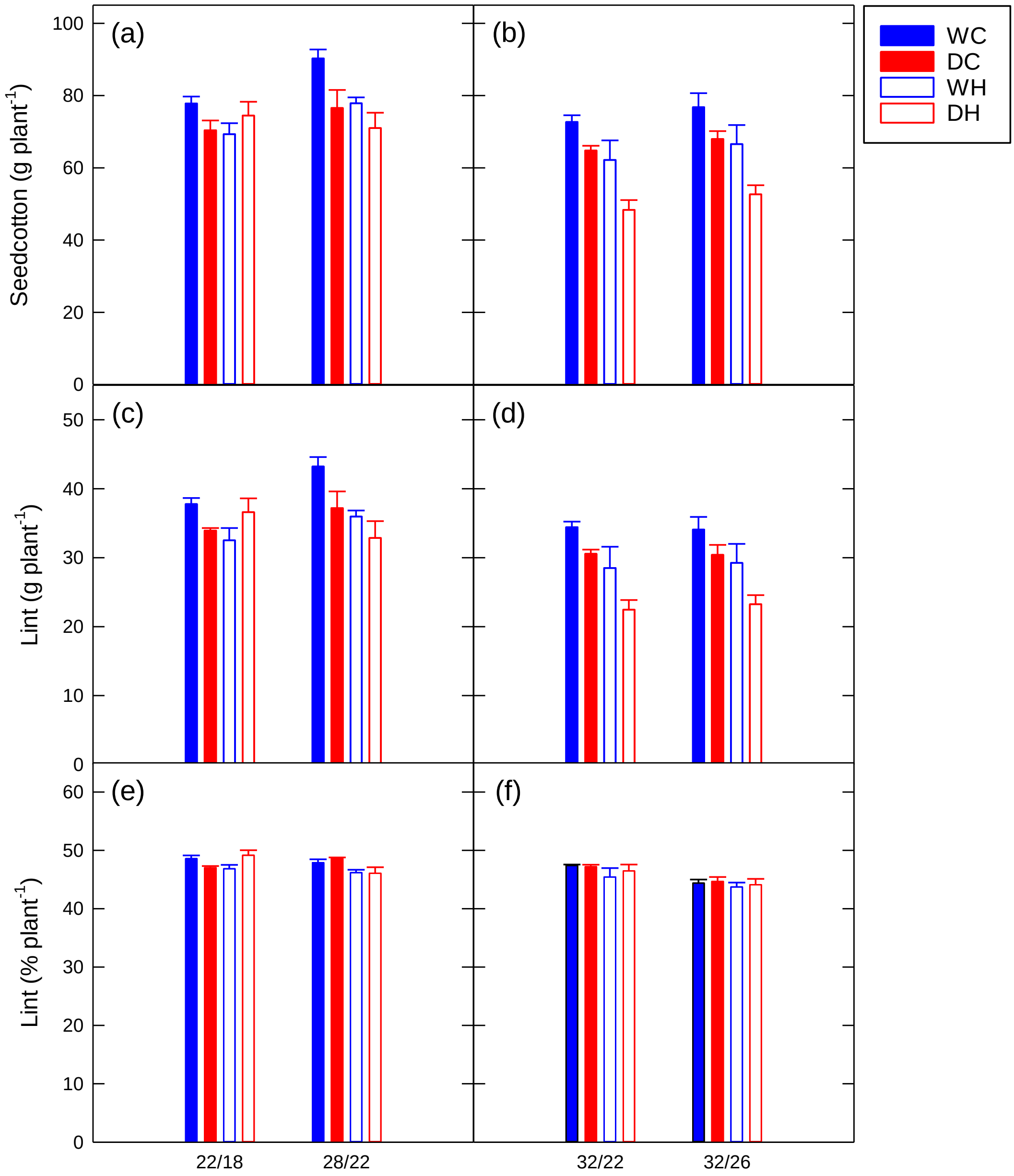

Figure 18: Mean seed cotton mass (a and b), lint mass (c and d) and lint percentage (e and f). WC is well-watered control, DC is water-limited control, WH is well-watered exposed to the heatwave event, and DH is water-limited exposed to the heatwave event.

Table 23: Results of analysis of variance (ANOVA) showing the effect of growth temperature (28/18, 28/22, 32/22, 32/26 ºC), water treatment (50% vs 100% field capacity), heatwave event and their interactions on photosynthesis (A_{sat}), stomatal conductance (g_s) and dark respiration (R_{dark}). Values are probability with significant at P<0.05.

	Main effects			Interactions			
Parameters	Temperature	Water	Heatwave	TxW	TxHW	WxHW	TxWxHW
Baseline							
A_{sat}	**0.001**	--	--	--	--	--	--
g_s	0.426	--	--	--	--	--	--
R_{dark}	**0.001**	--	--	--	--	--	--
Water limitation							

A_{sat}	**0.001**	0.416	--	**0.001**	--	--	--
g_s	**0.001**	0.088	--	0.337	--	--	--
R_{dark}	**0.001**	0.323	--	0.207	--	--	--
Heatwave							
A_{sat}	**0.001**	0.500	**0.001**	**0.043**	**0.001**	**0.006**	0.090
g_s	**0.001**	**0.039**	**0.001**	0.925	**0.001**	0.836	0.171
R_{dark}	**0.001**	0.149	0.332	0.428	**0.001**	0.552	0.395
Recovery							
A_{sat}	**0.001**	0.906	0.305	**0.005**	**0.001**	0.344	0.234
g_s	**0.001**	0.320	0.412	0.714	0.329	0.712	0.762
R_{dark}	**0.001**	**0.007**	**0.016**	0.694	**0.038**	0.637	0.305

Leaf gas exchange

Well-watered period (Baseline): Compared to the cooler daytime temperature treatments, warmer daytime temperatures increased photosynthetic rates (A_{sat}). Night warming increased A_{sat} in the 32/26^{o}C treatment compared with the 32/22^{o}C, however, warmer night temperatures did not increase A_{sat} of plants grown at cooler daytime temperatures (i.e. 28/18 and 28/22^{o}C). Plants grown at 32/26^{o}C had the greatest A_{sat} and the lowest dark respiration rates (R_{dark}) compared with plants grown in all other temperature treatments during the well-watered period. There was no difference in stomatal conductance (g_s) across all temperature treatments during this phase of measurement (Table 23).

During water-deficit and heatwave events: Averaged across all treatments, A_{sat} of plants exposed to the heatwave was reduced by 11.3%. Within each temperature treatment, A_{sat} of plants grown under stress, for instance, drought (DC) or heatwave (WH), or both drought and heatwave (DH), were similar with A_{sat} of non-stressed plants at temperature 28/18, 28/22 and 32/22^{o}C (Figure 19). In 32/26^{o}C treatment, heatwave reduced A_{sat} by 27.4% and 47.1% in well-watered and water-limited plants, respectively. Furthermore, water deficit significantly decreased A_{sat} by 25% in heatwave plants grown at 32/26^{o}C (Figure 19). Water-limited and heatwave plants grown at 32/26^{o}C had the lowest A_{sat} (14.8 µmol m^{-2} s^{-}1).

The heatwave significantly increased g_s of plants grown in cooler day temperatures by 49.5% and 67% at 28/18 and 28/22^{o}C, respectively. g_s of heatwave plants grown at 32/26^{o}C was 61.47% lower than the control plants (Figure 19). Similar to A_{sat}, water deficit decreased g_s of heatwave plants at 32/26^{o}C (Figure 19).Water-limited, heatwave plants grown at 32/26^{o}C had the lowest g_s across all treatments (0.19 µmol m^{-1} s^{-1}).

The heatwave decreased R_{dark} in plants grown at 28/22^{o}C by 31.1% and 22.8% in well-watered and water-limited plants respectively, while in 32/22^{o}C treatment, heatwave increased R_{dark} in both well-watered and water-limited plants (by 41.2% and 20.2% respectively; Figure 19).

Recovery: On average, there was no continuing heatwave effects on A_{sat}, g_s or R_{dark} during recovery. Cotton grown at warmer day temperatures had higher A_{sat} (18.63%), g_s (46.79%) and R_{dark} (28.4%) than plants grown at cooler day temperatures (Figure 20). Drought effects on R_{dark} were observed in plants grown at 28/18^{o}C (Figure 20).

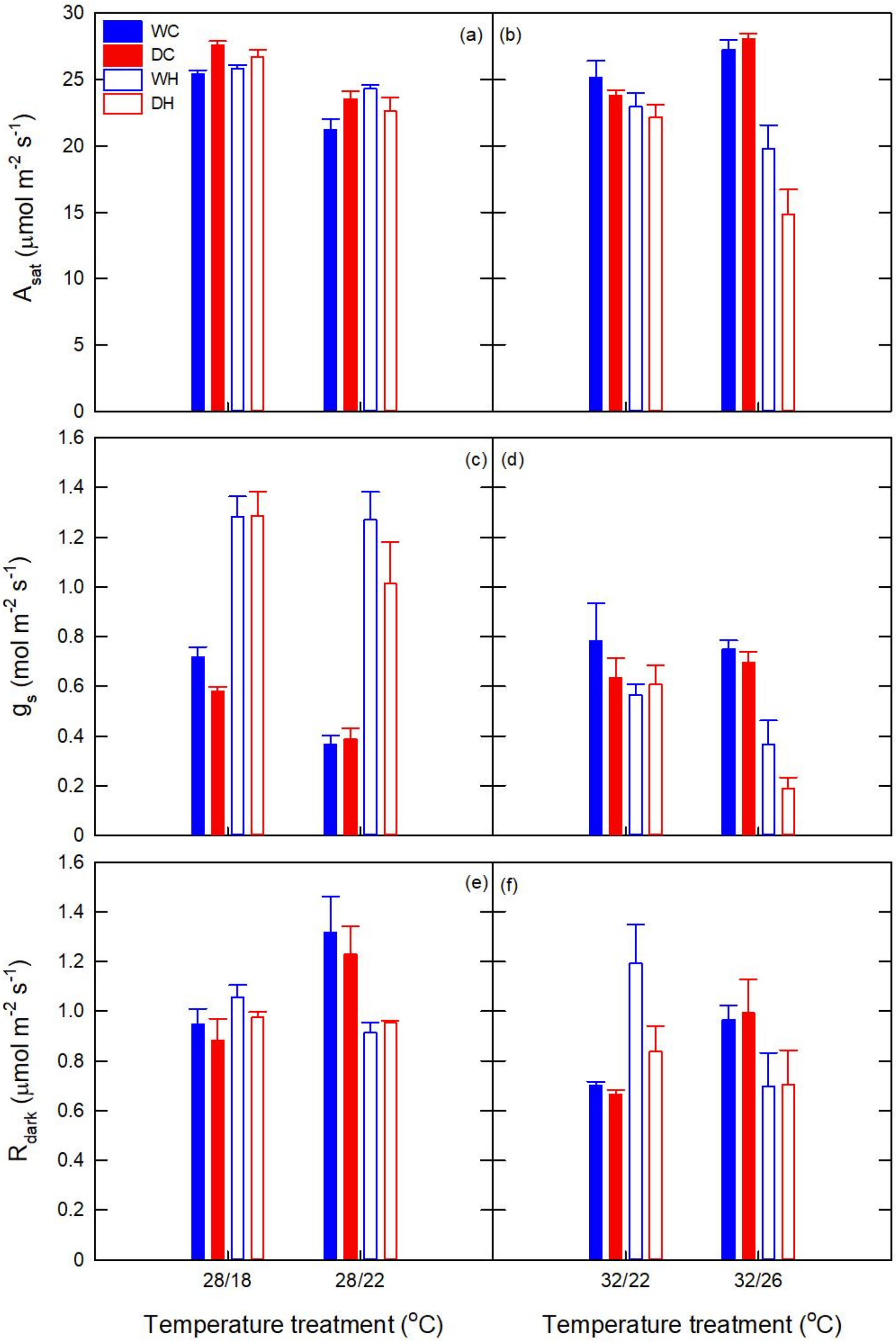

Figure 19: Effect of growth temperature, water limitation and heatwave treatment on (a,b) Photosynthesis at saturating light (A_{sat}), (c,d) stomatal conductance (g_s) and (e,f) dark respiration (R_{dark}) during drought and well-watered conditions. Blue, solid bars are well-watered, control plants (WC); red, solid bars are water-limited, control plants (DC); blue, open bars are well-watered, heatwave plants (WH); red, open bars are water-limited heatwave plants (DH). Values represent means ± SE (n=5).

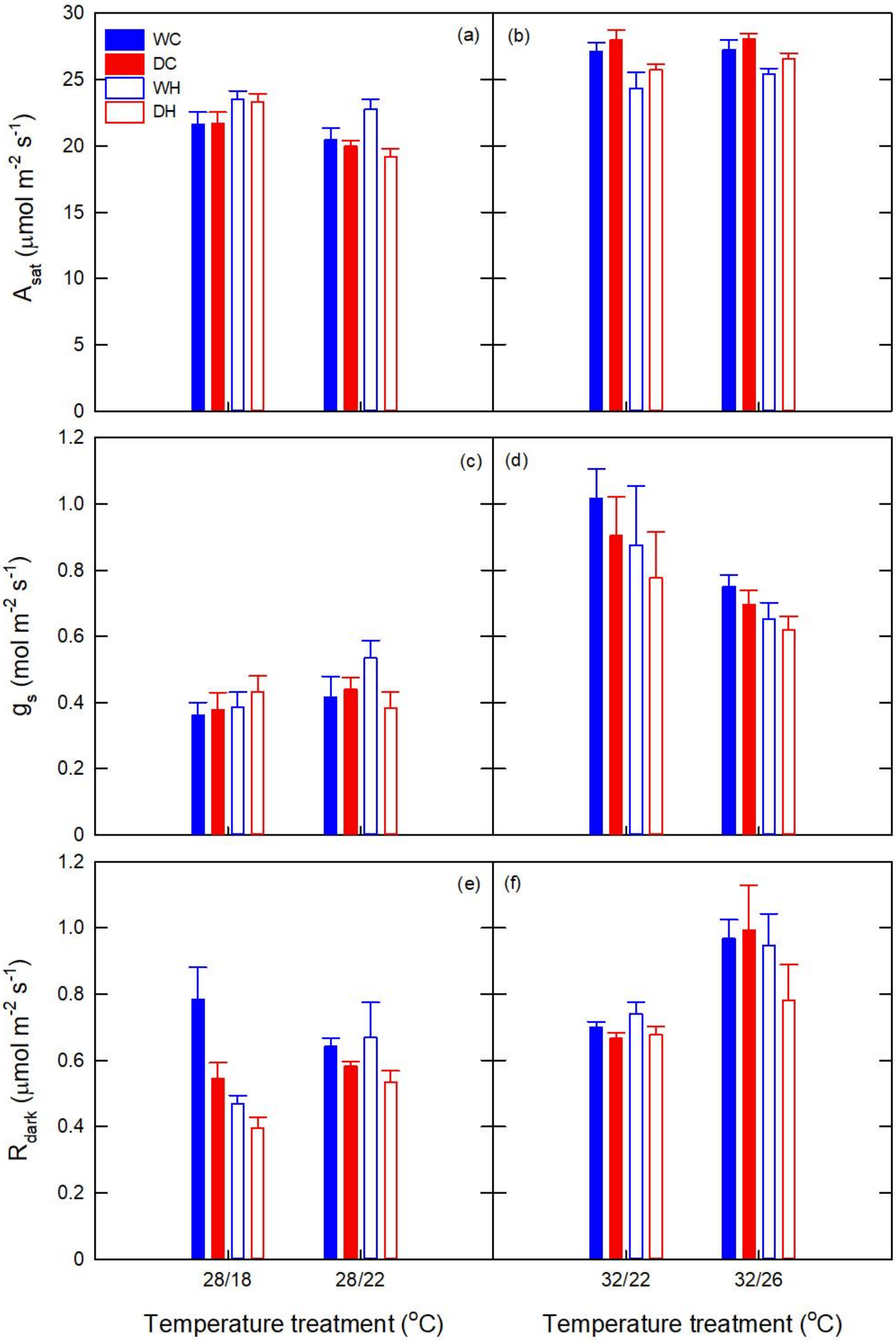

Figure 20: Effect of growth temperature, water limitation and heatwave treatment on (a,b) Photosynthesis at saturating light (A_{sat}), (c,d) stomatal conductance (g_s) and (e,f) dark respiration (R_{dark}) during recovery. Blue, solid bars are well-watered, control plants (WC); red, solid bars are water-limited, control plants (DC); blue, open bars are well-watered, heatwave plants (WH); red, open bars are water-limited heatwave plants (DH). Values represent means ± SE (n=5).

Identify management practices that mitigate the effects of interactive effects of climate change (Milestone 4).

A glasshouse experiment was conducted at UWS, Richmond to identify potential management practices that mitigate the effects of interactive effects of climate change. The outcomes of this experiment are summarised in Table 24. Our data suggest that vegetative biomass of cotton may be reduced with mepiquat chloride under some environmental conditions, but may not be effective in controlling vegetative growth of plants grown at both high temperature (36/22°C) and elevated [CO_2] (640 ppm). Therefore, further studies are required to explore management strategies for cotton grown in high temperature, high CO_2 environments.

Table 24: Key outcomes of glasshouse experiments conducted to quantify the impacts of more extreme climate change scenarios of higher average temperatures and more severe weather limitations.

Key outcomes: Identify management practices that mitigate the effects of interactive effects of climate change	
Growth and biomass	Compared with control plants, aminoethoxyvinylglycine (AVG) consistently increased vegetative biomass whereas applications of mepiquat chloride had a more variable effect. Our data show that vegetative biomass of cotton may be reduced with either a single application (600 ml ha^{-1}) or double application (900 ml ha^{-1}) of mepiquat chloride under some environmental conditions. However, for cotton grown at both high temperatures and elevated CO_2, mepiquat chloride may not be effective in controlling vegetative biomass.

Plant growth, biomass and fruit production

Plant height was monitored throughout the experiment and is shown in Figure 21.

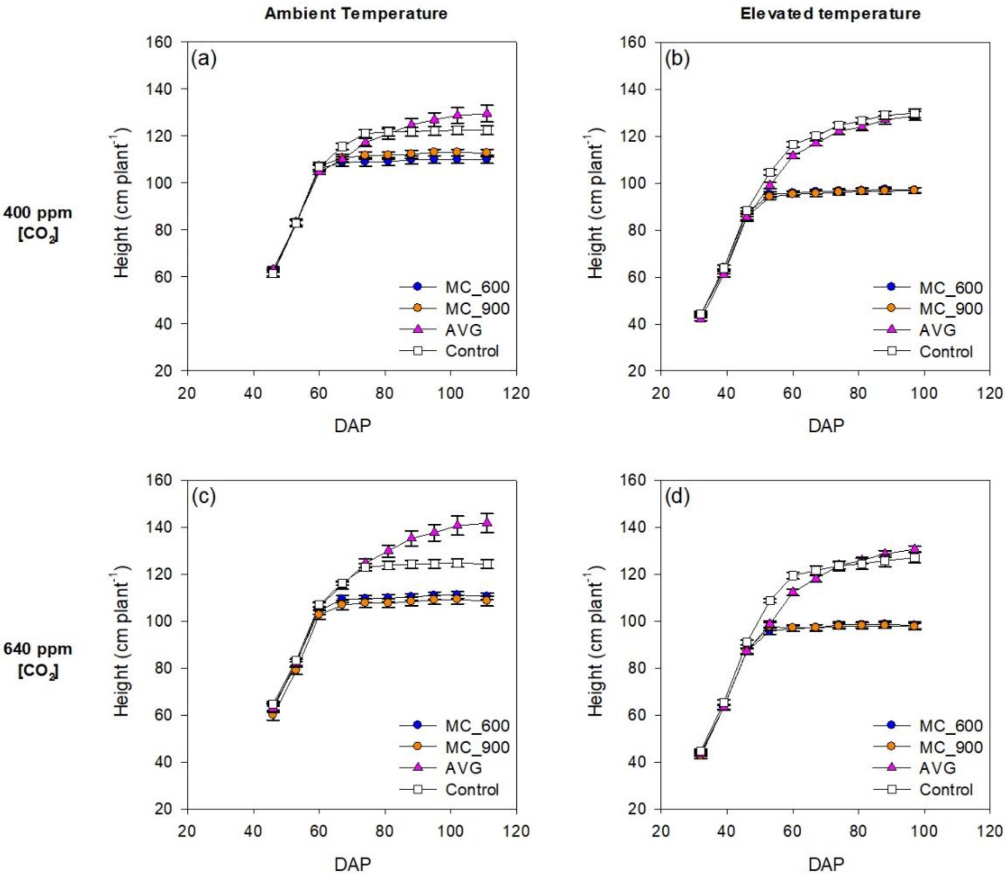

Figure 21: Plant height of cotton applied with a single mepiquat chloride treatment (MC_600, blue), two mepiquat chloride applications (MC_900, orange), aminoethyoxyvinylgline (AVG, pink) and control plants (white). Cotton was grown at (a) ambient temperature and elevated [CO_2], (b) elevated temperature and ambient [CO_2], (c) ambient temperature and elevated [CO_2], and (d) elevated temperature and elevated [CO_2]. Values represent the mean ± SE of 8 plants.

In each temperature and CO_2 treatment, cotton applied with AVG consistently had greater vegetative biomass than the control plants (Figure 22, Table 25). The effects of the MC applications for each temperature and CO_2 treatment were more variable. Compared with control plants, a single application of MC (MC_600) reduced vegetative biomass only in the ambient temperature (C_AT_A and C_ET_A) treatments, with no significant difference at elevated temperature. Compared with control plants, two applications of MC (MC_900) reduced vegetative biomass of cotton in the C_ET_A and C_AT_E treatments.

Table 25: REML analysis of leaf area and plant biomass harvested at the end of the experiment on the 23rd May 2018 (111 DAP for T_A and 97 DAP for T_E). Values in bold represent F probabilities significant at $P \leq 0.05$.

Parameter	**Temp**	**CO_2**	**Chemical**	**Temp x CO_2**	**Temp x Chem**	**CO_2 x Chem**	**Temp x CO_2 x Chem**
Leaf area (cm^2 $plant^{-1}$)	**0.001**	**0.001**	**0.001**	**0.001**	0.178	**0.001**	0.250
Vegetative biomass (g $plant^{-1}$)	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.050**	**0.001**
Fruit biomass (g $plant^{-1}$)	**0.001**	0.091	**0.001**	**0.001**	**0.001**	0.197	0.650
Total biomass (g $plant^{-1}$)	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.103	**0.001**

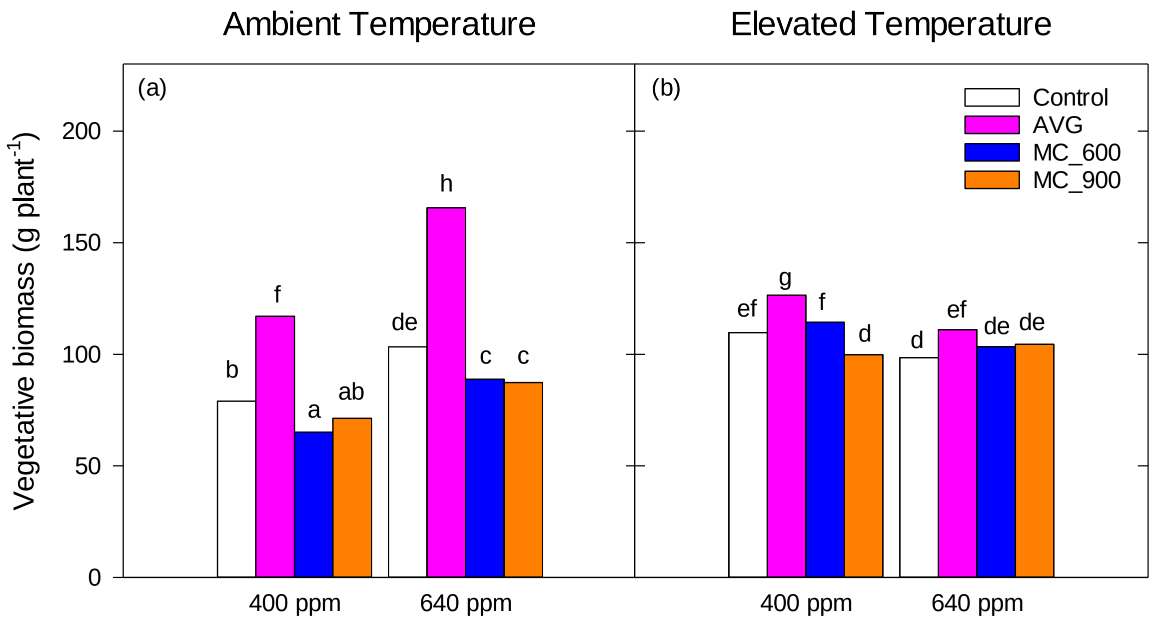

Figure 22: Vegetative biomass (g $plant^{-1}$) of cotton grown at (a) ambient temperature and (b) elevated temperature at both ambient (400 ppm) and elevated (640 ppm) [CO_2]. Chemical treatments included aminoethyoxyvinylgline (AVG, pink), a single mepiquat chloride treatment (MC_600, blue), two mepiquat chloride applications (MC_900, orange), and a control (white). Letters above the bars represent significant differences at P<0.05, with no differences between bars with the same letter.

For fruit biomass there was a significant temperature x CO_2 interaction (P=0.001, Table 25). For cotton grown at each CO_2 treatment, warmer temperatures reduced fruit biomass, however there was a greater negative effect of high temperature in an elevated CO_2 environment. Cotton grown at C_AT_E had 58% less fruit biomass than cotton grown at C_AT_A, whereas cotton grown at C_ET_E had 71% less fruit biomass than cotton grown at C_ET_A. The effect of elevated CO_2 on fruit biomass was variable depending on temperature treatments. Averaged across all chemical treatments, plants grown in the C_ET_A treatment had 16% greater fruit biomass cotton grown in the C_AT_A treatment, but cotton grown at C_ET_E had a 19% reduction in fruit biomass compared with plants grown at C_AT_E.

For fruit biomass, there was also a significant temperature x chemical interaction (P=0.001, Table 25). Cotton grown with each chemical treatment and elevated temperature had reduced fruit biomass, when compared with the ambient temperature treatment.

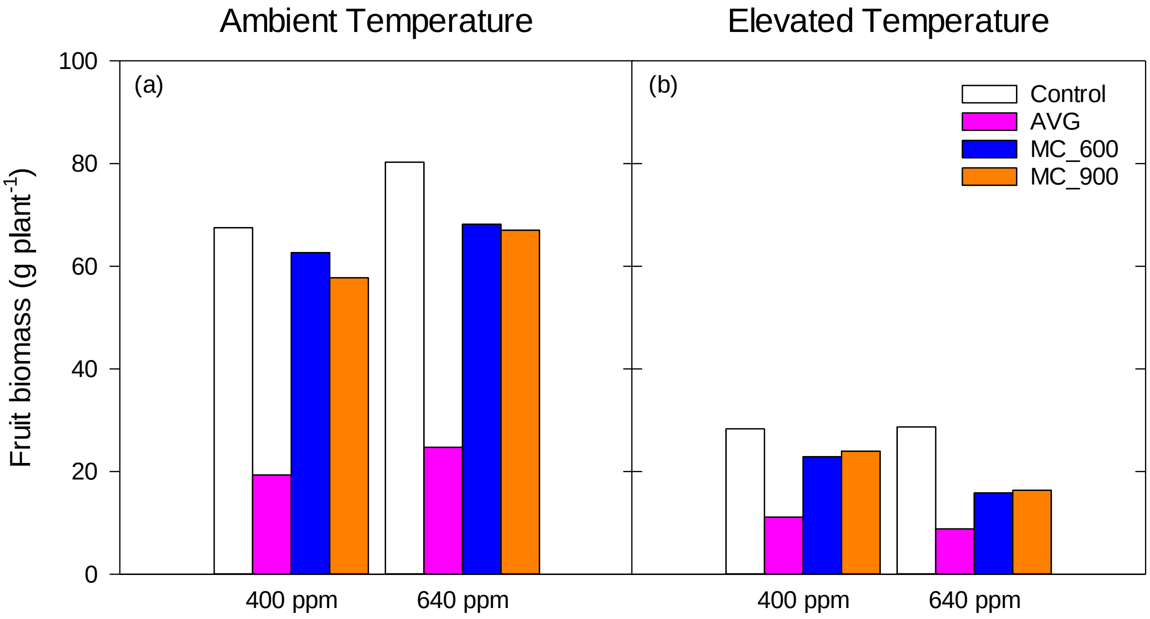

Figure 23: Fruit biomass (g plant^{-1}) of cotton grown at (a) ambient temperature and (b) elevated temperature at both ambient (400 ppm) and elevated (640 ppm) [CO_2]. Chemical treatments included aminoethyoxyvinylgline (AVG, pink), a single mepiquat chloride treatment (MC_600, blue), two mepiquat chloride applications (MC_900, orange), and a control (white). Values represent treatment means of approx. 8 plants.

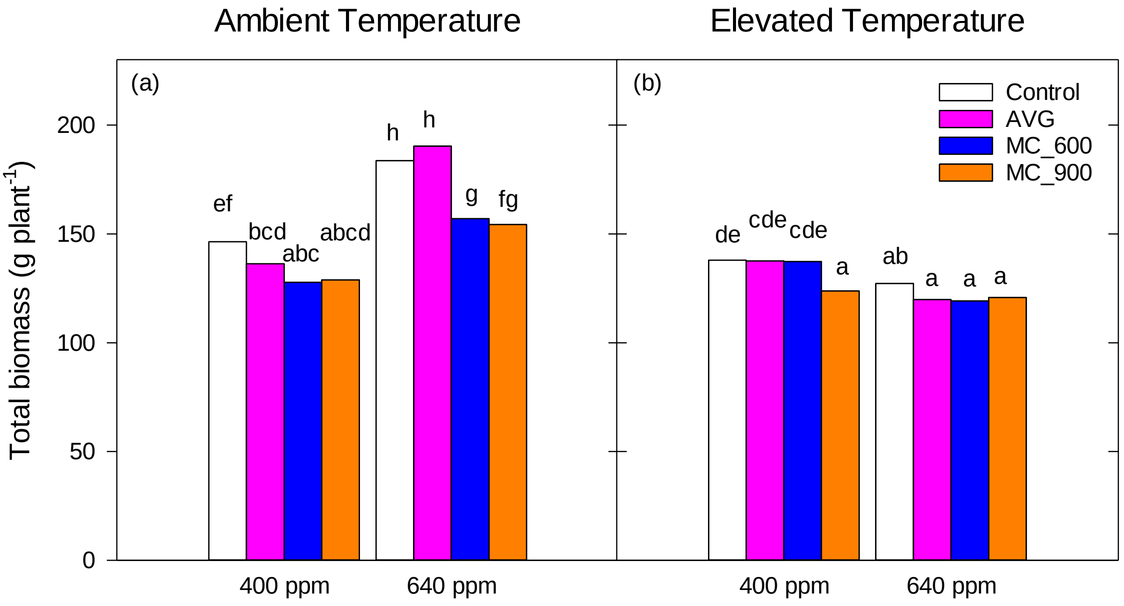

Figure 24: Total biomass (g plant^{-1}) of cotton grown at (a) ambient temperature and (b) elevated temperature at both ambient (400 ppm) and elevated (640 ppm) [CO_2]. Chemical treatments included aminoethyoxyvinylgline (AVG, pink), a single mepiquat chloride treatment (MC_600, blue), two mepiquat chloride applications (MC_900, orange), and a control (white). Letters above the bars represent significant differences at $P<0.05$, with no differences between bars with the same letter.

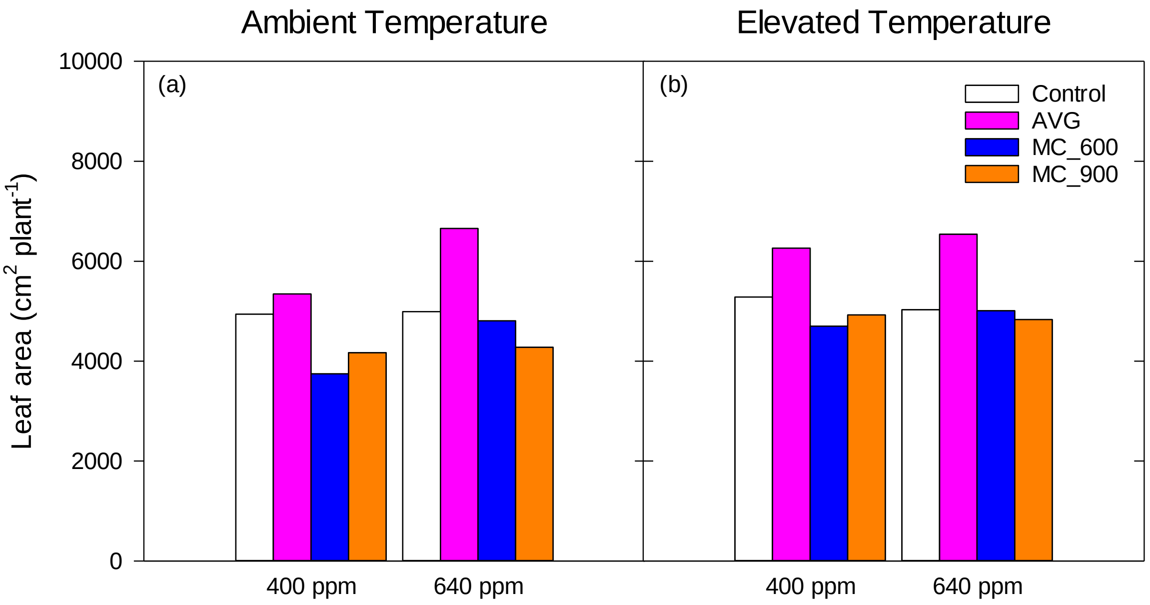

Figure 25: Leaf area (cm^2 plant^{-1}) of cotton grown at (a) ambient temperature and (b) elevated temperature at both ambient (400 ppm) and elevated (640 ppm) [CO_2]. Chemical treatments included aminoethyoxyvinylgline (AVG, pink), a single mepiquat chloride treatment (MC_600, blue), two mepiquat chloride applications (MC_900, orange), and a control (white). Values represent treatment means of approx. 8 plants.

Leaf gas exchange

There were significant temperature x CO_2 interactions (Table 26) for V_{cmax} and J_{max}/V_{cmax} whereby at ambient temperature, elevated CO_2 decreased V_{cmax} and increased J_{max}/V_{cmax}, however, atmospheric [CO_2] did not affect either V_{cmax} or J_{max}/V_{cmax} of plants grown at warmer temperatures (Figure 26). At each CO_2 treatment, cotton grown at elevated temperature consistently had lower V_{cmax} and higher J_{max}/V_{cmax} than cotton grown in the corresponding ambient temperature treatment.

Table 26: REML analysis of leaf level physiology. Values in bold represent F probabilities significant at P ≤ 0.05.

Parameter	**Temp**	**CO_2**	**Chemical**	**Temp x CO_2**	**Temp x Chem**	**CO_2 x Chem**	**Temp x CO_2 x Chem**
Vcmax	**0.001**	0.057	**0.001**	**0.003**	0.298	0.444	0.444
Jmax	**0.009**	**0.044**	**0.001**	0.728	**0.004**	0.892	0.661
Jmax/Vcmax	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.579	0.080

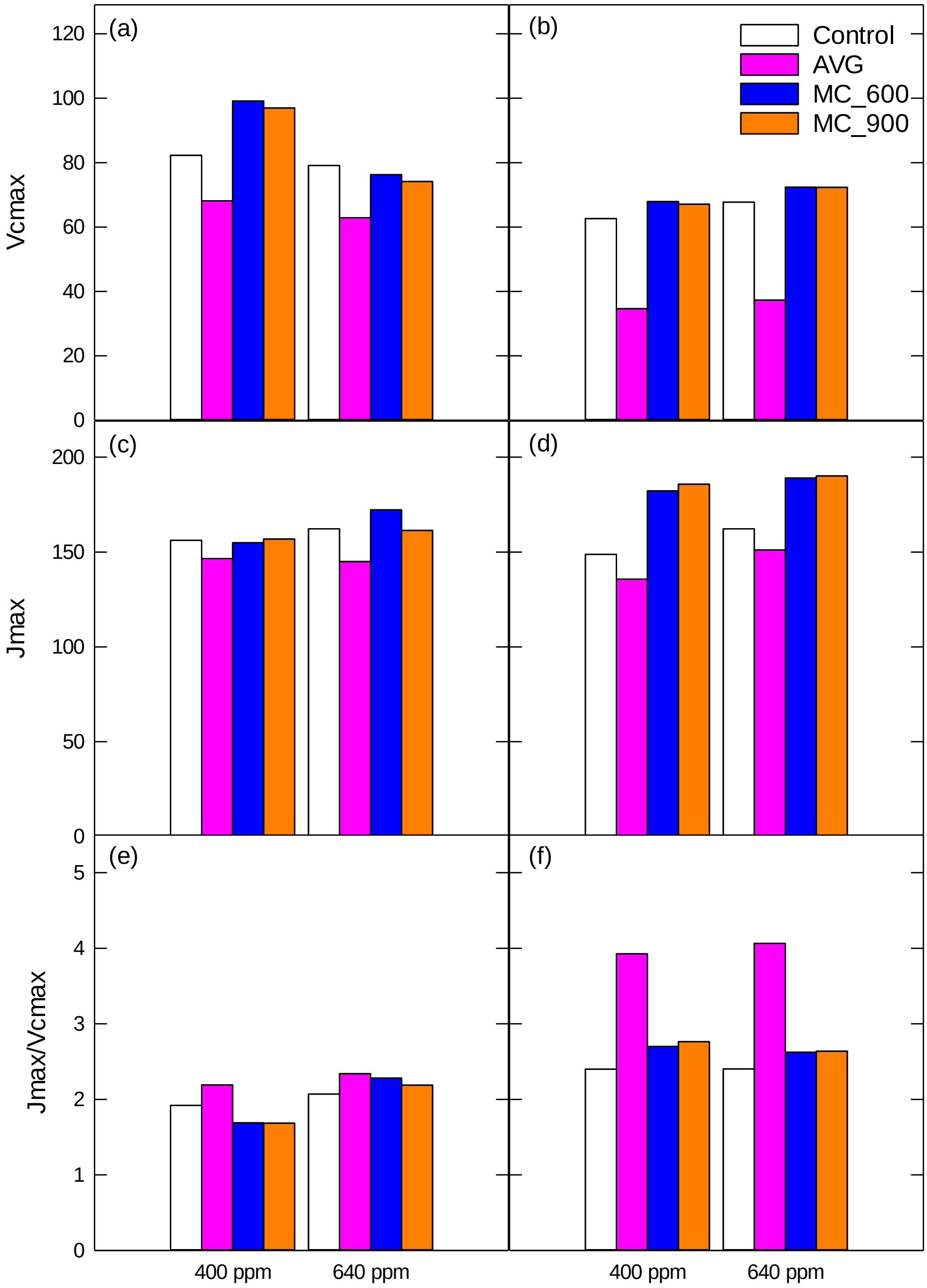

Figure 26: V_{cmax} (a and b), J_{max} (c and d) and J_{max}/V_{cmax} (e and f) of cotton grown at ambient temperature (a, c and e) and elevated temperature (b, d and f) and at both ambient (400 ppm) and elevated (640 ppm) [CO_2]. Chemical treatments included aminoethyoxyvinylgline (AVG, pink), a single mepiquat chloride treatment (MC_600, blue), two mepiquat chloride applications (MC_900, orange), and a control (white).

Improve the external parameters of the OZCOT crop simulation capacity to better capture interactive effects of climate change (Milestone 5)

The OZCOT model (version 2014) was used to explore how changing the CO_2 parameters alters yield and water use efficiency predictions of cotton grown at Narrabri, NSW. CO_2 levels were originally set at 330 ppm in the OZCOT model, but current atmospheric [CO_2] is closer to 400 ppm. The differences in the model output between the two atmospheric [CO_2] tested are shown in Figure 27. This objective was not completed during the course of this project cycle, however, a documented approach to model assessment and initial model sensitivity analysis will be provided as part of a progress report of the ongoing project: *Improving water use efficiency in a changing climate* (CSP1804).

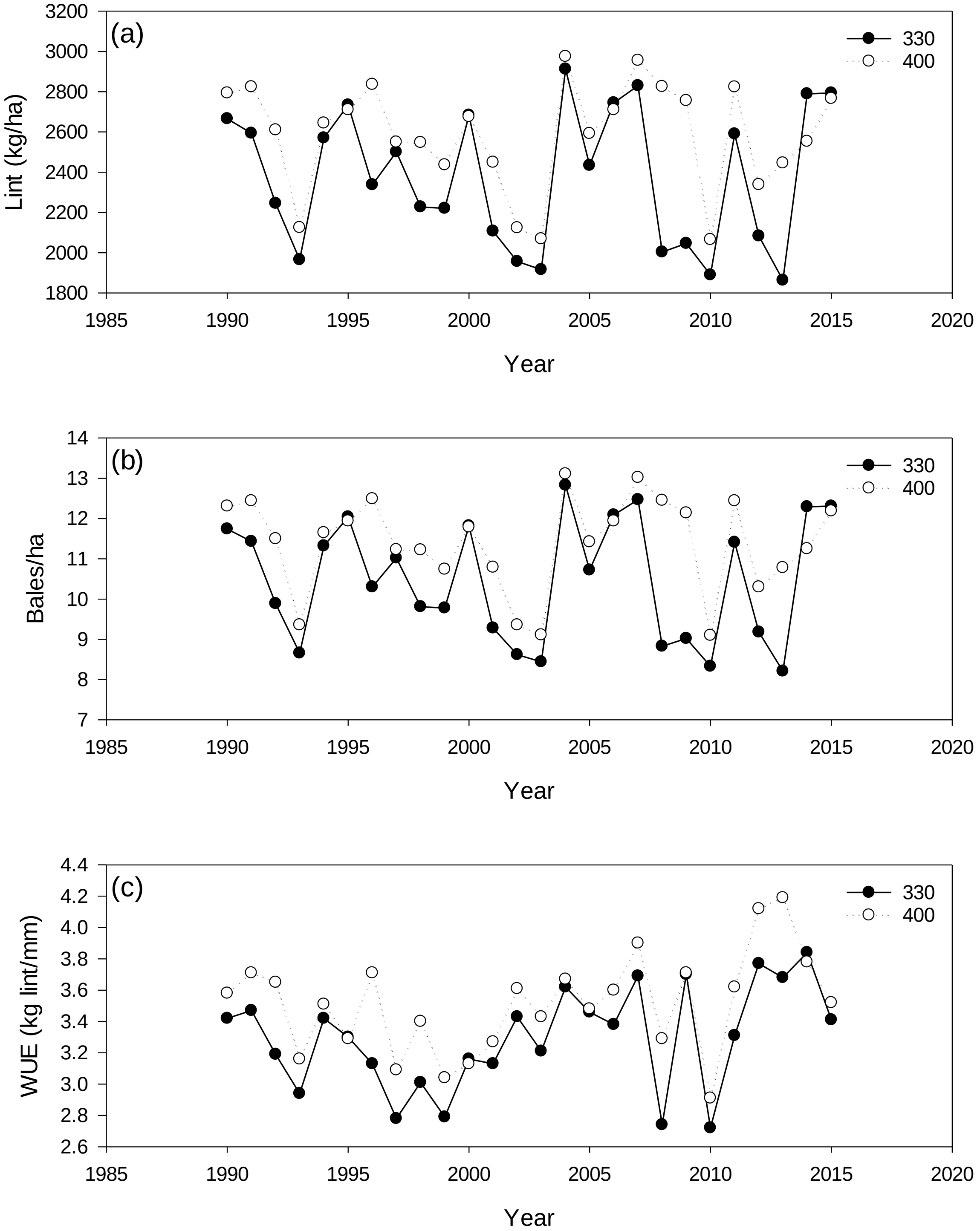

Figure 27: Comparison of (a) lint production, (b) bales of cotton per hectare, and (c) water use efficiency (WUE) of cotton grown at 330 and 400 ppm [CO_2], using the OZCOT model.

Outcomes

5. **Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.**

   It was identified in the project application that this project will generate new knowledge on the combined effects of elevated CO_2, warmer temperature, lower humidity (higher

VPD), variable soil water, their interactions on growth, yield, quality, and crop water use efficiency of Australian cotton, as well as early investigations into adaptation strategies. Field experiments using the National Facility for Climate Change Research have been successfully conducted over three consecutive cotton seasons in Narrabri, and a number of glasshouse experiments have been conducted at Western Sydney University in Richmond. This research has enabled the collection of valuable data that has provided information on physiology, growth and water use of cotton in projected climatic conditions. Thereby, this project has, and will continue to contribute to the ‘farmers’ program with the theme of profitable futures system by:

- Improving knowledge of cotton growth and agronomy by quantifying the effects of future climate conditions on crop productivity and capacity to recover from these events; and
- Improving management guidelines that enable growers to produce high-quality cotton lint, while simultaneously avoiding strategies that are mal-adaptive and reducing the environmental footprint under future climatic conditions.

6. **Please describe any:-**
   a) **technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);**
   b) **other information developed from research (eg discoveries in methodology, equipment design, etc.); and**
   c) **required changes to the Intellectual Property register.**

Not applicable.

Conclusion

7. **Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?**

The cotton production in a future climate project (CSP1501) has contributed to generating a better understanding of the response of cotton physiology, growth, water use, and cotton production systems to the multiple environmental variables that are projected to alter with climate change. This project has greatly increased our knowledge of climate effects on cotton growth and yield, which will assist producers to remain viable under changing climatic conditions. Field experiments have suggested that elevated atmospheric CO_2 does not mitigate the negative effects of higher temperatures on fruit retention. Our studies showed that cotton grown in warmer temperature and high [CO_2] environments may have increased vegetative biomass and reduced fruit retention than cotton grown in current temperature and CO_2 field conditions, despite non-significant differences in total aboveground biomass. This led to a large reduction in plant-level water use efficiency. Therefore, it is important to consider management strategies to reduce excess vegetative growth and improve resource use efficiencies for cotton grown in future climatic scenarios.

Extension Opportunities

8. **Detail a plan for the activities or other steps that may be taken:**
   (a) to further develop or to exploit the project technology.
   (b) for the future presentation and dissemination of the project outcomes.
   (c) for future research.

The chambers used in this study could continue to be used in field-based climate change research to develop a better understanding of the management strategies for cotton grown in projected climates. Given observed plant responses to elevated CO_2 and warmer temperature environments, we intend to use these chambers to investigate strategies to manage excessive vegetative growth, and improve plant water use efficiency of cotton in projected climates.

9. A. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

A manuscript, nominally titled "*The role of day and night warming on cotton response to soil water deficit and a heatwave event*" has been prepared on results from the temperature x drought x heatwave experiment conducted at UWS. It is anticipated that this manuscript will be ready for submission to a journal in 2018.

Furthermore, a review/summary paper of the climate change research that has been conducted within the Australian cotton industry is currently in preparation, nominally titled "*Where are we up to with climate change research in cotton? An overview of recent research into the effects of climate change on cotton systems in Australia*". It is anticipated that this review article will be completed in 2018.

During the course of this project we have presented our data at numerous conferences and seminars:

Conference presentations

Australian Cotton Research Conference, Toowoomba, September 2015

Australian Agronomy Conference, Hobart, September 2015

CSIRO Cotton Stream Seminar, ACRI, October 2015

Australian Cotton Conference, Gold Coast, August 2016

CSIRO Cotton Stream Seminar, ACRI, September 2016

Agriculture and Climate Change Conference, Spain, March 2017

Wageningen University and Research Centre Seminar, The Netherlands, March 2017

ANU Research School of Biology Lab Seminar, Canberra, April 2017

Australian Cotton Research Conference, Canberra, September 2017

CSIRO Cotton Stream Seminar, ACRI, September 2017

Australian Agronomy Conference, Ballarat, September 2017

Industry articles/presentations

CRDC Spotlight magazine: Autumn 2016 "Leading the world in cotton future climate studies" (http://www.crdc.com.au/publications/spotlight-magazine-autumn-2016)

In addition, I have also highlighted our climate change research at the Upper Namoi Farm Tour (15th December 2016) and spoken to numerous tour groups visiting the research station (e.g. Cotton Australia Textile Brands visit, 2nd May 2017).

B. Have you developed any online resources and what is the website address?

No.

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Rising atmospheric CO_2, higher air temperatures, lower humidity (high Vapour Pressure Deficit (VPD)) and reduced water availability as a consequence of climate change is likely to affect cotton production. However, there had previously been little research to assess the real world interaction of rising CO_2, temperature, VPD and reduced water availability.

To enhance the current understanding of cotton system adaptation to climate variability and plan for projected climate change, a combination of controlled environment glasshouse and field-based studies were conducted to assess the integrated effects of warmer temperatures and elevated atmospheric $[CO_2]$ on cotton growth, physiology and water use.

Glasshouse studies were conducted at Western Sydney University, Richmond to investigate the impact of more extreme climate change scenarios on cotton growth and physiology. These experiments suggest that warmer air temperatures may accelerate growth rates of cotton plants, but not necessarily equate to greater yields. Furthermore, cotton grown at warmer air temperatures may be more susceptible to water deficits and heatwave conditions.

Field based studies were conducted in Narrabri over three consecutive cotton seasons (2014/15 to 2016/17) utilising the National Facility for Climate Change Research. For each season, the experiment consisted of three treatments; warmer temperature and elevated CO_2, warmer temperature and ambient CO_2, and ambient temperature and ambient CO_2. These experiments showed that warmer temperatures may increase stomatal conductance and transpiration, resulting in reduced leaf-level water use efficiency. It was also found that increased photosynthesis and increased vegetative biomass under scenarios of projected future climatic conditions will not necessarily equate to higher yields. Results from the field experiments also indicate that elevated CO_2 does not mitigate the negative effects of higher temperatures on fruit retention, and furthermore, elevated CO_2 may potentially exacerbate the negative effects of warmer temperature in cotton, thus leading to large reductions in water and resource use efficiencies.

Further glasshouse studies were conducted to assess the effectiveness of using mepiquat chloride, applied according to current Australian cotton industry standards, to manage excessive vegetative growth. These experiments showed that vegetative biomass of cotton may be reduced with mepiquat chloride under some environmental conditions, but may not be effective in controlling vegetative growth of plants grown at both high temperature (36/22°C, day/night) and elevated $[CO_2]$ (640 ppm). Therefore, further studies are required to explore management strategies for cotton grown in high temperature, high CO_2 environments thereby enabling sustainable and efficient cotton production systems in the future.