FINAL REPORT

For Public Release

Part 1 - Summary Details

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Part 3 – Final Report

Background

1. Outline the background to the project.

Rising atmospheric CO_2, warmer air temperatures, higher Vapour Pressure Deficit (VPD) and reduced water availability as a consequence of climate change is likely to affect cotton production. Previous research from field-based climate change studies conducted in Narrabri indicated that there were no significant differences in total plant biomass between three environmental treatments (outside unchambered control, 400 ppm CO_2 and +3°C air temperature, and 550 ppm CO_2 and +3°C air temperature), but that cotton grown at warmer temperatures in the chambers produced greater vegetative biomass and less fruit biomass than cotton grown in control plots. Thus, cotton yield in future climatic scenarios may be significantly reduced compared with the current environment, resulting in large reductions in agronomic water use efficiency. Therefore, management strategies need to be explored to reduce excessive vegetative growth and improve resource use efficiencies for cotton grown in future climatic scenarios.

Furthermore, glasshouse and field research have shown that early-season growth benefits due to elevated atmospheric CO_2 may occur at ambient temperatures. However, elevated CO_2 may not mitigate negative effects, such as increased water use, on cotton growth and physiology in warmer temperatures expected in future environments. Similarly, the benefit of elevated CO_2 may be greatly reduced when cotton is grown in soil water deficit conditions. These studies have clearly indicated that the magnitude of cotton response to elevated CO_2 is largely dependent upon air temperature and water availability, which therefore must be included as environmental factors in experimental designs to accurately predict cotton response to future climates.

Our research has shown that cotton is strongly responsive to changes in leaf-to-air vapour pressure deficit (VPD), which accounts for a large proportion of environmental control on stomatal conductance, and hence photosynthetic rates. Similarly, soil water deficit strongly affects stomatal conductance and photosynthesis. However, very few studies have explicitly explored the independent and interactive effects of soil water deficit and atmospheric water deficit (VPD) on the physiological response of cotton in a changing climate, which is a crucial component of modelling cotton response to the environment.

Glasshouse experiments as part of CRDC project CSP 1501 investigated the effects of increased day and/or night temperatures, soil water deficit and heatwave events on the physiology and growth of cotton. Increased day temperatures reduced cotton growth and physiological responses, but these negative effects were greater when night temperature was also higher. Heatwaves further exacerbated the negative effects, indicating that warmer average day/night temperatures will be particularly damaging to cotton during expected heatwave events and soil water deficit. Few studies address the independent effect of night-time warming or the additive effect with day-time warming, but both are crucial components of the general rise in air temperature, which in turn affects cotton response to subsequent heat wave events.

Therefore, management strategies need to be explored to reduce excessive vegetative growth and improve resource use efficiencies for cotton grown in future climate scenarios. Adjusting management strategies may provide an opportunity to capitalise on the potential benefits of elevated CO_2 and warmer temperatures, while minimising the negative effects. This project aimed to contribute a better understanding of the role that plant growth regulators may play in managing crop growth conditions of warmer temperatures and elevated atmospheric [CO_2], and improved modelling capacity.

Objectives

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

Milestone Description	Extent to which these have been achieved

1. Research question: Assess the use of plant growth regulators to manage excessive vegetative growth of cotton grown in environments of elevated CO_2 and warmer temperatures in the field.	
1.1 *Investigate the use of growth regulators on mitigating the effects of climate change:* One season of field experiments completed in Narrabri where yield, quality and water use were measured.	Fully achieved. Field experiments were conducted over three consecutive cotton seasons (2017/18, 2018/19 and 2019/20) to investigate the effects of using mepiquat chloride to mitigate the effects of excessive vegetative growth of cotton grown in future climate scenarios. These experiments indicate that mepiquat chloride, applied according to current Australian recommendations, may suppress excessive vegetative growth of cotton grown in warmer temperature and elevated atmospheric CO_2.
1.2 *Identify optimal management strategies to improve resource use efficiency of cotton grown under future climate scenarios:* Two seasons of field experiments completed in Narrabri, where yield, quality and water use were measured.	Fully achieved. Field experiments were conducted over two consecutive cotton seasons (2018/19 and 2019/20) to compare growth responses of more determinate and more indeterminate cotton cultivar to future climate scenarios, with the purpose of identifying management strategies that may improve resource use efficiency under future climatic scenarios. Despite a short season in 2018/19 due to severe hail damage, yield and quality data were obtained in the 2019/20 season. These experiments indicate that there were no overall differences in yield, quality or water use between the two varieties tested. Cotton varieties may require careful management (such as the use of growth regulators) to ensure optimal growth in future climatic scenarios, but the selection between current cotton varieties may not have enough diversity to respond differently to future climatic scenarios.
2 Research question: Assess the use of plant growth regulators to manage excessive vegetative growth of cotton grown in elevated [CO_2] and warmer temperatures in the glasshouse.	
2.1 *Identify management practices that mitigate the effects of warmer temperatures and elevated atmospheric [CO_2] on cotton growth, physiology and water use:* Two glasshouse experiments completed at WSU where detailed measurements of cotton physiology were undertaken.	Fully achieved. Two experiments were conducted utilising one comprehensive glasshouse study at Western Sydney University to understand the relative effects of elevated CO_2 and warmer temperature on source/sink dynamics by manipulating sink strength and activity. This large study consisted of two sub-experiments to (1) assess the use of mepiquat chloride to mitigate the negative effects of fruit loss and associated excessive vegetative growth and (2) compare the growth and physiological responses to fruit loss of two cultivars with differing determinacy. These experiments show that (1) mepiquat chloride applications may be a successful method of controlling excessive vegetative growth in warmer temperature and elevated CO_2 scenarios; and (2) there were no significant differences in growth or physiology responses between the two cultivars tested.
3 Research question: Scaling leaf- to canopy-level responses	

3.1 *Improved understanding of scaling leaf- to canopy-level responses to determine water use efficiency dynamics in the field:* Two seasons of field experiments completed in Narrabri	Partially achieved. To improve understanding of scaling leaf- to canopy-level responses in warmer temperature and elevated CO_2 conditions, field experiments were conducted during the 2016/17 and the 2019/20 cotton seasons. These experiments tested the hypotheses that (1) canopy CO_2 and H_2O flux can be used to calculate canopy photosynthetic rates; (2) CO_2 drawdown is consistent across all four field-based chambers (The National Climate Change Facility for Cotton Research); and (3) CO_2 drawdown changes throughout the day as rates of gas exchange change. Our ability to complete this milestone was affected by the severe damage to the field chambers in the 2018/19 cotton season, which resulted in us having to rebuild the chambers and start again.
3.2 *Improved understanding of carbon and water fluxes of cotton grown under soil water deficits and high atmospheric vapour pressure deficit (VPD) in the glasshouse:* Two glasshouse experiments completed at WSU where detailed measurements of cotton physiology were measured.	Fully achieved. For efficiency, two experiments were combined into one. A comprehensive glasshouse experiment was conducted to investigate the interactive effects of atmospheric VPD and soil water deficit on the physiology of cotton. The objective of this experiment was to determine the relative impact of soil water deficit (50% field capacity) and atmospheric vapour pressure deficit on the physiology of cotton. Increasing VPD stimulated stomatal closure all temperatures measured. Soil water deficit reduced stomatal conductance of cotton at all temperatures measured, particularly at lower VPD compared with well-watered plants at the same VPD.
4. Research question: Ability to better model and predict the impact of future climate scenarios on water use and yield	
4.1 *Improve the external parameters of models to better capture interactive effects of climate change on water use and yield using potentially two different cotton simulation models:* Undertake a simulation study that uses models to assess the ability to capture the effects measured in field studies investigating the interactive effects of climate change.	Fully achieved. To address this milestone, we have used a multi-faceted approach. We used Cottassist to explore the climatic changes that have already occurred at key locations throughout the Australian cotton industry. Our findings may help define adaptation strategies by linking regions with similar past and present climatic conditions. We also adjusted the external parameters of the OZCOT model to better understand how the model responds to changing atmospheric CO_2 concentration. Our study shows that increased CO_2 predicted increased lint yield, as demonstrated in field experiments. However, further research is required to link simulations and field data.

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
AEDT: Australian Eastern Daylight Time
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®
DAFR: days after fruit removal
DAP: days after planting
DOY: day of year
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
WSU: 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 2017/18, 2018/19 and 2019/20 cotton seasons. For the 2017/18 and the 2019/20 cotton seasons, the experiment consisted of (a) warmer temperature and ambient CO_2, and (b) ambient temperature and ambient CO_2. For the 2018/19 season, the experiment 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 objectives of these experiments were to (1) assess the use of growth regulators on mitigating the effects of climate change; (2) identify optimal management strategies to improve resource use efficiency of cotton grown under future climatic scenarios; and (3) improved understanding of scaling leaf- to canopy- level responses to determine water use efficiency dynamics in the field.

Field-season measurements

Cotton seeds were planted into the field using standard farming practices. Chambers were moved into the field after the cotton had emerged. The injection of CO_2 into the chambers commenced between 22 and 63 DAP for two seasons (Table 1). The commercially available cultivar, Sicot 74B3F was grown each of the three seasons. In addition, a CSIRO breeding line representing a more determinate variety, CSX 3301, was grown in the 2018/19 and 2019/20 seasons. The 2018/19 season experiment was a short season experiment (harvested at 85 DAP), and both the 2017/18 and 2019/20 season experiments were conducted over the full cotton season (harvested at 177 DAP and 182 DAP, respectively).

Table 1: Details of field experiments conducted using the National Facility for Cotton Climate Change Research, ACRI Narrabri.

Cotton Season	Date Sown	Cultivars	CO_2 injection in chambers	Date Harvested
17/18	14/11/17	Sicot 746B3F	N/A	10/5/18 (177 DAP)

18/19	04/02/19	Sicot 746B3F CSX 3301	26/02/19 (22 DAP)	30/04/19 (85 DAP)
19/20	02/12/19	Sicot 746B3F CSX 3301	03/02/20 (63 DAP)	01/06/20 (182 DAP)

Plants were monitored weekly for growth and development (i.e. heights and nodes). Plant physiology was measured using a Licor 6400 XT or the newer model Licor 6800 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 green light red light (GLRL) capacitance sensors (Odyssey Dataflow Systems, Christchurch, NZ). Biomass samples were collected at final harvest.

Mepiquat chloride (MC) application

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. Details of mepiquat chloride applications for the three cotton seasons from 2017 until 2020 are detailed in
Table 2.

Table 2: Mepiquat chloride applications for field experiments at ACRI, Narrabri.

Cotton Season	**MC Application Date (DAP)**	**Rate**	**Application Notes**
17/18	11/01/18 (58 DAP)	300 ml ha^{-1}	Applied inside chambers only
	18/02/18 (65 DAP)	300 ml ha^{-1}	Applied inside chambers only
	29/01/18 (76 DAP)	300 ml ha^{-1}	Applied inside chambers only
18/19	12/04/19 (67 DAP)	3 ml L^{-1}	Applied only to Sicot 746B3F
19/20	21/01/20 (50 DAP)	7 ml L^{-1}	Applied inside chambers only
	30/01/20 (59 DAP)	7 ml L^{-1}	Applied inside chambers only
	04/03/20 (93 DAP)	1.5 L ha^{-1}	Cut-out rate applied to all plots

Rebuilding the chambers following storm damage

The climate change chambers sustained severe storm damage on the 20th December 2018 (Figure 1). This occurred 49 DAP of the 2018/19 field experiment. Following the storm damage, the chambers had to be repaired. Cotton was re-sown in February 2019, and a short season field experiment was conducted.

Figure 1: Storm damage to the experiment and chamber facilities during the 2018/19 season (photo taken 2/1/2019).

Drawdown experiment to improve understanding of scaling leaf- to canopy-level responses to determine water use efficiency dynamics in the field

To improve understanding of scaling leaf- to canopy-level responses in warmer temperature, high CO_2 conditions, data were collected during the 2016-17 and the 2019/20 cotton seasons. Field chambers were used to modify the environment of cotton grown in the field at ACRI, Narrabri. This study explored the concept of using leaf-level physiology data collected by the Licor 6400, canopy CO_2 and H_2O flux collected by the Licor 840-A and growth measurements. This experiment tested the hypotheses that (1) canopy CO_2 and H_2O flux can be used to calculate canopy photosynthetic rates for the purpose of crop-level simulation models; (2) CO_2 drawdown is consistent across all four chambers; and (3) CO_2 drawdown changes throughout the day as rates of gas exchange change.

The closed-system CO_2 drawdown experiments were conducted on the 14th February 2017 (110 DAP), the 17th March 2020 (106 DAP), and the 19th March 2020 (108 DAP). This coincided with the beginning of boll opening of plants grown inside the chambers. Air conditioner units were run for 10 minutes to ensure that each chamber was cool prior to starting the drawdown period of the experiment. The air conditioner units and CO_2 supply were manually turned off for between five and ten minutes. After five to ten minutes of being shut down, air conditioners and CO_2 delivery were turned back on and run for a further ten minutes. Three cycles of CO_2 drawdown and temperature cooling were run for each of three measurement periods; approximately 9am, 12 and 3pm, AEDT.

CO_2 and H_2O concentrations inside each of the chambers were sampled using the Licor 840-A gas analyser and recorded by LabVIEW software. Air temperatures inside the chambers were also recorded by LabVIEW. Linear regressions were fit to the CO_2 and temperature data to determine the rate of change in CO_2 concentration, and rate of change in temperature per second.

Leaf level physiology measurements were measured using a Licor 6400XT and Licor 6800 (2019/20 season). Leaf-level gas exchange was measured on two consecutive days during the 2016/17 cotton season at 118 DAP (22/3/17) and 119 DAP (23/2/17). During the 2019/20 cotton season, leaf- level gas exchange was measured on 107 DAP (18/3/20), 109 DAP (20/2/20), and 112 DAP (23/3/20).

Plant growth and development (including height and the number of nodes) was monitored regularly throughout the season. Light interception (%) was also measured throughout the season using solarimeters. At the beginning of the season solarimeters were placed on the diagonal across the row both above the canopy and below the canopy. The solarimeters remained in place throughout the season, and data were recorded using LabVIEW. Light interception was calculated from solar noon measurements (between 12pm – 1pm, AEDT).
Plant biomass (half a metre from each plot) was harvested on the 7th February 2017 (103 DAP) and the 24th March 2020 (113 DAP), mapped, and partitioned into stems, leaves and fruit. Leaf area was measured using a leaf area meter (Licor 3100-C). Biomass was dried at 65°C for a minimum of 7 days then weighed.

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

1. *<u>Experiment: SWC x VPD-</u> Improved understanding of carbon and water fluxes of cotton grown under soil water deficits and high atmospheric vapour pressure deficit (VPD) in the glasshouse*

An experiment was conducted to investigate the interactive effects of atmospheric VPD and soil water deficit on the physiology of cotton (Figure 2). This experiment was conducted in the controlled environment glasshouse at Western Sydney University from October 2018 to December 2018. The objective of this experiment was to determine the relative impact of soil water deficit (50% field capacity) and atmospheric vapour pressure deficit on the physiology of cotton.
Cotton (Sicot 746B3F) was planted on the 22nd October 2018 and grown at in a naturally lit CO_2 and temperature-controlled glasshouse (Temperature: 32/21°C and CO_2: 420 ppm). Plants were watered daily to field capacity, ensuring that the plants were well-watered. Plants were grown for four weeks under fully watered conditions, prior to a drought treatment (50% field capacity; FC) applied to half

the plants at 28 days after planting. The two water treatments (100% FC and 50% FC) were maintained for the remainder of the experimental period.

The physiological response to altered VPD and soil water deficit commenced on the 3rd December 2018 (42 DAP) and was measured over 10 days (42-46 DAP and 49-53 DAP). Each plant was measured once. On days of measuring VPD responses, air temperature of the glasshouse was stepped with the following increments throughout the day: 22, 26, 30, and 34°C. Plants were allowed time to acclimate to each temperature change for 30 minutes before gas exchange measurements were made using the new CSIRO Licor 6800 (Figure 2). An auto-programme was used to set the VPD inside the leaf cuvette with the following increments: 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 kPa for measurements at each temperature. These measurements were taken on well-watered and water-deficit plants, enabling us to determine the impact of soil water status on plant response to variable VPD.

Figure 2: (a) The new Licor 6800 was used to measure leaf-level physiological response to altered temperature and VPD conditions in well-watered and water-stressed cotton; and (b) L-R Candy Theerasutthikul (WSU), Renee Smith (WSU) and Katie Broughton (CSIRO) taking physiology measurements in the glasshouses at Western Sydney University.

2. *<u>Experiment: Source/Sink dynamics-</u> Assess the use of plant growth regulators to manage excessive vegetative growth of cotton grown in elevated CO_2 and warmer temperature in the glasshouse*

Several questions were addressed in conducting a single, very large glasshouse experiment at WSU to assess the use of cultivar and plant growth hormones to mitigate the effects of climate change on cotton growth and fruit losses. The objective of this experiment was to gain a more refined understanding of source and sink dynamics of cotton grown in future climatic conditions, particularly quantifying the relativity of the effects when all the factors (air temperature, atmospheric [CO_2], fruit retention and MC application) were considered together.

Comparing the effect of fruit removal on vegetative biomass production of two modern cotton cultivars

Seeds of two cotton cultivars (cv. Sicot 746B3F and CSX3301; Bollgard III, CSIRO) were planted into 9L pots at the beginning of January 2019 (i.e. during a long photoperiod) and grown at ambient temperature (32/21°C; day/night) and ambient CO_2 (420 ppm) in a single glasshouse bay until the beginning of flowering. Sicot 746B3F represented a more indeterminate cultivar and CSX 3301 represented a more determinant cultivar. At first flower (66 DAP), plants were moved into four glasshouse bays with a combination of temperature and CO_2 treatments and were grown in these environmental conditions until the end of the experiment. Two glasshouse compartments were set to simulate average temperatures (T_A: 32/21°C; day/night, the ambient temperature treatment) and two compartments were set at a daily temperature cycle that was 4°C higher than the ambient temperature regime (T_E: 36/25°C; day/night, the high temperature treatment). Temperature was changed five times over 24 h to simulate the daily temperature cycle in the field. For each of the temperature treatments, there were two CO_2 treatments (C_A: 420 ppm; C_E: 640 ppm). At 70 DAP (14th March 2019), 50% of the fruit was removed (alternating one-on/one-off, starting from the base of the plant) from five plants of each cultivar, to alter the sink capacity. Five control plants of each cultivar did not have any fruit manually removed.

The effect of fruit removal and mepiquat chloride on the vegetative biomass of Sicot 746B3F
Five additional Sicot 746B3F plants in temperature and CO_2 treatment had 50% fruit removed, as outlined above. Two days after fruit removal (72 DAP) mepiquat chloride was applied. The mepiquat chloride was applied at a rate of 600 ml ha^{-1} (22.8 g ai ha^{-1}). The mepiquat chloride treatment was not applied to CSX 3301 plants as the objective of the study was to assess the effect of MC on physiology and growth responses of plants that had lost fruit, rather than exploring cultivar by MC interactions. The treatment structure of the Source/Sink experiment is shown in Figure 3.

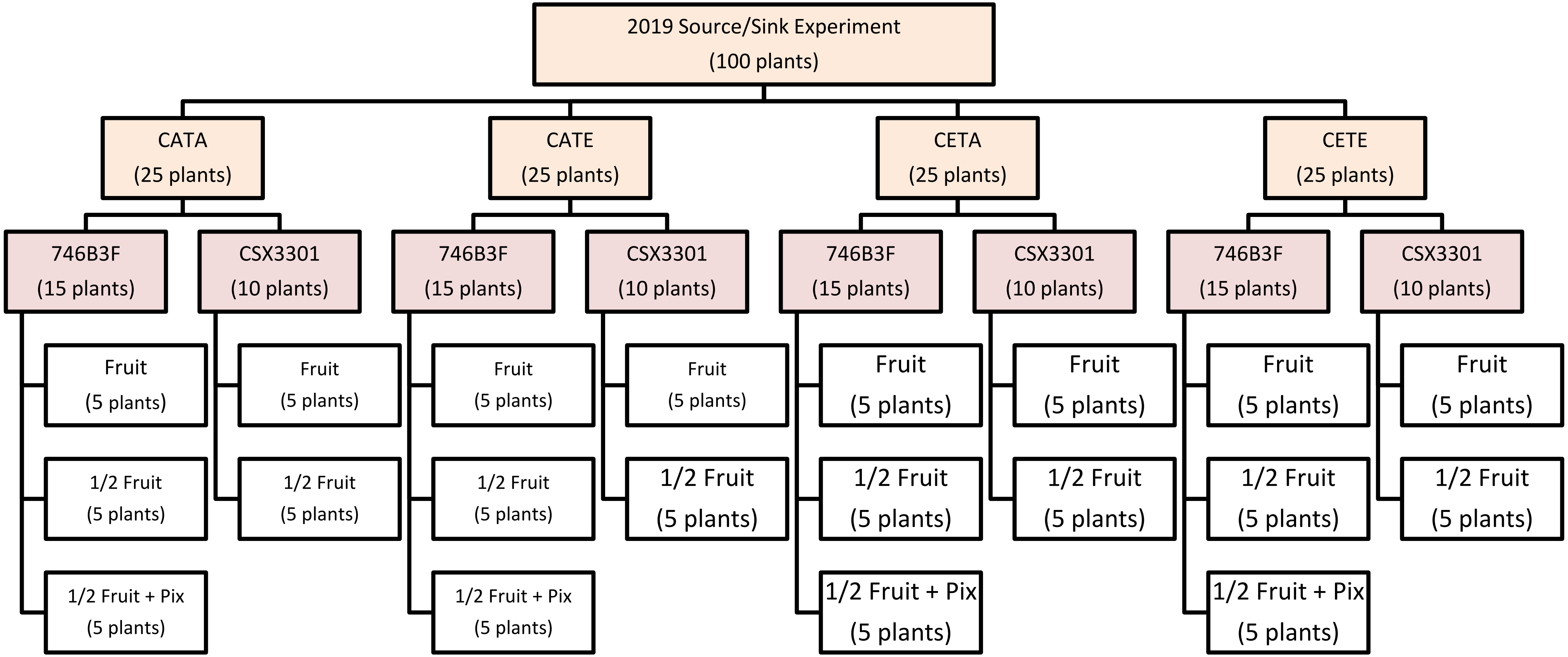


Figure 3: Treatment structure for the 2019 Source/Sink experiment at WSU, Richmond.

Plant growth measurements

Plant growth (heights and nodes) was monitored on a weekly basis throughout the experimental period (from 66 DAP to 99 DAP; mid-March to mid-April). Vegetative growth rate (VGR, cm/node) was calculated as the difference in height divided by the difference in the number of nodes, monitored weekly, with a total of four VGR measurements between 77 DAP and 99 DAP. At the end of the experiment, four weeks after the fruit removal treatment (99 DAP, 16th April 2019, just prior to first open boll), plants were mapped (to determine final fruit number, total fruit lost/removed and thus calculate retention), harvested and separated into vegetative (stems and leaves) and reproductive (squares, flowers, and bolls) organs. Total leaf area was measured using a portable leaf area meter (LI-3100A, LI-COR). Harvested samples were oven dried at 80°C for a minimum of 48 h and weighed.

Leaf gas exchange measurements

Photosynthetic rates at saturating light (A_{sat}) were measured on recently fully expanded leaves using a portable open gas exchange system (LI-6400XT, LI-COR). Leaf gas exchange measurements were taken at 67 DAP before fruit removal, and then once per week for four weeks after fruit removal (at 77, 84, 91, 98 DAP) to monitor plant physiological responses to temperature, atmospheric CO_2 and fruit treatments for each cultivar.

Carbohydrate sampling

At 5pm on the days following leaf gas exchange measurements, the same leaf that was measured for gas exchange was harvested and dried for carbohydrate analysis. Total non-structural carbohydrates (TNC; starch and soluble sugar) were calculated according the methods outlined in Tissue and Wright (1995). In summary, leaves were dried at 80°C for a minimum of three days, ground into a fine powder and then extracted three times with 2ml of a methanol:chloroform:water (12:5:3 v/v) solution to separate the soluble sugars from the pellet fraction containing starch. The pellet was treated with 5ml of perchloric acid (35% v/v) for 1 h to hydrolyse the starch. Soluble sugar and starch concentration were determined colorimetrically using the phenol-sulfuric acid method. TNC was calculated as the sum of starch (mg g^{-1}) and soluble sugar (mg g^{-1}) concentrations (Tissue and Wright, 1995). Starch, soluble sugar and TNC were used to determine availability of carbohydrates for vegetative and reproductive growth to link growth and physiology measurements.

Statistical analyses

We were limited to four glasshouse bays for our experiment, so we did not have replicates bays for the growth temperature and atmospheric CO_2 treatments. Therefore, acknowledging pseudo-replication as a function of our complex experimental design, we were limited to considering individual plants within each bay as independent experimental units (i.e. replicates) and tested the treatment effects with a general linear model, as has been done in previous studies (Apgaua et al., 2019; Duan et al., 2014; Lewis et al., 2013). For the leaf gas exchange measurements, one leaf from each plant was measured each day. For both leaf gas exchange and plant growth measurements, five plants in each treatment were measured.

Data were analysed by ANOVA using Genstat ver. 19 (VSN International Ltd). A four-way ANOVA was used to assess the effects of temperature, CO_2, cultivar and fruit load on growth and physiology variables. A three-way ANOVA was used to assess the effects of temperature, CO_2 and fruit treatment (including Pix) on growth and physiology variables of Sicot 746B3F. For leaf gas exchange and carbohydrates measured on multiple dates, each date was analysed separately. To assess the proportion of vegetative biomass of full fruit plants relative to the vegetative biomass of plants with half the fruit load, one-way ANOVAs were used to assess each temperature and CO_2 combination separately. The assumptions of normality and homogeneity of variance were met for all variables and transformations were applied where necessary. Means of treatments were compared using LSD at a 5% level of probability.

Improving the external parameters of models to better capture interactive effects of climate change on water use and yield using cotton simulation models

To improve our understanding of the external parameters of models to better capture interactive effects on water use and yield, temperature and CO_2 inputs into the OZCOT model (Version 2014) were altered to explore the effects of changing CO_2 concentration and growth temperatures in the model. Predictions were based on using SILO temperature data recorded from 1960 to 2019 at ACRI, Narrabri, NSW (-30.2083, 149.5967). Atmospheric CO_2 concentration inputs were tested at 300, 350, 400, 450, 500, 550, 600, and 650 ppm. Planting date was set to 1st October. Initial soil nitrogen was 300 kg/ha, with an additional application of N at 50 kg/ha on day of year (DOY) 354. Simulated water inputs were a pre-irrigation 12 days before sowing, irrigation at a soil profile deficit of 70mm, maximum water available for the season 1500mm, irrigation efficiency 80%, first irrigation 2 days after first square, and last irrigation at 60% open boll.

To better understand how the climate in Australian cotton regions has already changed, Cottassist has been used to access BOM climate data to explore the climate trends in representative cotton regions since 1957. Eight locations spanning the current Australian cotton industry have been assessed to explore the temperature trends over three periods: (a) 1957 to 2018 (61 years), (b) 1957 to 1996 (39 years); and (c) 1997 to 2018 (21 years). Regression lines were fit to each time period of season-long weather data (1st September to 30th April) at each location, with a trend significant at $P<0.05$. The eight representative cotton locations selected were Dalby, Emerald, Goondiwindi, Gunnedah, Griffith, Moree, Narrabri, and Narromine. Climatic trends assessed included day degrees, the frequency of cold shocks, and the frequency of hot days (greater that 35°C, 40°C and 45°C). These assessments using the OZCOT model and the Cottassist analyses help inform potential adaptation strategies of cotton to a changing climate by identifying locations that may have had similar climates in the past.

Results

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

Assess the use of plant growth regulators to manage excessive vegetative growth of cotton grown in environments of elevated CO_2 and warmer temperatures in the field.

Field experiments were conducted over three consecutive cotton seasons (2017/18, 2018/19 and 2019/20) to investigate the effects of various strategies to manage excessive vegetative growth of cotton grown in elevated atmospheric CO_2 and warmer temperature conditions in the field. Management strategies tested include (1) the use of mepiquat chloride to control vegetative growth, and (2) comparing the growth of a determinate and indeterminate cultivar to the different environmental scenarios. The outcomes of these experiments are summarised in Table 3. These experiments indicate that mepiquat chloride applied according to current Australian recommendations may suppress excessive vegetative growth of cotton grown in warmer temperatures and elevated CO_2 environments. Observations of increased difficulty of defoliating cotton plants grown in warmer temperatures and elevated CO_2, suggest that understanding defoliation application and timing in future climatic scenarios may be an important avenue of research. It is possible that optimal growth conditions dilute the effects of hormones involved in defoliation, but these concepts should be tested.

Table 3: Summary of results and outcomes for field experiments conducted at the Australian Cotton Research Institute, Narrabri.

Key outcomes: Assess the use of plant growth regulators to manage excessive vegetative growth of cotton grown in environments of elevated CO_2 and warmer temperatures in the field	
Growth and biomass	Final plant height and vegetative growth of cotton grown in environments of warmer temperature and elevated CO_2 were managed using the application of the plant growth regulator, mepiquat chloride, in two out of the three seasons.
Yield and quality	Seed cotton weights of plants grown in warmer temperature environments were lower than cotton grown in the control treatments. This suggests that although the balance between vegetative growth and reproductive growth has been improved through the application of mepiquat chloride,

	reductions in final yields may still occur in warmer temperature and high CO_2 environments.
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 most of the depths and irrigation periods, there were no consistent differences in ΔSWC between any of the environmental scenarios tested, indicating that there were no differences in plant water use between the treatments. No detectable difference in overall plant water use highlights that plant level water use efficiencies in future climates will be dictated by the ability of cotton to retain fruit and limit excessive vegetative growth, particularly in high temperature events. Therefore, it is important to understand if increased CO_2 or warmer temperatures are driving excessive growth and/or fruit loss to enable the development of better varieties, stronger management, or a combination of both.

Growing environment throughout the season

Chambers were used to elevate atmospheric CO_2 concentration and generate warmer temperatures in the field. Understanding the environmental conditions that the plants are responding to is an important aspect of this research. Minimum (Figure 4) and maximum (Figure 5) daily air temperatures, and mean atmospheric vapour pressure deficit (Figure 6) for the chamber and control treatments during each season are shown. Our data shows that daily air temperatures inside the chambers were generally similar to the control plots, with minimal spikes in temperature due to power failures. Mean VPDa inside the chambers were often lower than in the control plots, probably due to greater humidity inside the chambers.

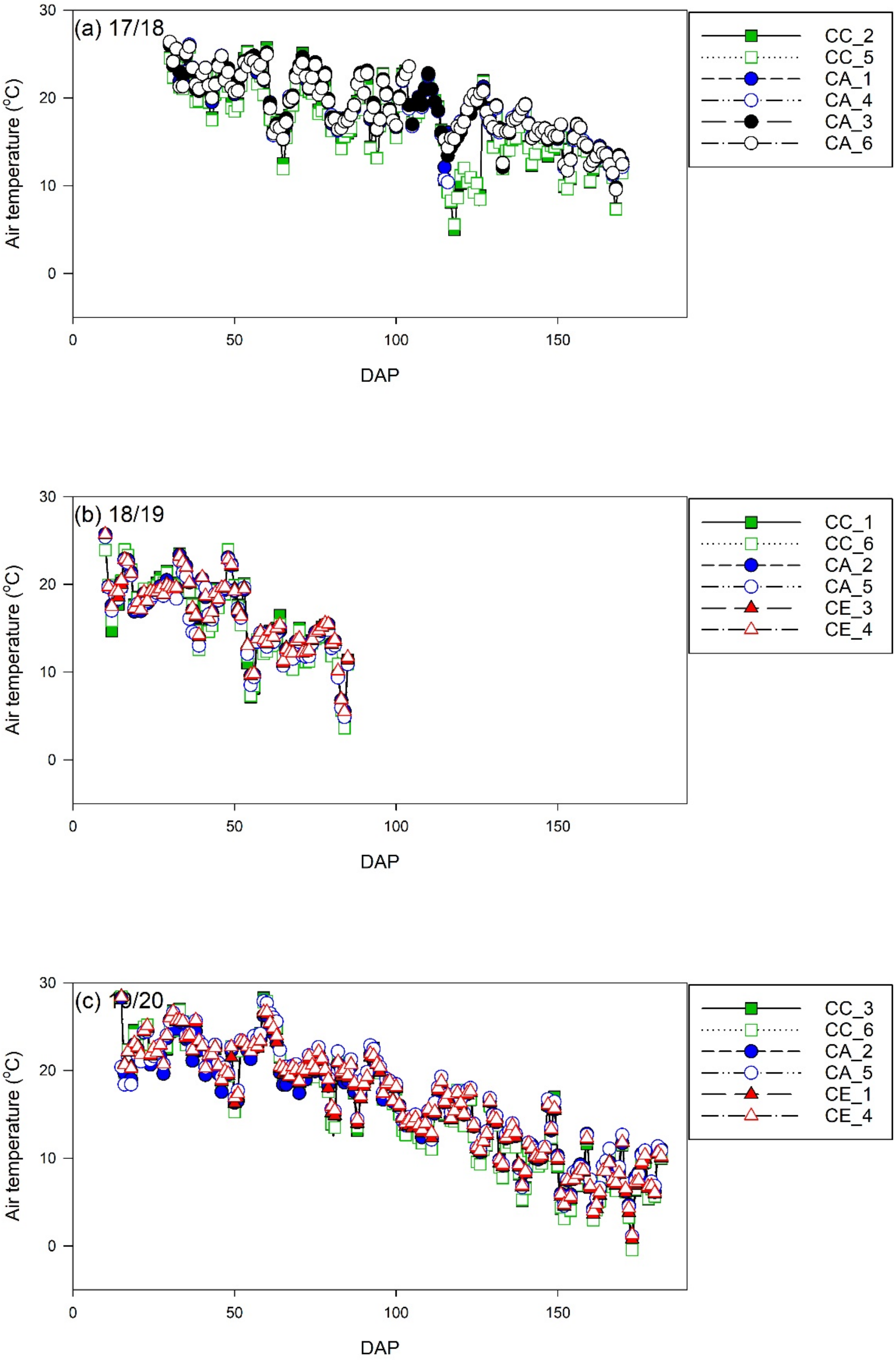
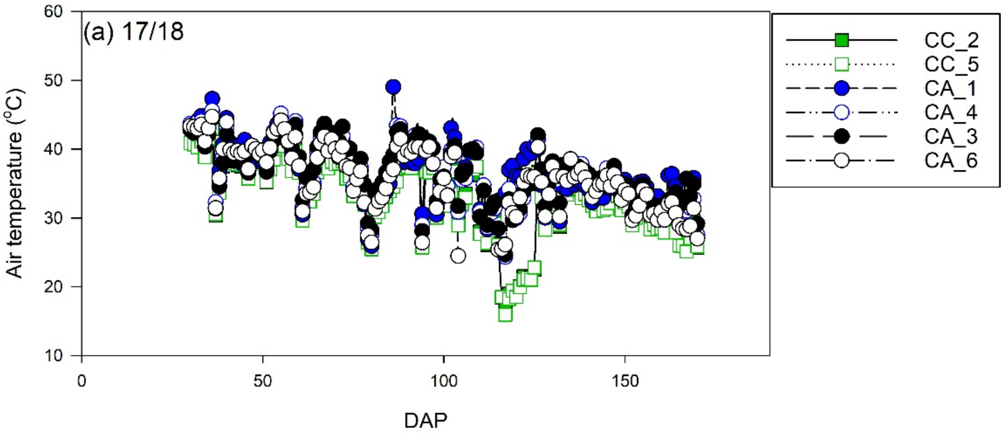
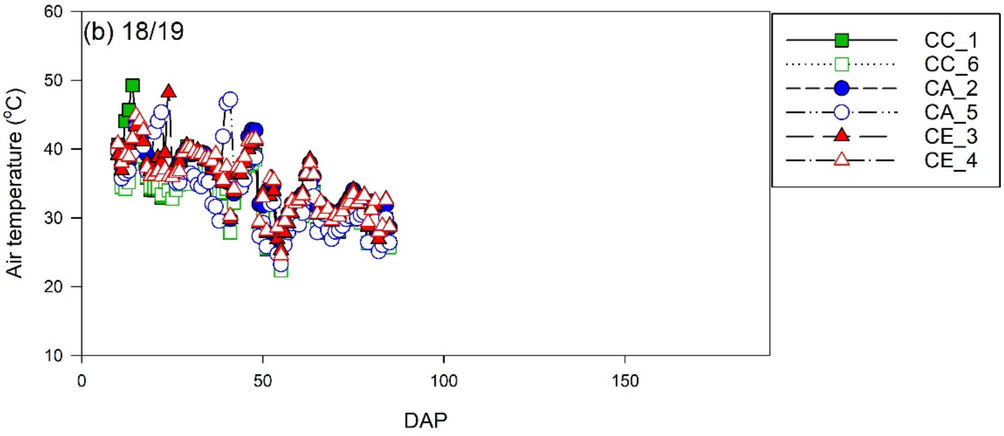


Figure 4: Minimum daily air temperature (°C) for each of the plots for (a) 2017/18, (b) 2018/19, and (c) 2019/20. CC represents control plots (squares), CA represents ambient CO_2 chambers (circles), and CE represents elevated CO_2 chambers.





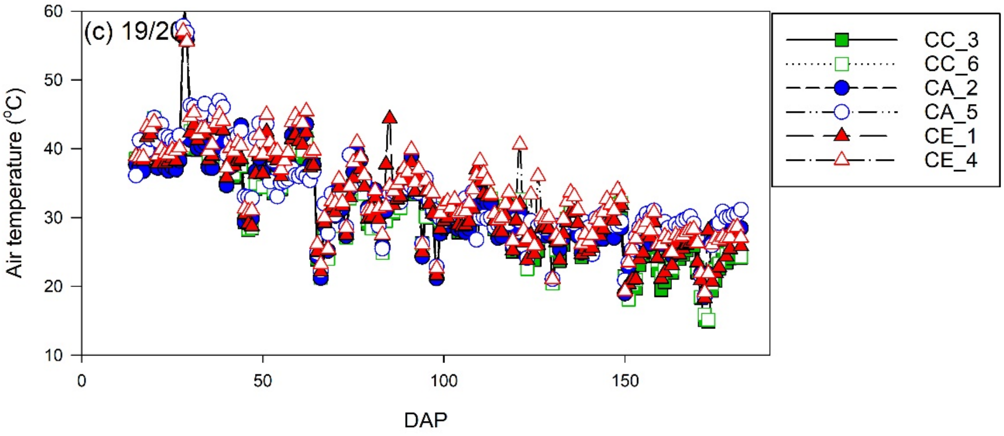


Figure 5: Maximum daily air temperature (°C) for each of the plots for (a) 2017/18, (b) 2018/19, and (c) 2019/20. CC represents control plots (squares), CA represents ambient CO_2 chambers (circles), and CE represents elevated CO_2 chambers.

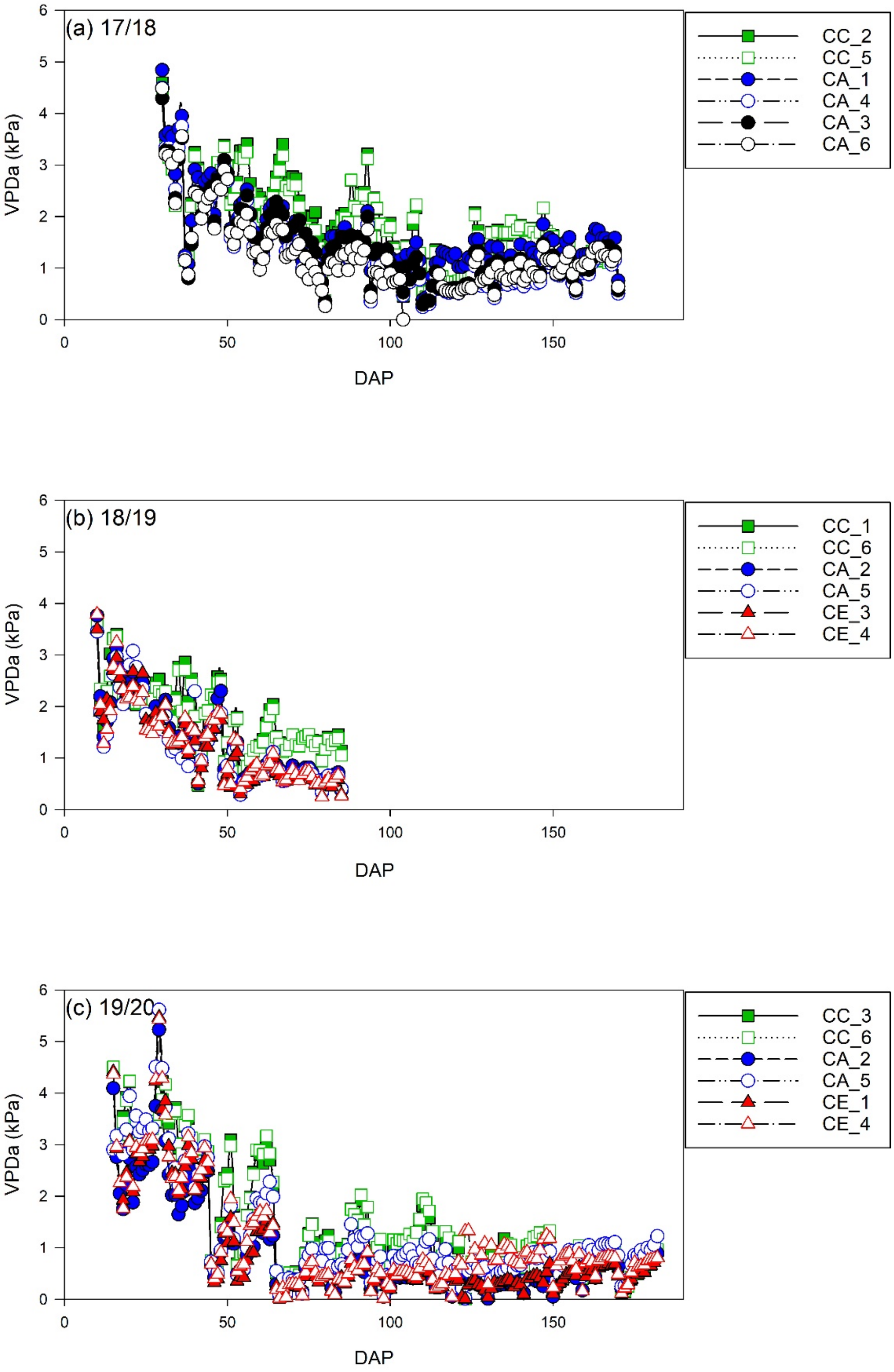


Figure 6: Mean daily vapour pressure deficit of the atmosphere (VPDa, kPa) for each of the plots for (a) 2017/18, (b) 2018/19, and (c) 2019/20. CC represents control plots (squares), CA represents ambient CO_2 chambers (circles), and CE represents elevated CO_2 chambers.

Plant growth and development throughout the season

Plant height and the number of nodes throughout each of the seasons are shown in Figure 7 (Sicot 746B3F) and Figure 8 (CSX 3301).

Chamber effect (CC compared with CA)

The effect of warmer temperature on final plant height was variable across the three seasons. At the end of the 17/18 season, plants grown at CA were 24% shorter than plants in the CC plots. This suggests that the mepiquat chloride treatment was effective in suppressing plant height and vegetative growth, potentially leading to greater yields than previous field experiments (as seen in CSP1501). For the 18/19 season (at 85 DAP), plants grown at CA were 10% taller than plants in the CC plots. For the 19/20 season, there was a significant Environment by Variety interaction. CSX cotton grown in the CA treatment was 23% taller than CSX plants grown at CC, whereas there was no difference in the height of Sicot cotton grown at CA or CC (Table 4). Warmer temperatures only increased the final number of nodes in one cotton season. In 19/20, CSX cotton grown at CA had 16% greater number of nodes at the end of the season than plants grown at CC. However, there was no difference in the final number of nodes of Sicot.

Effect of elevated [CO_2] (CA compared with CE)

Across the two seasons that had elevated CO_2 treatments, the effects of elevated CO_2 on final height and the number of nodes were inconsistent. For the 18/19 season, plants grown at CE were 7% taller than plants grown at CA. For the 19/20 season, Sicot plants grown at CE were 10% shorter than Sicot plants grown at CA. CSX plants grown at CE were 7% shorter than CSX plants grown at CA. There was no difference in the final number of nodes between CA and CE grown plants for either the 18/19 or 19/20 seasons. This suggests that pix treatments may be an effective strategy to control plant height and the number of nodes of cotton grown in a combination of warmer temperatures and elevated CO_2.

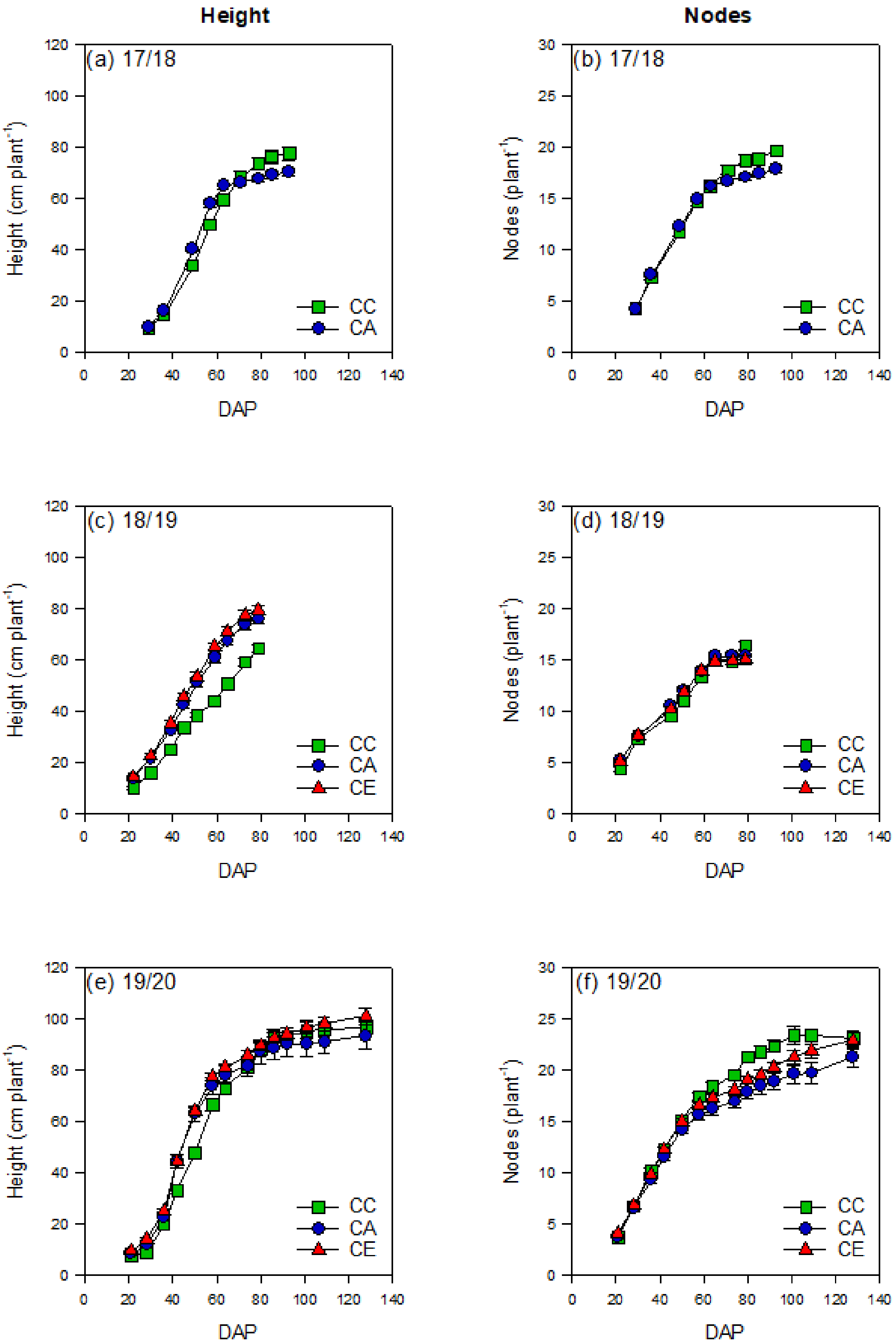


Figure 7: Height (cm plant^{-1}) and nodes (plant^{-1}) of Sicot 746B3F cotton grown in control plots (CC, green), ambient CO₂ (CA, blue) and elevated CO₂ (CE, red) chambers over each of the three cotton seasons: 2017/18 (a and b), 2018/19 (c and d) and 2019/20 (e and f).

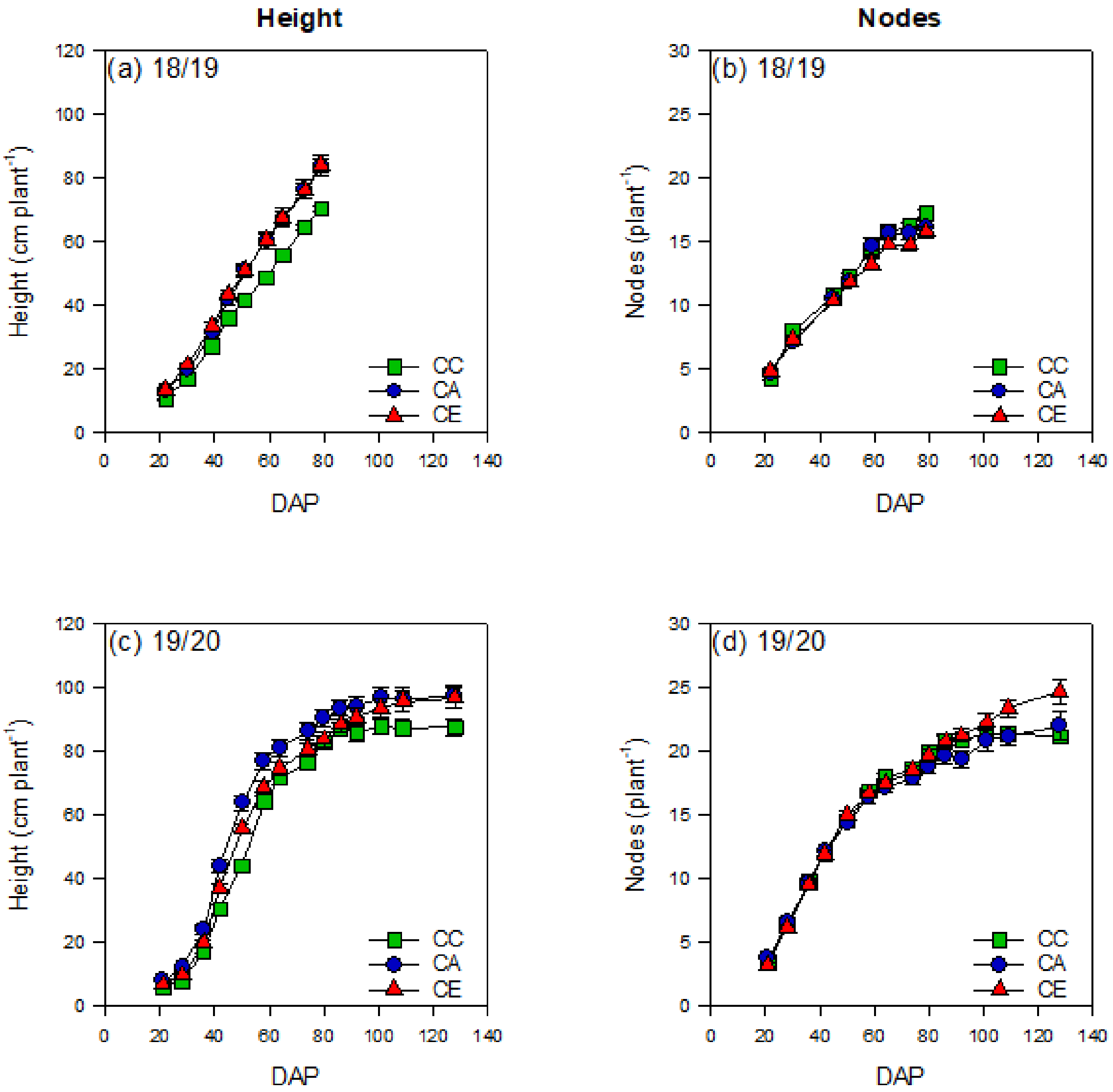


Figure 8: Height (cm plant^{-1}) and nodes (plant^{-1}) of CSX 3301 cotton grown in control plots (CC, green), ambient CO_2 (CA, blue) and elevated CO_2 (CE, red) chambers over two cotton seasons: 2018/19 (a and b) and 2019/20 (c and d).

Table 4: REML analyses for final height and nodes of cotton at the end of each season. Values represent F. probabilities. Values in bold are significant at P< 0.05. N/A indicates not applicable.

Cotton Season	Harvest	Parameter	Environment	Variety	Environment x Variety
17/18	177 DAP	Height	**0.001**	N/A	N/A
		Nodes	0.472	N/A	N/A
18/19	85 DAP	Height	**0.001**	0.145	0.832
		Nodes	0.086	0.575	0.382
19/20	182 DAP	Height	**0.001**	**0.001**	**0.018**
		Nodes	**0.002**	**0.001**	**0.010**

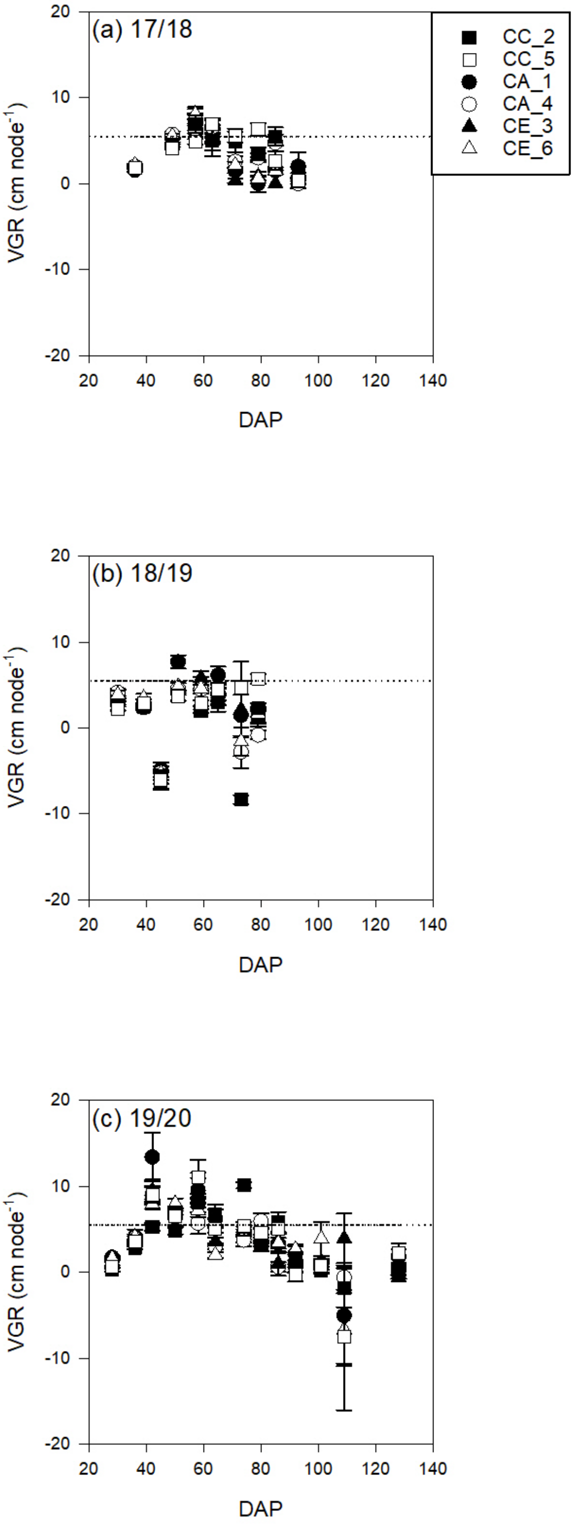


Figure 9: Vegetative growth rate (VGR) of Sicot 746B3F during the (a) 17/18, (b) 18/19, and (c) 19/20 cotton seasons. Symbols (CC: square, CA: circle, CE: triangle) represent mean ± SE. Numbers following treatment in the legend indicate plot number. Dotted line indicates where VGR is 5.5cm node^{-1}.

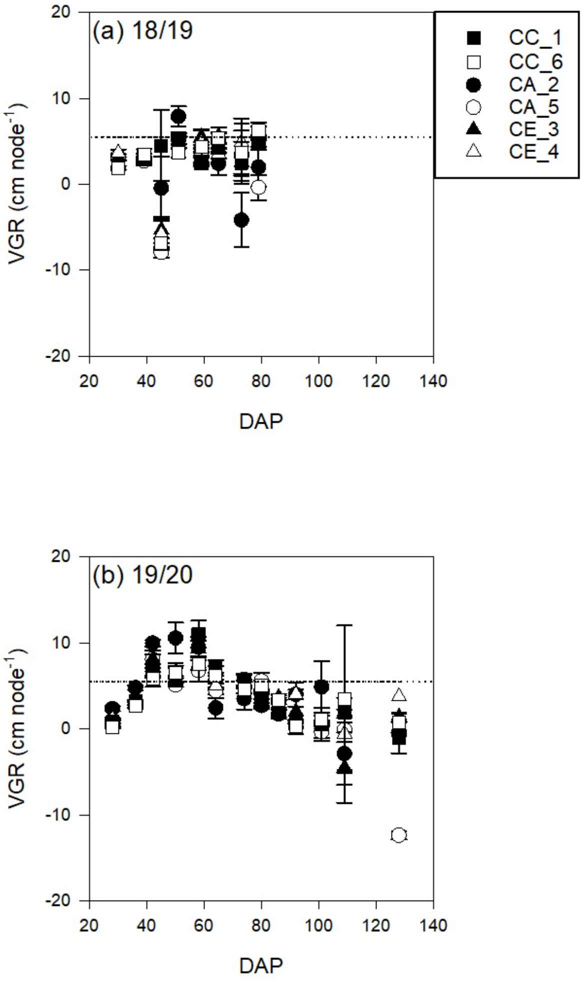


Figure 10: Vegetative growth rate (VGR) of CSX 3301 during the (c) 18/19, and (b) 19/20 cotton seasons. Symbols (CC: square, CA: circle, CE: triangle) represent mean ± SE. Dotted line indicates where VGR is 5.5 cm node^{-1}.

Plant biomass

- *Chamber effects (CC compared with CA):*

There were no differences in vegetative biomass of plants grown in ambient CO_2 (CA) and control (CC) across any of the three seasons tested (Figure 11, Table 5). In two of the three seasons, there were also no differences in fruit biomass between CA and CC treatments (Figure 12, Table 5). However, in the 2018-19 cotton season, plants grown at CA had 37% less fruit biomass than plants in the CC treatment. Although there was a trend for reduced fruit biomass for CA compared with CC treatments in the 2019/20 season, the difference was not significant. There was no difference in total biomass of plants grown at CC or CA during the 2019-20 cotton season.

- *Effects of elevated CO_2 (CA compared with CE):*

There were no significant differences in vegetative biomass of plants grown in ambient CO_2 chambers (CA) and plants grown in elevated CO_2 (CE) chambers across the two seasons. In the 2018/19 cotton season, plants grown at CE had 69% more fruit biomass than plants grown in the CA treatment, but there was no difference in the 2019/20 cotton season. Plants grown at CE had 12% less total biomass than plants grown at CA, but only for the 2019-20 cotton season. This was driven by a trend of lower fruit biomass in the CE treatment.

Findings from the field experiments of CSP1501 suggested that elevated atmospheric CO_2 does not mitigate the negative effects of warmer temperatures on fruit retention. They demonstrated that cotton grown in warmer temperature and high [CO_2] environments may have increased vegetative biomass and reduced fruit retention compared with current temperature and CO_2 field conditions, despite non-significant differences in total aboveground biomass.

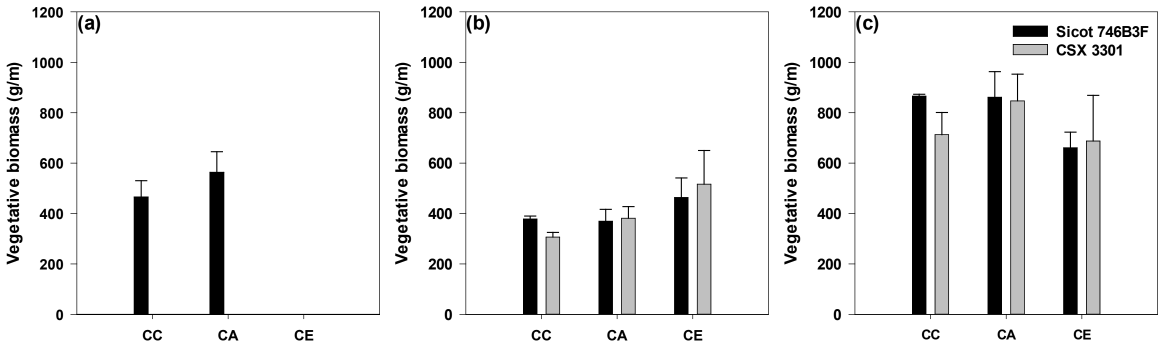


Figure 11: Vegetative biomass for (a) 2017/18, (b) 2018/19, and (c) 2019/20 cotton seasons for Sicot 746B3F (black) and CSX 3301 (grey). Plants were harvested at 121, 85 and 113 DAP, respectively. Values represent means ± SE of two plots, except CA treatment in the 2017/18 where n=4.

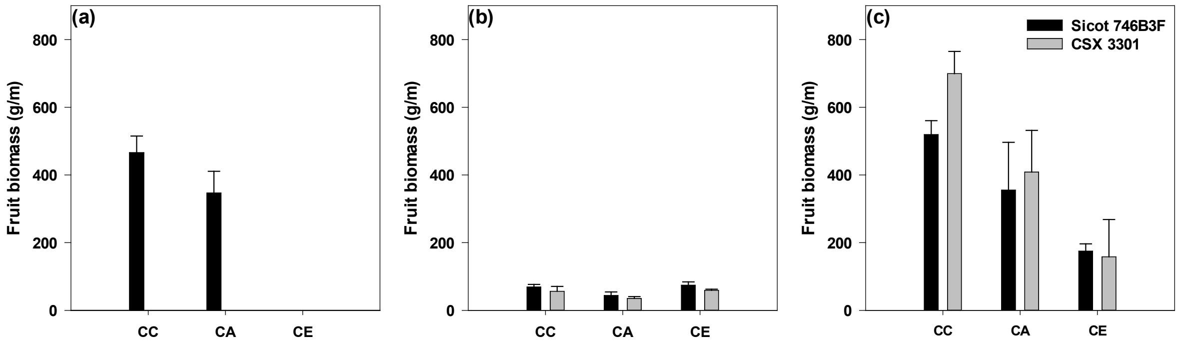


Figure 12: Total fruit biomass for (a) 2017/18, (b) 2018/19, and (c) 2019/20 cotton seasons for Sicot 746B3F (black) and CSX 3301 (grey). Plants were harvested at 121, 85 and 113 DAP, respectively. Values represent means ± SE of two plots, except CA treatment in the 2017/18 where n=4.

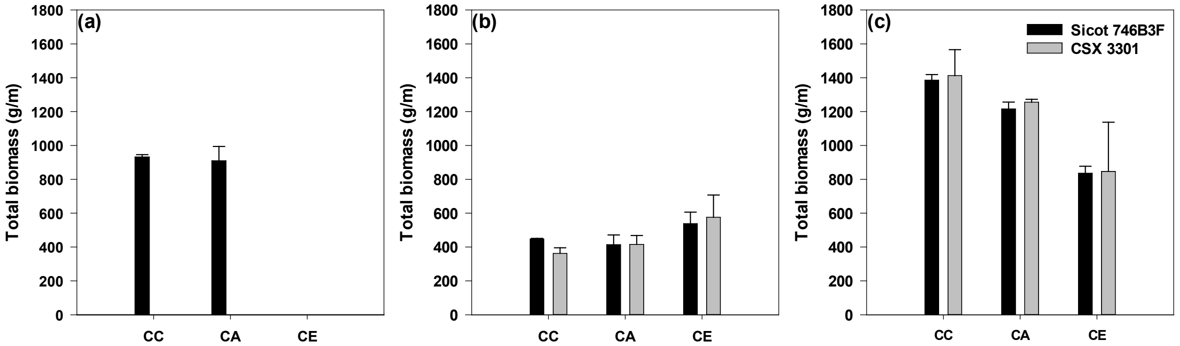


Figure 13: Total biomass for (a) 2017/18, (b) 2018/19, and (c) 2019/20 cotton seasons for Sicot 746B3F (black) and CSX 3301 (grey). Plants were harvested at 121, 85 and 113 DAP, respectively. Values represent means ± SE of two plots, except CA treatment in the 2017/18 where n=4.

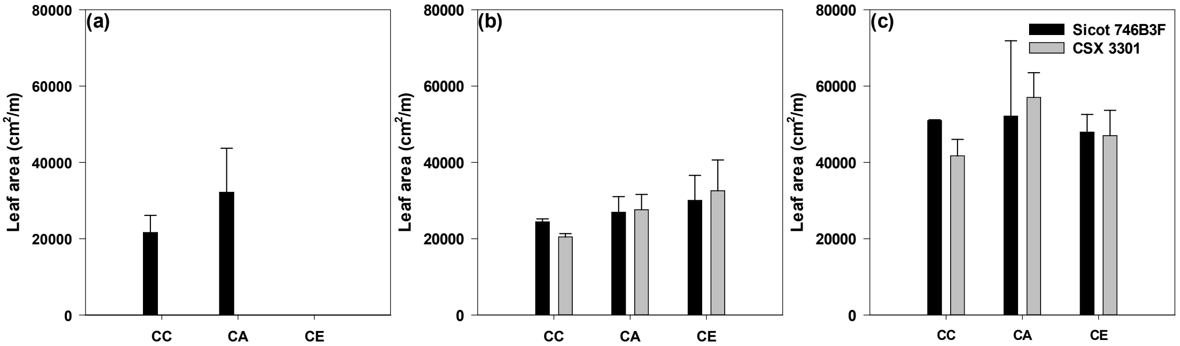


Figure 14: Leaf area for (a) 2017/18, (b) 2018/19, and (c) 2019/20 cotton seasons for Sicot 746B3F (black) and CSX 3301 (grey). Plants were harvested at 121, 85 and 113 DAP, respectively. Values represent means ± SE of two plots, except CA treatment in the 2017/18 where n=4.

Table 5: ANOVA and REML analyses of plant biomass and leaf area over three consecutive cotton seasons from 2017 to 2020. Values are F. probabilities, with values in bold significant at $P < 0.05$. N/A indicates not applicable, as there was not a variety treatment in the 2017/18 cotton season.

	Cotton Season	**Environment**	**Variety**	**Environment x Variety**	**CA compared with CC (%)**	**CE compared with CA (%)**
Vegetative biomass (g/m)	2017/18	0.497	N/A	N/A	0	0
	2018/19	0.163	0.964	0.676	0	0
	2019/20	0.295	0.603	0.685	0	0
Fruit biomass (g/m)	2017/18	0.304	N/A	N/A	0	0
	2018/19	**0.050**	0.155	0.946	↓ 37%	↑ 69%
	2019/20	**0.010**	0.387	0.602	0	0
Total biomass (g/m)	2017/18	0.874	N/A	N/A	0	0
	2018/19	0.125	0.802	0.680	0	0
	2019/20	**0.017**	0.829	0.994	0	↓ 12%
Leaf area (cm^2/m)	2017/18	0.583	N/A	N/A	0	0
	2018/19	0.266	0.949	0.799	0	0
	2019/20	0.648	0.823	0.756	0	0

The distribution of fruit on the plants at harvest

Distribution of the number of open bolls and the number of shed fruit at harvest during the 2017/18 and 2019/20 seasons were assessed. Overall, cotton grown in the CA treatment had 17 - 33% fewer open bolls than plants grown in the CC treatment. The distribution of boll retention varied across the two seasons. For plants grown at CA in the 2017/18 season, there was a reduction in the number of open bolls on the vegetative branches (-40%), nodes 11-15 (-46%) and nodes 16-20 (-39%) compared with plants grown in the control, with no difference in the number of open bolls for nodes 0-10 or nodes 21+ (Figure 15, Table 6). For plants grown at CA in the 2019/20 season, there was a reduction in the number of open bolls on nodes 0-10 (-53%), nodes 11-15 (-42%), and an increase in the number of open bolls at nodes 21+ (571%).

During the 2017/18 season, cotton in the CA treatment had less shedding at nodes 0-10 (-22%) and at nodes 21+ (-45%) compared with control plants (Figure 16, Table 7). However, in the 2019/20 season, cotton grown in the CA treatment had increased shedding of fruit at nodes 11-15 (+29%), nodes 16-20 (+39%), and nodes 21+ (+24%).

Cotton grown in the CE treatment had 36% fewer open bolls than cotton grown in the CA treatment, with reductions in open boll number across the whole plant. Cotton grown at CE had reductions at nodes 0-10 (-61%), nodes 11-15 (-27%), nodes 16-20 (-48%), and nodes 21+ (-79%). Our data shows that the total number of shed fruit in CE was 19% greater than the number of shed fruit in the CA treatment.

Overall, there was no difference in the number of open bolls between the two cotton varieties, however, CSX 3301 had 34% less fruit shed than Sicot 746B3F.

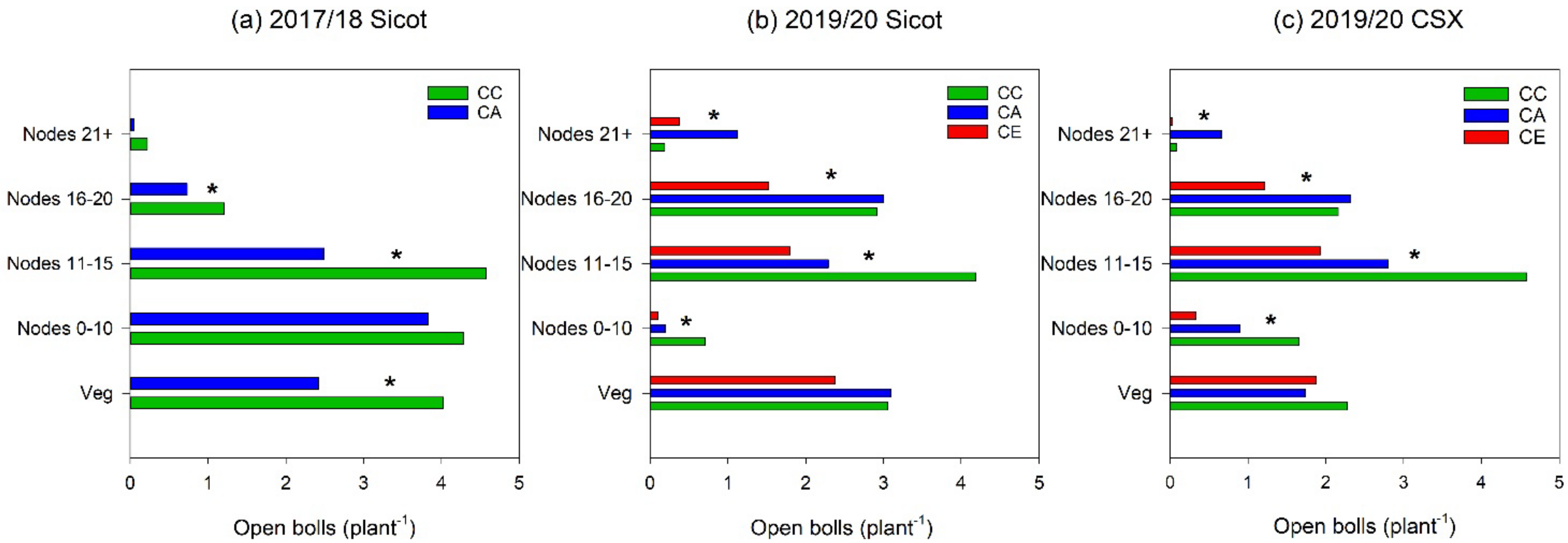


Figure 15: Number of open bolls per metre for Sicot (a) 2017/18, (b) 2019/20, and CSX (c) 2019/20 plants grown in altered environmental conditions (CC is shown in green, CA is shown in blue, and CE is shown in red). * represents significant difference at P<0.05.

Table 6: The number of open bolls at each range of nodes for cotton grown in the chambers and control plots during the 2017/18 and 2019/20 field experiments. Values in bold represent significant difference in F probability at P<0.05.

Open bolls	**Season**	**Environment**	**Variety**	**Environment x Variety**
Vegetative	17/18	**0.009**	N/A	N/A
	19/20	0.649	**0.037**	0.696
Nodes 0-10	17/18	0.333	N/A	N/A
	19/20	**0.001**	**0.001**	**0.030**
Nodes 11-15	17/18	**0.001**	N/A	N/A
	19/20	**0.001**	0.139	0.798
Nodes 16-20	17/18	**0.026**	N/A	N/A
	19/20	**0.001**	**0.021**	0.749
Nodes 21+	17/18	0.132	N/A	N/A
	19/20	**0.001**	**0.009**	0.452

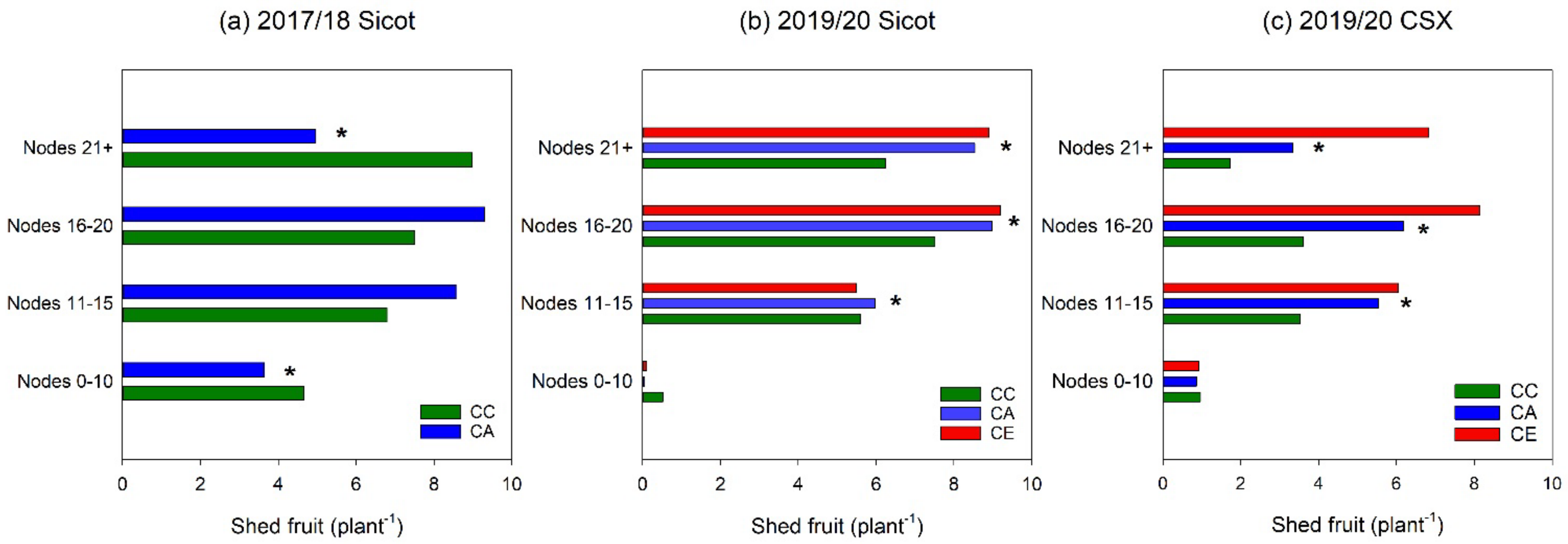


Figure 16: Number of shed fruit per metre for Sicot (a) 2017/18, (b) 2019/20, and CSX (c) 2019/20 plants grown in altered environmental conditions (CC is shown in green, CA is shown in blue, and CE is shown in red). * represents significant difference at P<0.05.

Table 7: The number of shed fruit at each range of nodes for cotton grown in the chambers and control plots during the 2017/18 and 2019/20 field experiments. Values in bold represent significant difference in F probability at P<0.05.

	Season	Environment	Variety	Environment x Variety
Nodes 0-10	17/18	**0.022**	N/A	N/A
	19/20	0.081	**0.001**	0.285
Nodes 11-15	17/18	0.068	N/A	N/A
	19/20	**0.004**	0.085	**0.022**
Nodes 16-20	17/18	0.110	N/A	N/A
	19/20	**0.001**	**0.001**	0.102
Nodes 21+	17/18	**0.001**	N/A	N/A
	19/20	**0.001**	**0.001**	0.208

Figure 17: Darin showing the distribution on fruit on a plant grown in the chambers during the 2015/16 cotton season (left) compared with the 2019/20 cotton season, where vegetative growth was managed using mepiquat chloride (right).

Figure 18: Observations of cotton grown at warmer temperatures and higher CO_2 indicates that improved understanding of defoliation efficacy under future climates may be an important avenue for future research. It is possible that optimal conditions for growth dilute the effects of the hormones involved in defoliation.

Plot 1 – CE

Plot 2 - CA

Plot 3 – CC

Plot 4 - CE

Plot 5 – CA

Plot 6 – CC

Figure 19: Comparison of final yield of two cotton varieties (CSX 3301 and Sicot 746B3F) grown in the 2019/20 cotton season. In each photo, CSX 3301 is on the left and Sicot 746B3F is on the right. Note that Lint was harvested from CSX in plots 1 and 4. Final yield photos CSX 3301 on Left, Sicot on Right. Note: Plot 1 and 4 Lint was harvested from CSX 3301 plants.

Gin Data

Seed cotton of plants grown in the CA treatment was 33 - 36% lower than plants grown in the CC treatment (Table 8). Consequently, lint weight of plants grown in the CA treatment was 42% lower than plants grown in the control in both the 17/18 and 19/20 cotton seasons (Table 8). There were no differences in either seed cotton weight or lint weight between plants grown in the CA and CE treatments.

Table 8: Gin data for cotton grown during the 2017/18 and 2019/20 field experiments. Values in bold represent significant difference in F probability at P<0.05. NA represents not applicable as there was only one variety tested during the 2017/18 cotton season.

Parameter	Season	Environment	Variety	Environment x Variety	CC mean (g/m)	CA mean (g/m)	CE mean (g/m)	CA compared with CC (%)	CE compared with CA (%)
seed cotton weight (g/m)	17/18	**0.001**	NA	NA	539	348	NA	-36	NA
	19/20	**0.002**	0.908	0.638	473	318	173	-33	0
lint weight (g/m)	17/18	**0.001**	NA	NA	265	153	NA	-42	NA
	19/20	**0.001**	0.895	0.784	188	109	62	-42	0

Fibre test results

Analyses of HVI data showed an increase in length (2-5%) in CA compared with CC across both the 2017/18 and 2019/20 seasons. Strength (13%) and length uniformity index (1%) were also higher for cotton grown in the chambers compared with the control only during the 2017/18 season (Table 9). HVI data showed there was a reduction in elongation (7-13%), moisture content (3%) and fineness (24%) for cotton grown in the chambers compared with the control (Table 9) only in the 2017/18 cotton season. However, despite these differences in HVI data, cotton grown in the chambers and control plots generally fall into the same ranges: long fibre length, average maturity ratio, and very high length uniformity index. Strength was high for cotton grown in the CC plots and very high for cotton grown in the CA plots (Table 10). Elongation was low for cotton grown in the CC treatment and average for cotton grown in the CA treatment.

Table 9: Results from HVI testing for cotton grown in climate change chambers and control plots during the 2017/18 and 2019/20 field experiments. NA indicates not applicable as there was only one variety for the 17/18 cotton season. Values in bold represent significant difference in F probability at P<0.05. n.s. indicates no significant difference at P<0.05.

Parameter	**Season**	**Environment**	**Variety**	**Environment x Variety**	**CA compared with CC (%)**
Length (inches)	17/18	**0.022**	**NA**	**NA**	2
	19/20	**0.012**	0.159	0.848	5
Elongation (%)	17/18	**0.001**	**NA**	**NA**	-13
	19/20	**0.002**	**0.001**	0.079	-7
Maturity ratio	17/18	0.316	**NA**	**NA**	n.s.
	19/20	0.651	0.072	0.379	n.s.
Micronaire	17/18	0.092	**NA**	**NA**	n.s.
	19/20	0.205	**0.007**	0.22	n.s.
Moisture (%)	17/18	**0.017**	**NA**	**NA**	-3
	19/20	0.721	0.132	0.708	n.s.
Short fibre index (%)	17/18	**0.001**	**NA**	**NA**	-24
	19/20	0.073	0.127	0.891	n.s.
Strength (grams per tex)	17/18	**0.001**	**NA**	**NA**	13
	19/20	0.685	0.398	0.698	n.s.
Length uniformity index (%)	17/18	**0.001**	**NA**	**NA**	1
	19/20	0.835	0.676	0.743	n.s.

Table 10: Summary of values for the interpretation of CSIRO fibre testing

Length	Elongation	Maturity Ratio	Micronaire	Moisture	Strength	Length Uniformity index
<1.00 very short	<5.0 very low	< 0.7 immature	3.5-5.0 accepted without penalty	6-8% ideal	<24 very low	<75 very low
1.00-1.14 medium	5.0-5.8 low	0.7-0.8 below average			24-26 low	75-78 low
1.15-1.29 long	5.9-6.7 average	0.81-0.95 average			27-30 average	79-82 average
>1.29 extra long	6.8-7.6 high	>0.95 above average			31-34 high	83-85 high
	>7.6 very high				>34 very high	>85 very high

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 2017/18 season, there were no significant differences in ΔSWC (Table 11). There were some significant differences in the ΔSWC during the 2018/19 and 2019/20 cotton seasons. Overall, across most of the depths and irrigation periods, there were no consistent differences in ΔSWC between any of the environmental scenarios tested, indicating that there were no differences in plant water use between the treatments.

Table 11: 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.

	10cm			**30cm**			**50cm**			**70cm**			**90cm**		
DAP	**Env.**	**Var.**	**Env. X Var.**	**Env.**	**Var.**	**Env. X Var.**	**Env.**	**Var.**	**Env. X Var.**	**Env.**	**Var.**	**Env. X Var.**	**Env.**	**Var.**	**Env. X Var.**
2017/18 season															
28-33	0.568	-	-	0.425	-	-	0.653	-	-	0.063	-	-	0.185	-	-
35-36	0.528	-	-	0.422	-	-	0.187	-	-	0.139	-	-	0.241	-	-
38-50	0.081	-	-	0.051	-	-	0.057	-	-	0.844	-	-	0.702	-	-
52-65	0.471	-	-	0.973	-	-	0.890	-	-	0.181	-	-	0.629	-	-
67-75	0.668	-	-	0.978	-	-	0.378	-	-	0.303	-	-	0.542	-	-
77-85	0.864	-	-	0.571	-	-	0.783	-	-	0.115	-	-	0.163	-	-
87-181	0.165	-	-	0.165	-	-	0.166	-	-	0.166	-	-	0.166	-	-
2018/19 season															
12-24	0.388	0.331	0.388	0.388	0.332	0.388	0.388	0.331	0.388	0.389	0.331	0.388	0.389	0.331	0.388
26-31	0.390	0.333	0.390	0.387	0.330	0.391	0.387	0.333	0.389	0.385	0.334	0.388	0.384	0.332	0.388
33-41	0.266	0.483	0.606	0.266	0.483	0.605	0.266	0.483	0.606	0.267	0.483	0.606	0.267	0.483	0.607
43-52	0.461	0.676	0.346	0.148	0.647	0.609	0.564	0.843	0.825	0.662	0.343	0.694	0.324	**0.041**	0.152
54-66	0.899	0.624	0.666	0.071	0.843	0.839	0.386	0.339	0.390	0.397	0.328	0.388	0.390	0.334	0.393
68-101	0.389	0.333	0.389	0.385	0.332	0.390	0.382	0.333	0.387	0.384	0.332	0.391	0.390	0.337	0.395
2019/20 season															
18-31	0.390	0.345	0.376	0.258	0.981	0.931	0.627	0.646	0.723	0.394	0.339	0.381	0.359	0.303	0.342
33-44	0.458	0.446	0.321	0.387	0.334	0.388	0.510	0.599	0.652	0.456	0.449	0.318	0.389	0.332	0.389
46-65	**0.020**	0.832	0.055	0.579	0.967	0.330	0.384	0.341	0.498	0.160	0.838	0.305	0.399	0.336	0.391
67-94	0.250	0.993	0.318	0.357	0.428	0.807	0.391	0.337	0.389	0.245	0.520	0.642	0.387	0.334	0.387
96-99	**0.001**	0.758	0.222	**0.001**	0.051	0.118	0.386	0.334	0.388	**0.001**	0.924	0.226	0.388	0.331	0.388
101-183	0.019	0.191	0.455	0.582	0.533	0.638	0.240	0.776	0.196	0.086	0.242	0.642	0.251	0.328	0.251

Assess the use of plant growth regulators to manage excessive vegetative growth of cotton grown in elevated [CO_2] and warmer temperatures in the glasshouse (Milestone 2).

A glasshouse experiment was conducted at WSU, Richmond to identify potential management practices that mitigate the interactive effects of climate change. The outcomes of this experiment are summarised in Table 12. Our data show that fruit loss in cotton may greatly increase VGR of cotton, potentially leading to greater vegetative biomass and greater leaf area. Our data showed that mepiquat chloride applications reduced the VGR of cotton that had lost fruit, suggesting that mepiquat chloride applications may be a successful method of controlling excessive vegetative growth in future climates of warmer temperatures and elevated CO_2. Our data showed that there were no differences in the growth or physiological responses between the two cultivars tested.

Table 12: Key outcomes of glasshouse experiments conducted to assess the use of plant growth regulators to manage excessive vegetative growth of cotton grown in elevated [CO_2] and warmer temperatures in the glasshouse.

	Key outcomes: Identify management practices that mitigate the effects of warmer temperature and elevated atmospheric [CO_2] on cotton growth, physiology and water use.
Growth and biomass	Mepiquat chloride applied at current Australian industry standards reduced the VGR of cotton that had lost fruit, suggesting that mepiquat chloride may be a successful method of controlling excessive vegetative growth in future climates of warmer temperatures and elevated CO_2.
Leaf physiology	There do not appear to be any direct effects of fruit removal on the physiology of cotton, however, there may be some interactive effects of the combination of fruit removal and the addition of Pix on cotton physiology 3 to 4 weeks after the fruit removal.

The effects of fruit removal and plant growth regulators (comparison of Sicot only)

Averaged across all environment and cultivar treatments, cotton with fruit removed had consistently greater VGR than cotton with a full fruit load, from 14 DAFR until 29 DAFR (Figure 20 and Table 13). Cotton with fruit removed had 57%, 110% and 219% greater VGR than full fruit plants at 14 DAFR, 22 DAFR and 29 DAFR, respectively. There was no difference in VGR of cotton with a full fruit load and cotton with fruit removed at 7 DAFR.

Averaged across all environment and cultivar treatments, VGR of cotton in the Remove+Pix treatment was consistently lower than both cotton with fruit removed and cotton in the full fruit treatment, from 14 DAFR until 29 DAFR (Figure 20 and Table 13). Cotton in the Remove+Pix treatment had an 81%, 107% and 100% reduction in VGR compared with fruit removed cotton at 14 DAFR, 22 DAFR and 29 DAFR, respectively. There was no difference in VGR of cotton with fruit removed and cotton with fruit Removed+Pix at 7 DAFR.

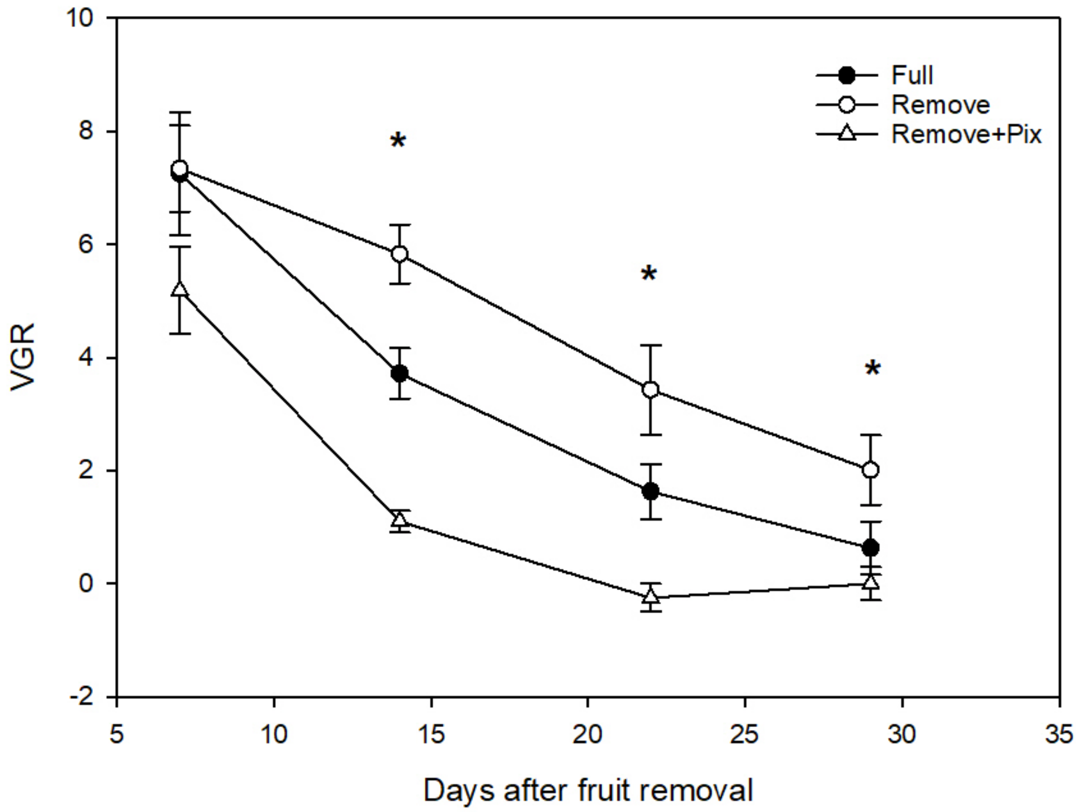


Figure 20: Vegetative growth rate (VGR) of plants with three different fruit treatments for Sicot. Values represent means ± SE, averaged across CO_2, temperature and cultivar treatments. * represent significant difference at P<0.05.

Table 13: ANOVA table of VGR for comparison of fruit load treatments in Sicot 746B3F. Values in bold represent significant difference at P < 0.05. DAFR is days after fruit removal.

	7 DAFR	14 DAFR	22 DAFR	29 DAFR
CO_2	0.702	0.928	0.066	0.514
Temperature	0.177	0.678	0.595	0.397
Fruit treatment	0.159	**0.001**	**0.001**	**0.020**
CO_2 x Temperature	0.429	0.710	0.451	0.632
CO_2 x Fruit	0.779	0.668	0.344	0.630
Temperature x Fruit	0.877	0.549	0.587	0.731
CO_2 x Temperature x Fruit	0.064	0.895	0.935	0.493

Averaged across all temperatures and CO_2, the fruit removal treatment generally only affected the physiology of Sicot 746B3F at 21 and 28 DAFR (Figure 21 and Table 14). At 28 DAFR, gs-sat and E of cotton in the Pix treatment were 39% and 21% lower, respectively, compared with cotton in the Full fruit treatment. The reduction in E led to a 30% increase in A/E of cotton in the Pix treatment compared with the Full fruit treatment. These data indicate that there do not appear to be any direct effects of fruit removal on the physiology of cotton, but that there may be some effects of the combination of fruit removal and the addition of Pix on cotton physiology that starts to play out 3 to 4 weeks after the fruit removal has occurred.

Table 14: Three-way ANOVA for comparison of fruit load treatments in Sicot 746B3F. Values in bold represent significant difference at P < 0.05. DAFR is days after fruit removal.

DAFR	Parameter	CO_2	Temp	Fruit	CO_2 x Temp	CO_2 x Fruit	Temp x Fruit	CO_2 x Temp x Fruit
8	A	**0.001**	0.116	0.359	0.223	0.425	**0.002**	0.876
	gs	**0.050**	0.730	0.746	0.058	0.536	0.063	0.390
	E	**0.001**	**0.001**	0.621	**0.001**	0.504	0.414	0.217
	A/gs	**0.012**	**0.002**	0.787	**0.001**	0.845	0.171	0.678
	A/E	0.435	**0.001**	0.928	**0.001**	0.622	0.891	0.617
14	A	**0.001**	0.104	0.692	0.509	0.700	0.621	0.697
	gs	0.569	0.099	0.282	0.411	0.833	0.938	0.929
	E	0.968	**0.001**	0.362	0.887	0.866	0.892	0.956
	A/gs	0.068	0.357	0.607	0.246	0.831	0.762	0.892
	A/E	**0.001**	**0.009**	0.495	0.735	0.713	0.787	0.872
21	A	**0.001**	**0.004**	0.902	0.521	0.496	0.399	0.483
	gs	0.463	**0.001**	**0.001**	**0.001**	0.389	**0.011**	0.361
	E	0.137	**0.001**	0.056	**0.001**	0.867	0.318	0.424
	A/gs	**0.001**	**0.001**	**0.004**	**0.001**	0.389	0.367	0.372
	A/E	**0.001**	**0.001**	0.312	**0.003**	0.996	0.325	0.729
28	A	**0.001**	**0.001**	0.461	0.097	0.805	**0.012**	0.774
	gs	0.314	**0.001**	**0.003**	**0.001**	0.668	0.287	0.833
	E	0.820	**0.001**	**0.016**	**0.001**	0.813	0.465	0.890
	A/gs	0.377	**0.001**	**0.001**	**0.001**	0.551	0.161	0.698
	A/E	**0.027**	**0.001**	**0.013**	**0.012**	0.497	0.527	0.976

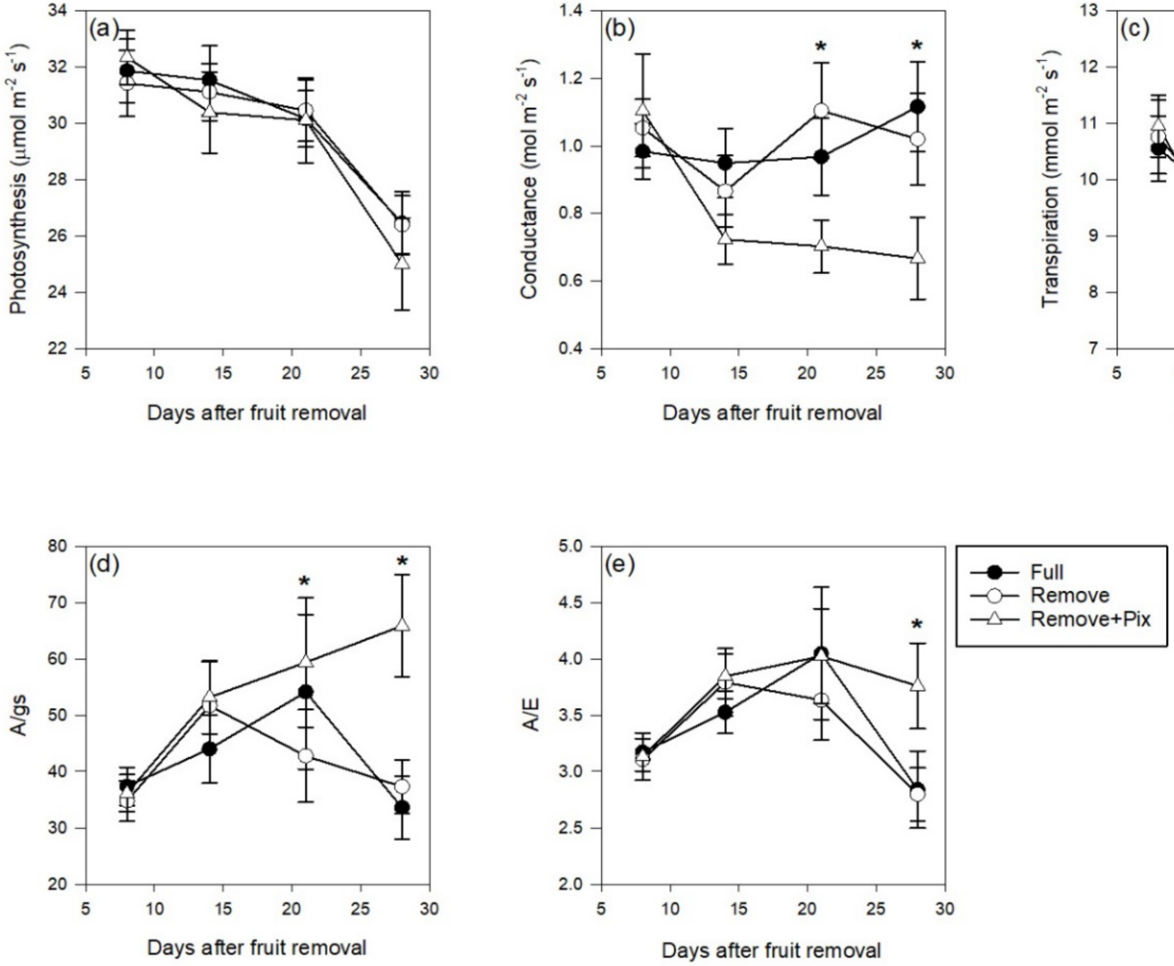


Figure 21: Physiology of plants with three different fruit treatments for Sicot 746BRF. Values represent means ± SE, averaged across CO_2, temperature and cultivar treatments. * represent significant difference at $P<0.05$.

Were there any differences between the two varieties?

Overall, there were generally no interactions for effects of temperature, CO_2, cultivar and fruit removal treatments on VGR, other than a CO_2 by cultivar interaction 29 DAFR (Table 15). This indicates that the VGR responses of the two cultivars to fruit removal and environmental conditions were similar. Averaged across both cultivars and all temperature and CO_2 treatments, cotton with fruit removed consistently had increased VGR (107% - 221%) than the full fruit treatment at 14 DAFR, 22 DAFR and 29 DAFR (Figure 22).

Table 15: Vegetative Growth Rate (VGR) of plants with fruit removed and the full fruit treatment for Sicot 746BRF and CSX 3301 cotton grown at two temperature and two CO_2 environmental conditions. Values represent the F probability. Effects significant at $P<0.05$ are shown in bold. DAFR is days after fruit removal.

	7 DAFR	**14 DAFR**	**22 DAFR**	**29 DAFR**
CO_2	0.757	1.000	**0.016**	0.381
Temperature	0.837	0.413	0.619	**0.041**
Cultivar	**0.001**	0.079	0.528	0.666
Fruit treatment	0.095	**0.001**	**0.001**	**0.018**
CO_2 x Temperature	0.998	0.898	0.742	0.475
CO_2 x Cultivar	0.859	0.630	0.803	**0.032**
Temperature x Cultivar	0.101	0.147	0.854	0.513
CO_2 x Fruit	0.383	0.850	0.333	0.706
Temperature x Fruit	0.201	0.794	0.511	0.843
Cultivar x Fruit	0.116	0.108	0.218	0.765
CO_2 x Temperature x Cultivar	0.632	0.501	0.575	0.945
CO_2 x Temperature x Fruit	0.250	0.549	0.438	0.796
CO_2 x Cultivar x Fruit	0.955	0.914	0.316	0.192
Temperature x Cultivar x Fruit	0.371	0.363	0.398	0.562
CO_2 x Temperature x Cultivar x Fruit	0.902	0.850	0.807	0.166

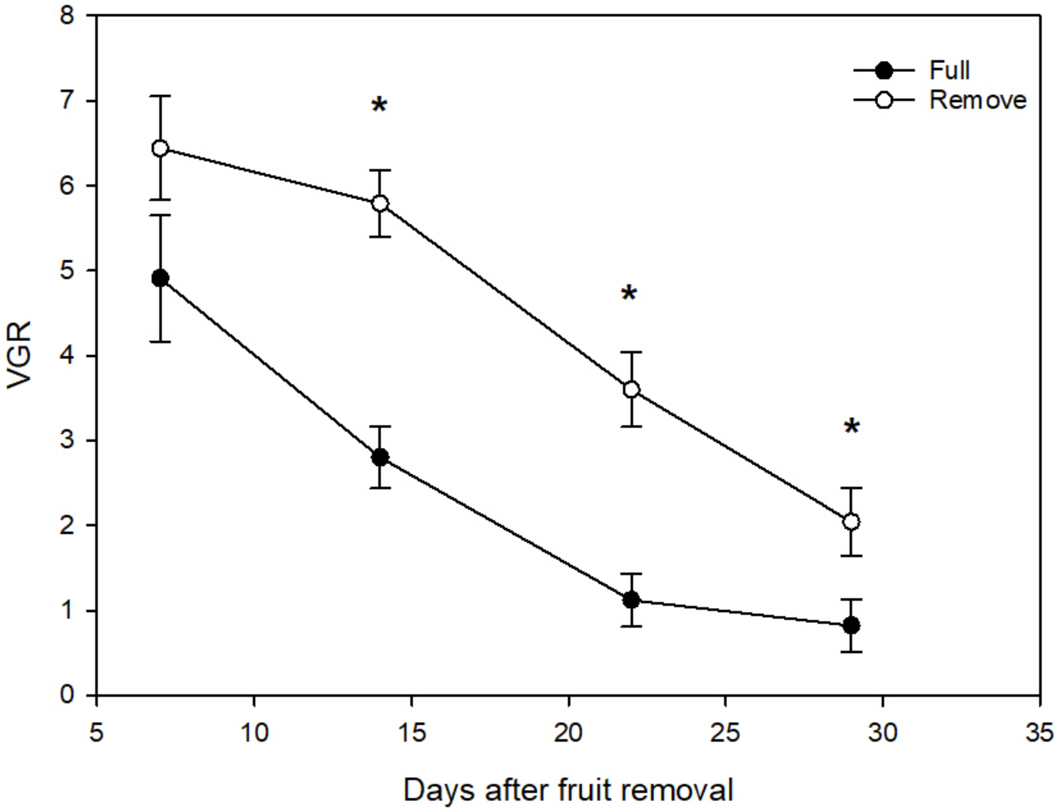


Figure 22: Vegetative growth rate (VGR) of plants with fruit removed and the full fruit treatment for Sicot 746B3F and CSX 3301 cotton grown at ambient and warmer temperatures (TA and TE, respectively) and ambient and elevated CO_2 (CA and CE, respectively). Values represent means ± SE. * indicates significant at $P<0.05$.

Scaling leaf- to canopy- level responses (Milestone 3).

To improve understanding of scaling leaf- to canopy-level responses in warmer temperature and elevated CO_2 conditions, field experiments were conducted during the 2016/17 and the 2019/20 cotton seasons. These experiments tested the hypotheses that (1) canopy CO_2 and H_2O flux can be used to calculate canopy photosynthetic rates; (2) CO_2 drawdown is consistent across all four chambers; and (3) CO_2 drawdown changes throughout the day as rates of gas exchange change.

A glasshouse experiment was conducted to investigate the interactive effects of atmospheric VPD and soil water deficit on the physiology of cotton. The objective of this experiment was to determine the relative impact of soil water deficit (50% field capacity) and atmospheric vapour pressure deficit on the physiology of cotton. Increasing VPD stimulated stomatal closure at any given temperature. Soil water deficit appeared to reduce stomatal conductance of cotton at all temperatures measured, but particularly at lower VPD compared with well-watered plants at the same VPD.

The outcomes of the field and glasshouse experiments investigating leaf and canopy level responses of cotton to altered environmental conditions are shown in Table 16.

Table 16: Summary of results and outcomes for field and glasshouse experiments addressing the scaling of leaf- to canopy-level responses of cotton.

Key outcomes: Scaling leaf- to canopy-level responses		
	Field	**Glasshouse**

Canopy level responses	At the canopy-level, there were no differences in the rate of change of CO_2 between the CA and CE treatments. Canopy-level measurements were affected by the time of day, with the rate of CO_2 drawdown 30% greater at mid-day compared with the rate of drawdown at 9am.	Not applicable.
Leaf-level responses	Cotton leaves grown in the chambers had a greater percentage of carbon than leaves grown in control plots. Furthermore, younger leaves had a greater percentage of carbon (43%) than older leaves (42%).	Increasing VPD stimulated stomatal closure at any given temperature. Although water deficits (50% field capacity) reduced stomatal conductance and consequently transpiration, there were no changes to photosynthetic rates.

Milestone 3.1: Improved understanding of scaling leaf- to canopy- level responses to determine water use efficiency dynamics in the field

Field experiments using the climate chambers were conducted over two seasons (2016/17 and 2019/20) to test the hypotheses: (1) canopy CO_2 and H_2O flux can be used to calculate canopy photosynthetic rates; (2) CO_2 drawdown is consistent across all four chambers; and (3) CO_2 drawdown changes throughout the day as rates of gas exchange change.

In both seasons leaf-level and canopy-level responses were measured. We had planned to improve our understanding of leaf to canopy scaling however malfunctioning gas exchange equipment (Licor 840-A and Licor 6800), resulted in reduced ability to conduct the planned experiments throughout the 2019/20 cotton season. Although we measured both leaf- and canopy-level responses, modelling work still needs to be done to scale water use efficiency dynamics in the field. This is an area for further research. We investigated various methodologies for improving canopy-level measurements, and it is possible that technology such as Image J and Lidar can be used to obtain non-destructive canopy measurements.

Leaf-level responses

Leaf-level physiology was measured on two consecutive days at 118 DAP and 119 DAP. ACi curves are shown in (Figure 23).

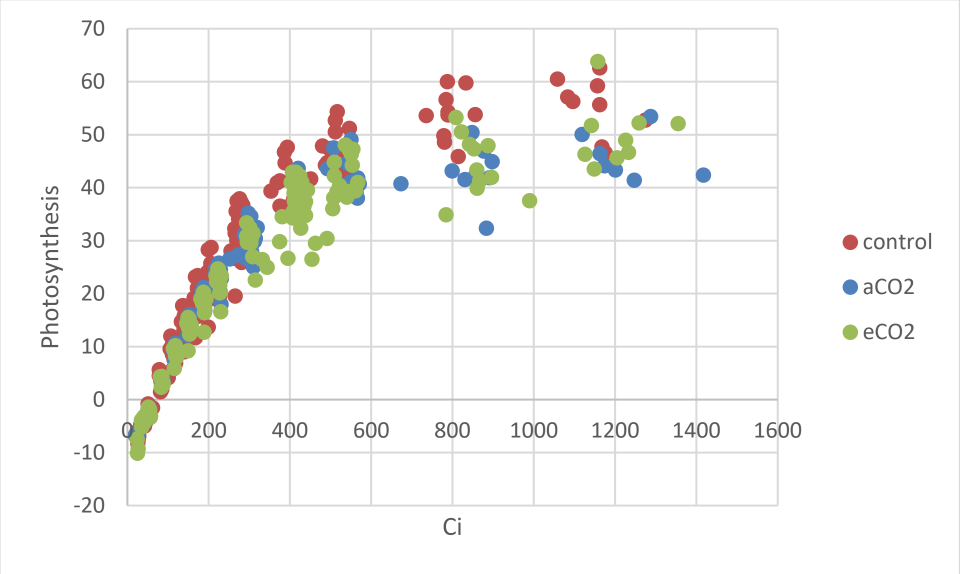


Figure 23: ACi curves of cotton grown in control, ambient CO_2 and elevated CO_2 treatments. Measurements were taken over two consecutive days at 118 (22/2/17) and 119 DAP (23/2/17).

Leaf-level physiology was measured (temperature response curves) at 107 DAP (18/3/20) and 109 DAP (20/3/20). Photosynthesis and transpiration responses to leaf temperature are shown in Figure 24. Photosynthesis and transpiration responses comparing chamber and control data are shown in Figure 25. As the leaves in the control plots were beginning to show signs of aging, we elected to investigate the differences between older and younger leaves both in a representative chamber and in the control plots (measured at 112 DAP (23/3/20)). Although we attempted to measure gas exchange on leaves of different ages, we had equipment malfunction and the data in the file was not saved.

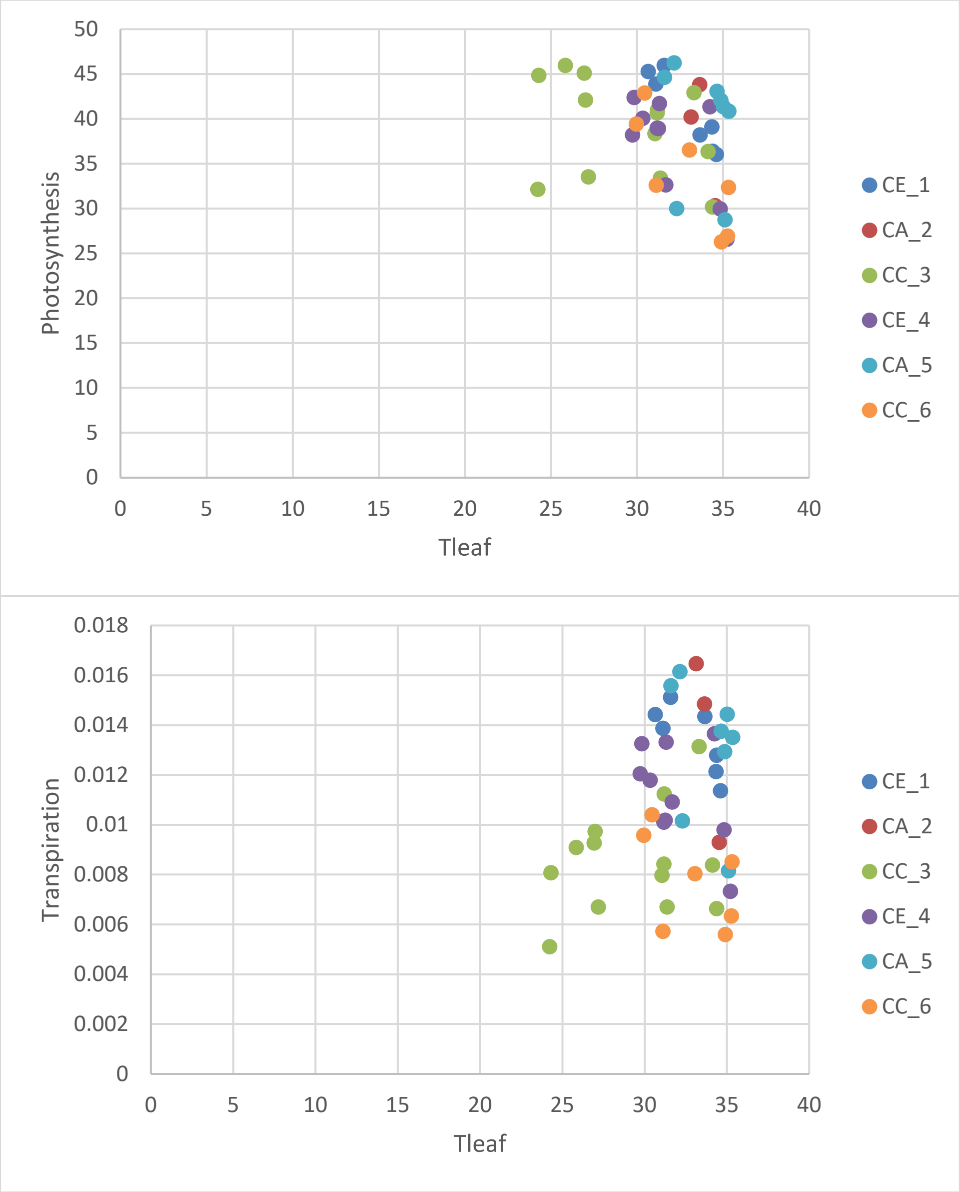
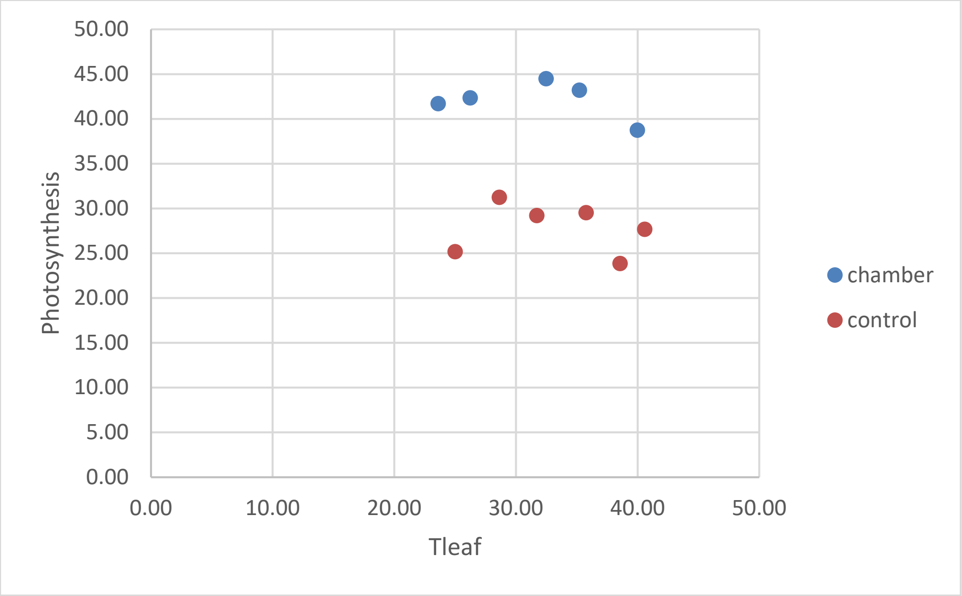


Figure 24: Photosynthesis and transpiration responses to leaf temperature (Tleaf) for chamber and control treatments. Measurements were taken 107 DAP (18/3/20).



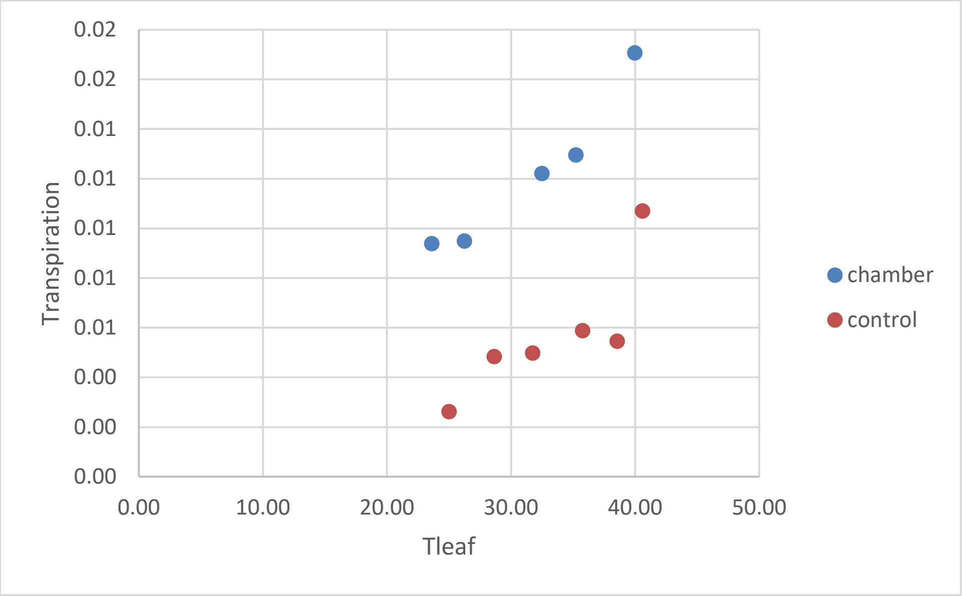


Figure 25: Photosynthesis and transpiration responses to leaf temperature (Tleaf) for chamber and control treatments. Measurements were taken 109 DAP (20/3/20).

There was no difference in the carbon content of cotton leaves grown in the control or chamber treatments (Table 17). However, leaves grown inside the chambers had a greater nitrogen content (4.48%) than leaves grown in the control (4.11%).

Table 17: REML analysis of carbon and nitrogen content of cotton leaves grown in control and chamber environments. Values in bold are F probabilities significant at P<0.05. Leaf samples were collected 113 DAP (24/3/20).

	F. pr.	**Control**	**Chamber**
Carbon (%)	0.217	41.41	41.97
Nitrogen (%)	**0.036**	4.11	4.48

Plants grown in the chambers had a greater percentage of carbon in the leaves (43%; Table 18) than cotton grown in the control plots (42%). Younger leaves also had a greater percentage of carbon (43%) than older leaves (42%). However, there was no effect of either environment or age on the nitrogen in the leaves (Table 18).

Table 18: ANOVA analysis of Environment and Age effects on carbon and nitrogen in older and younger leaves. Values are F. pr. Values in bold are significant at P<0.05. Leaf samples were collected 113 DAP (24/3/20).

	Environment	Age	Environment x Age
Carbon (%)	**0.001**	**0.010**	0.846
Nitrogen (%)	0.645	0.700	0.124

Canopy-level growth responses

Plant height and the number of nodes were measured throughout each season (Figure 26). There was no difference in the plant height or the number of nodes at 113 DAP (24/3/20).

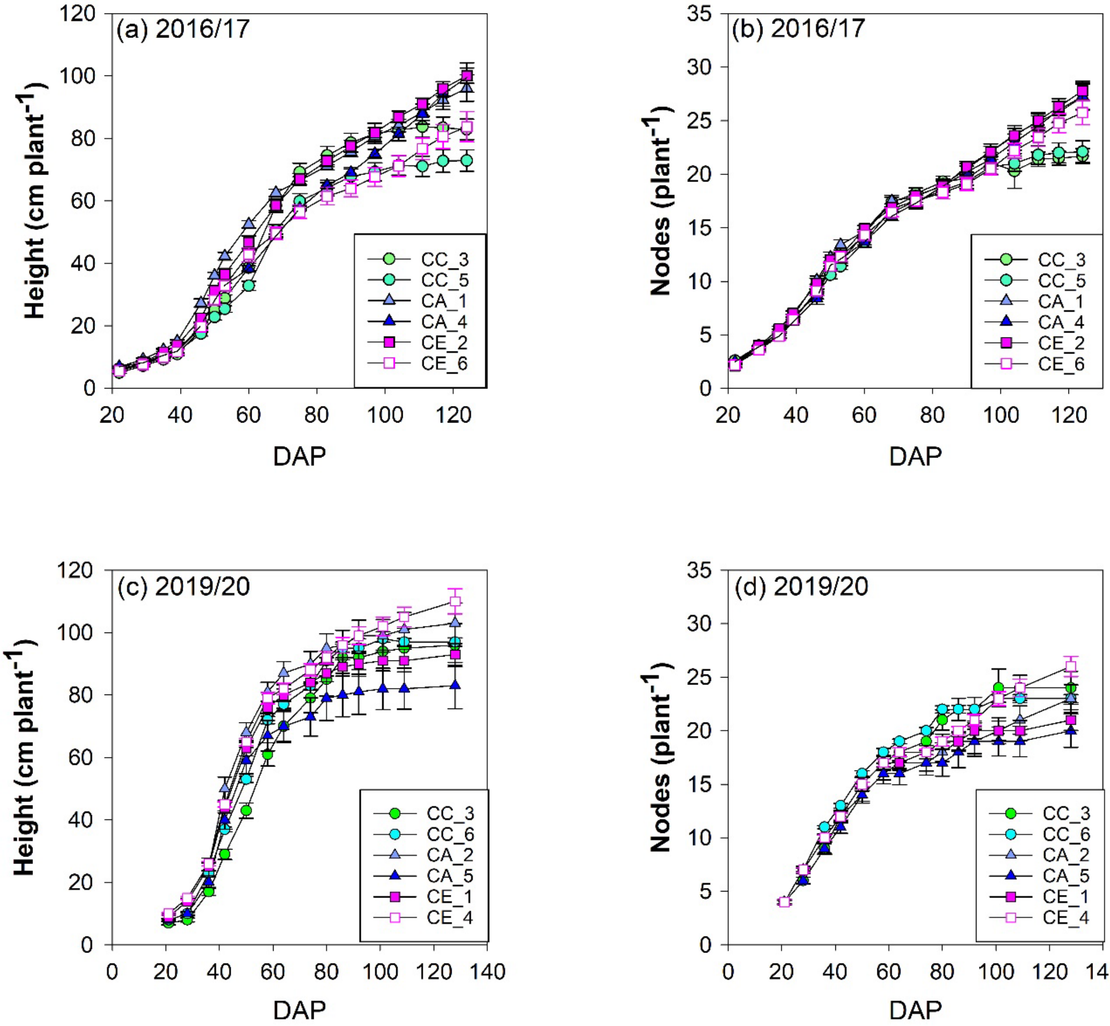


Figure 26: Height (a and c) and number of nodes (b and d) of cotton grown in control (CC), ambient CO_2 (CA) and elevated CO_2 (CE) conditions during the 2016/17 and the 2019/20 cotton seasons. Numbers following CO_2 treatments indicates plot number.

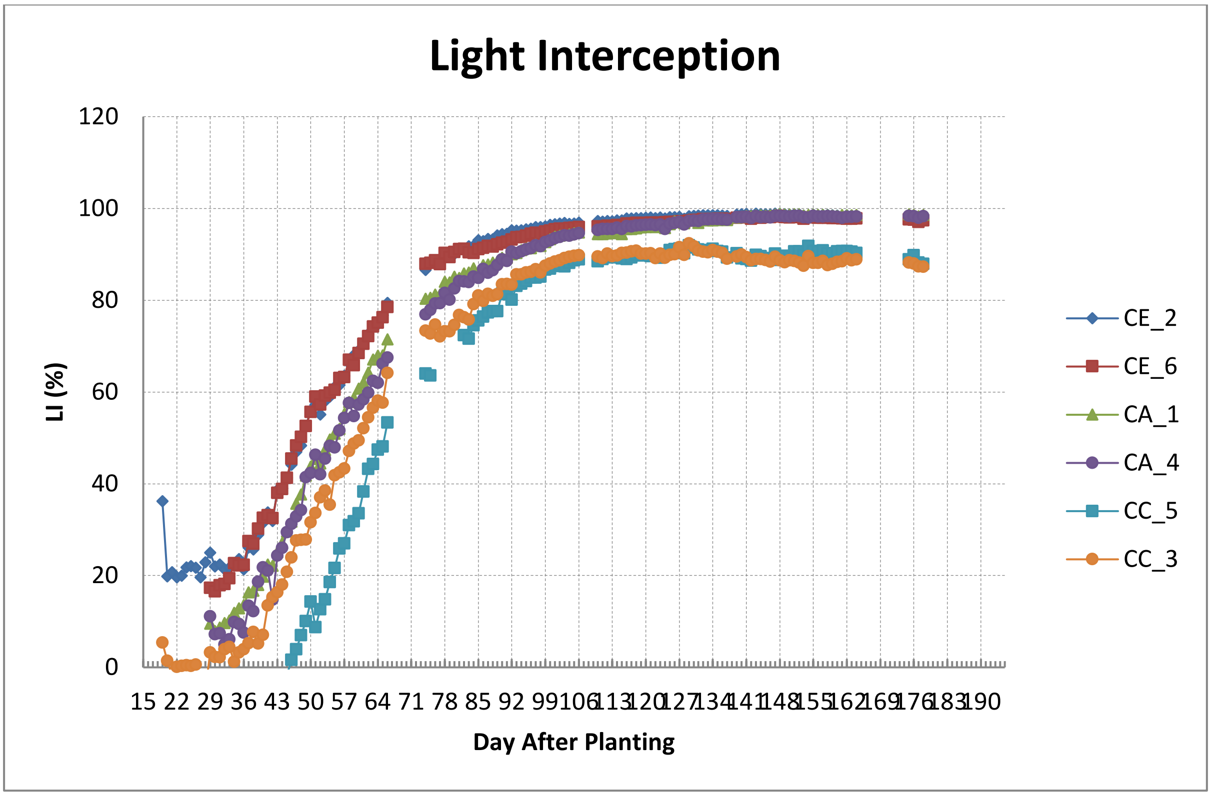


Figure 27: Light interception (%) of cotton grown in control (CC), ambient CO_2 (CA), and elevated CO_2 (CE) conditions. Numbers following CO_2 treatment indicates plot number.

Table 19: Growth measurements for cotton in each plot at the time of the CO_2 drawdown study.

Plot	CC_3	CC_5	CA_1	CA_4	CE_2	CE_6
Plants per half metre	8	7	7	8	6	7
LA (cm^2 per half metre) @ 103 DAP	19305	19946	23159	18538	28143	23203
%LI @ 103 DAP	89	87	94	94	97	96
%LI @ 111 DAP	89	89	94	95	97	96
Height per plant @ 104 DAP	82.6	71.4	84.1	81.5	86.9	78.7
Nodes per plant @ 104 DAP	20.3	21	23.7	23	23.6	22.2
Height per plant @ 111 DAP	83.7	71.1	88.4	87.8	91.1	80.8
Nodes per plant @ 111 DAP	21.4	21.8	24.9	24.5	25	23.8

Table 20: Calculation of chamber leaf area measured at 103 DAP.

Plot	CC_3	CC_5	CA_1	CA_4	CE_2	CE_6
LAROW (cm2)	154440	159568	185272	148304	225144	185624
LAChamber (cm2)	617760	638272	741088	593216	900576	742496
LAROW (m2)	15.444	15.9568	18.5272	14.8304	22.5144	18.5624
LAChamber (m2)	61.776	63.8272	74.1088	59.3216	90.0576	74.2496

Canopy-level responses measured using the chamber Licor 840-A

For the 2019/20 cotton season we were limited to only two functioning Licor 840-A systems, because the equipment had reached end of life. Therefore, if these experiments were to be repeated, new gas exchange monitoring systems will have to be purchased. The Licor 840-A system has been superseded by the Li-850.

Changes in CO_2 and air temperature over each measurement period are shown in

Table 21. The rate of CO_2 drawdown ranged from 0.102 – 0.370 ppm/sec. The rate of temperature increase in the chambers ranged from 0.015 – 0.040 ^{o}C/sec.

Table 21: CO_2 and temperature changes over three time-periods measured in the chambers during the drawdown experiment.

Date	DAP	Node	CO_2 Trt	Δ CO_2 (ppm/sec)			Δ Temperature (°C/sec)		
				9am	12pm	3pm	9am	12pm	3pm
14/02/2017	110	1	CE	-0.292	-0.290	-0.370	0.026	0.031	0.036
		2	CE	-0.233	-0.245	-0.245	0.024	0.026	0.030
		3	CA	-0.203	-0.239	-0.270	0.015	0.021	0.021
		4	CA	-0.260	-0.247	-0.247	0.026	0.040	0.023
17/03/2020	106	1	CE	-0.215	-0.263	-0.199	0.018	0.023	0.018
		2	CE	-0.102	-0.351	-0.248	0.019	0.031	0.025
19/03/2020	108	1	CE	-0.234	-0.299	-0.291	0.020	0.024	0.023
		2	CE	-0.170	-0.296	-0.243	0.017	0.024	0.025

Our data indicates that there were no differences in the rate of change of CO_2 between the different CO_2 treatments (P=0.670, Table 22). However, the time of day did affect the rate of change in CO_2 (P=0.049, Table 22). The rate of CO_2 drawdown was the greatest at 12pm, averaging a rate of decline of 0.279 ppm/sec (P= 0.049). This was 30% greater than the rate of drawdown at 9am. There was no difference in the rate of CO_2 drawdown at 12pm and 3pm. CO_2 treatment and the time of day had no effect on the rate of air temperature changes inside the chambers during measurement (Table 22).

Table 22: REML analysis of CO_2 treatment and Time effects on the rates of change in CO_2 and temperature inside the chambers. Values are F. pr. Values in bold are significant at P<0.05.

	CO_2Trt	**Time**	**CO_2 x Time**
Δ CO_2	0.670	**0.049**	0.493
Δ temperature	0.956	0.073	0.447

Using other technologies to improve our understanding of leaf- to canopy- level scaling

We explored the options of using Image J and LiDAR technology to gain information on canopy level responses. We tested Image J, in collaboration with Viv Rolland (CSIRO), and found that the shadows in the photographs prevented proper segmentation. It was determined that this system requires quite a bit of set-up to work but could potentially be used in future experiments. Viv Rolland indicated that he would be interested in assisting, if we decided to pursue this avenue of research.

Milestone 3.2: Improved understanding of carbon and water fluxes of cotton grown under soil water deficits and high atmospheric vapour pressure deficit (VPD) in the glasshouse

Soil water deficit reduced plant growth

The soil water deficit treatment decreased plant growth, including plant height and reducing the number of nodes. Cotton grown in the drought treatment were 8 – 13 % shorter than plants grown in the well-watered treatment at 14 and 21 DAT, respectively (Figure 28). At 7, 14 and 21 DAT, cotton grown in the drought treatment had between 4 - 6% fewer nodes than cotton grown in the well-watered treatment.

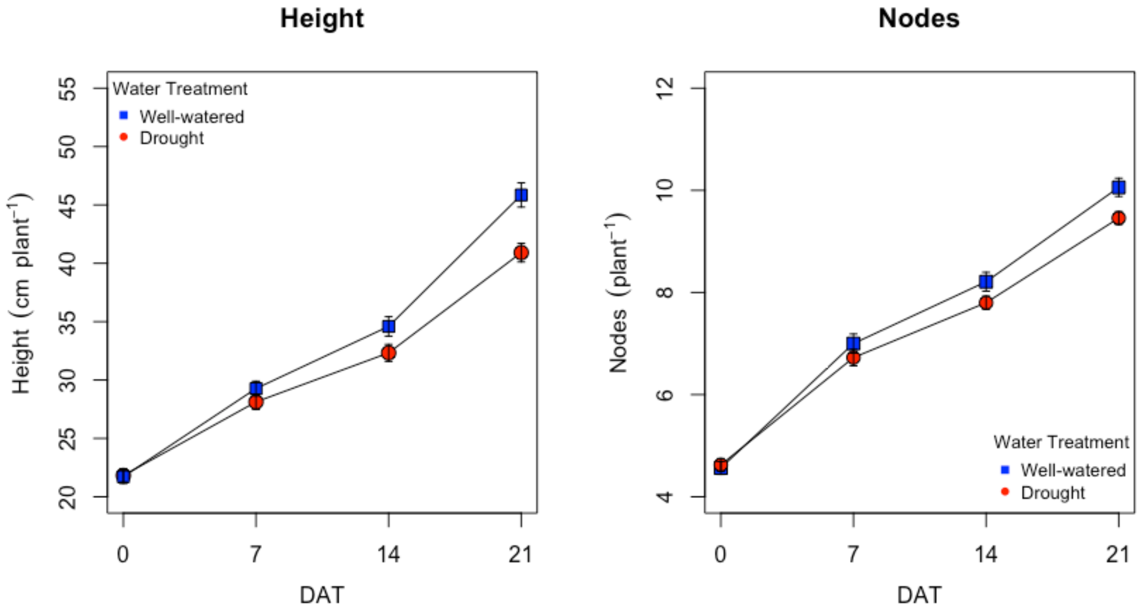


Figure 28: The effect of soil water deficit treatment on growth, height (left) and the number of nodes (right), measured on a weekly basis. Plots and bars are means and standard errors, respectively. DAT is days after water treatment commenced. Number of samples for height and number of nodes measured on days 0, 7, 14 and 21 were 22, 22, 22 and 18 plants for well-watered treatment and 21, 21, 21 and 17 for soil water deficit treatment, respectively.

The effect of SWD (and growth) on leaf water potential (Ψ_{leaf})

There was a weak relationship between Ψ_{leaf} and plant height ($R^2=0.20$), and the height decreased Ψ_{leaf} (P=0.012). The number of nodes also formed a weak relationship with Ψ_{leaf} ($R^2=0.14$), but it did not affect Ψ_{leaf} (P=0.160). Our results suggested that the soil water content at 50% field capacity was sufficient to decrease the growth, but insufficient to substantially alter the midday hydraulic status (Figure 29).

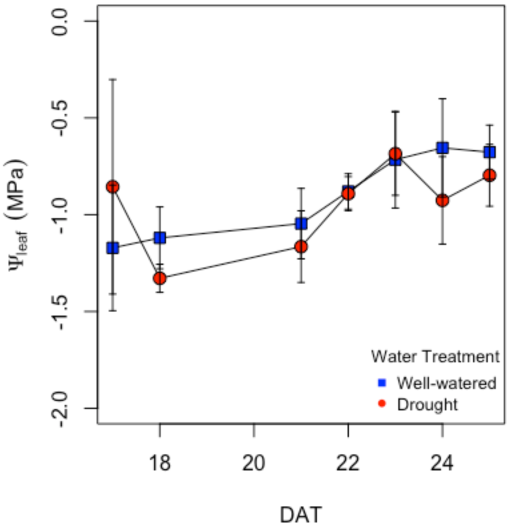


Figure 29**:** Midday leaf water potential (Ψ_{leaf}), under well-watered treatment and soil water deficit treatment, measured daily. DAT is days after water treatment commenced. Plots and bars are means and standard errors, respectively, of number of samples measured each day. For well-watered treatment, 17, 18 and 21 DAT n=2, 22 DAT n=6, 22 and 24 DAT n=3 and 25 DAT n=4. For soil water deficit treatment, 17, 18, 21 and 23 DAT n=2, 22 DAT n=6, 24 DAT n=3 and 25 DAT n=4.

The effect of SWD on plant physiology

We observed that g_s of cotton grown under SWD conditions was lower than g_s in well-watered soil (P<0.001). The SWD also decreased *E* (P=0.004), while there was no effect of soil water treatment on *A* (P=0.116). At any given DAT, Ψ_{leaf} was not affected by SWD (P=0.827) which suggested that the hydraulic system in cotton may cope with moderately to severely low soil water content.

The effect of increasing VPD and warmer temperature on plant physiology

Increasing VPD stimulated the stomatal closure at any given temperature (P<0.001). Our results indicated that RH was the main factor that contributed to the stomatal closure as we observed that the warmer temperature did not alleviate the reduction in g_s at increasing VPD, (Figure 30). The results also showed that the stomata did not shut completely under SWD (Figure 30) which did not lead to an embolism in the xylem. Therefore, the Ψ_{leaf} was not affected by the SWD (Figure 29).

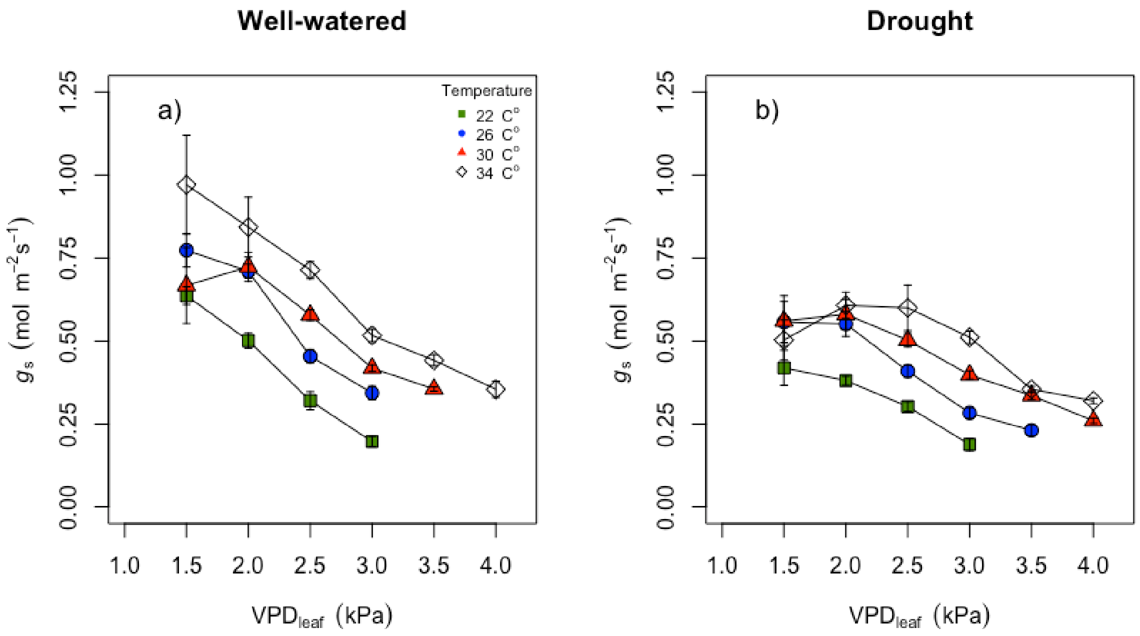


Figure 30: Effects of vapour pressure deficit and temperatures (22, 26, 30 and 34°C) on stomatal conductance (g_s) in well-watered (left) and soil water deficit (right) treatments. Points and bars are means and standard errors, respectively, of 14 plants for each water treatment.

We observed that the warmer temperature increased *E* at lower VPD then slightly decreased as the VPD continued to rise (Figure 31). This pattern was an indication that the stomatal closure had some effect on *E* as we observed the reduction in *E* occurred at approximately 2.5 and 3.0 kPa where the sharp reduction in g_s occurred. However, we also observed that g_s did not close completely, and it may allow the cotton to exhibit the high transpiration rate at increasing VPD. Our results suggest that this cotton variety has no limiting point for transpiration; the cotton will continue to use water, particularly under warmer climatic conditions.

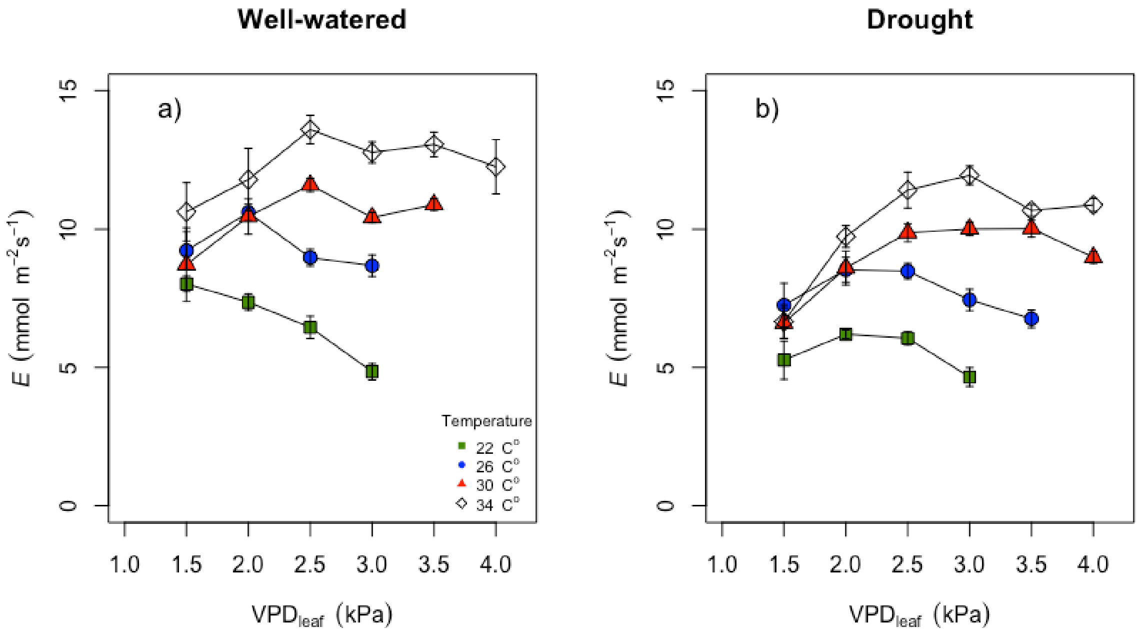


Figure 31: Effects of vapour pressure deficit and air temperature (22, 26, 30 and 34°C) on transpiration (*E*) in well-watered (left) and soil water deficit (right) treatments. Points and bars are means and standard errors, respectively, of 14 plants for each water treatment.

Warmer temperatures increased *A*, while increasing VPD decreased *A* (Figure 32). The results indicated that it could be the effect of stomatal closure or increasing temperature rather than the direct response to the increasing VPD. This assumption is supported by C_i/C_a ratio results as this ratio decreased at increasing VPD at any given temperature, (Figure 33).

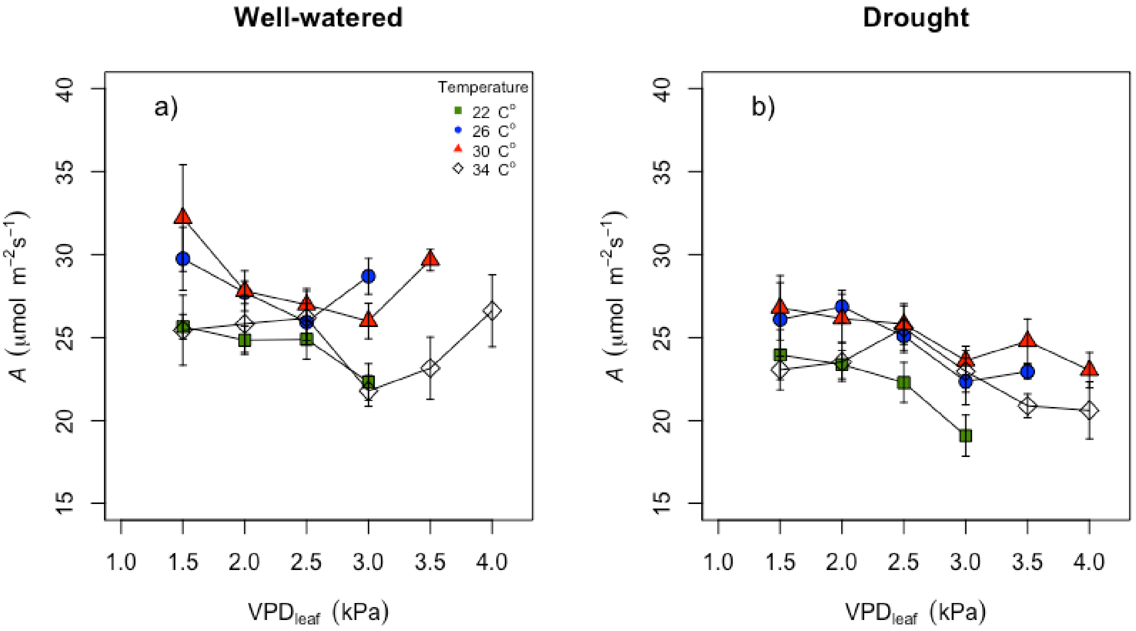


Figure 32**:** Effects of vapour pressure deficit and measurement temperature (22, 26, 30 and 34°C) on photosynthesis (*A*) in well-watered (left) and soil water deficit (right) treatments. Points and bars are means and standard errors, respectively, of 14 plants for each water treatment.

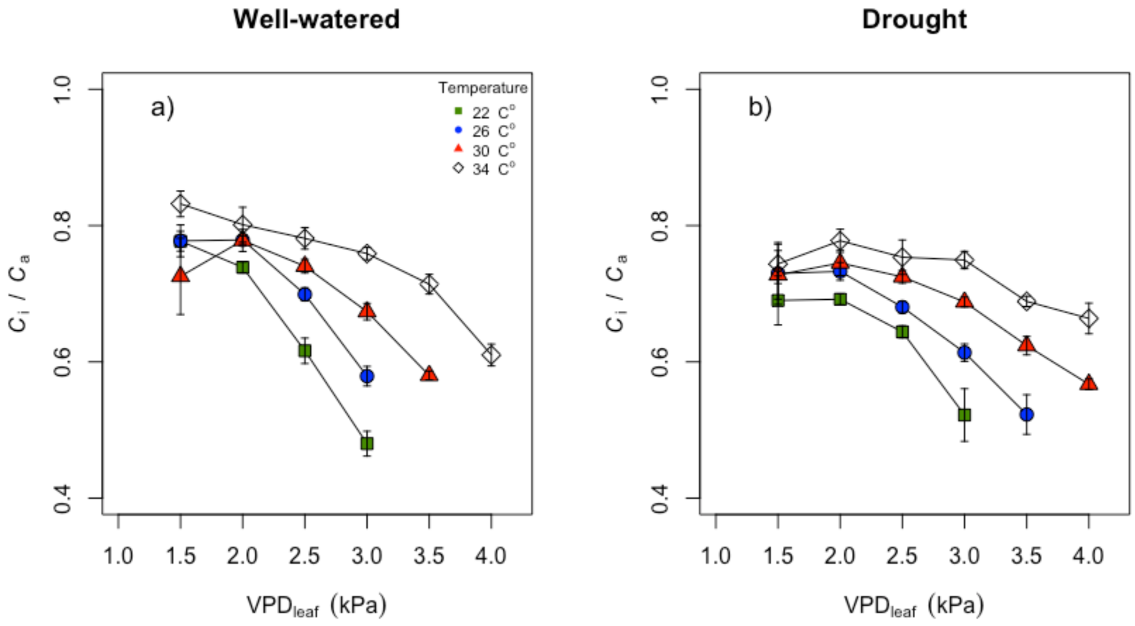


Figure 33**:** Response of intercellular CO_2 (C_i) to ambient CO_2 (C_a) ratio to vapour pressure deficit across different measurement temperatures (22, 26, 30 and 34°C) under well-watered (left) and soil water deficit (right) treatments. Points and bars are means and standard errors, respectively, of 14 plants for each water treatment.

Table 23: Analysis of variance (ANOVA) for the interactive effects of drought, vapour pressure deficit (VPD) and temperature on stomatal conductance (g_s), photosynthesis (*A*), transpiration (*E*), intercellular CO_2 (C_i) and ratio of intercellular CO_2 (C_i) to ambient CO_2 (C_a) in cotton. Values in the

table represent P-values; significant results (P<0.05) are indicated in bold type and italics indicate marginal significance. The results are based on 14 samples for each water treatment.

Factors	**Gas exchange parameters**			
	g_s	***A***	***E***	C_i/C_a
VPD	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Temperature	**<0.001**	**0.004**	**<0.001**	**<0.001**
Water treatment	**<0.001**	0.116	**<0.001**	0.238
VPD x Temperature	**<0.001**	0.250	**<0.001**	**<0.001**
VPD x Water treatment	**<0.001**	0.235	**0.008**	**0.008**
Temperature x Water treatment	**0.035**	0.789	**0.003**	0.105
Temperature x VPD x Water treatment	0.151	0.439	0.146	**<0.001**

The effect of SWD, increasing VPD and warmer temperature on plant physiology

There were no three-way interactive effects of SWD, VPD and temperature on g_s (P=0.151), *E* (0.146) and *A* (P=0.439). We also did not observe the two-way interactive effects between SWD and VPD or warmer temperature on *A* (P=0.250 and P=0.789, respectively). SWD reduced the sensitivity of g_s to increasing VPD as we observed that g_s in SWD cotton decreased at a slower rate at each increment of VPD, (Figure 30). This finding suggests that SWD of cotton may have a greater capacity to adapt to drought conditions than well-watered cotton. The results indicated that SWD may have limited or controlled water use efficiency in cotton as we observed that *E* under the SWD was lower than in the well-watered cotton. We also noted that the effect of SWD on WUE may be compromised by increasing temperatures. Overall results suggested that the cotton grown in well-watered soil (irrigated cotton) are likely to use more water under warmer and drier climate (i.e. high VPDa). This is based on the instantaneous response of cotton to increasing VPD, using the unified stomatal model by Medlyn et al. (2011) (Figure 34). Nevertheless, it is important to note that the increasing VPD exacerbated the reduction in g_s in cotton grown in SWD, which could result in higher water conservation and may reduce optimal growth.

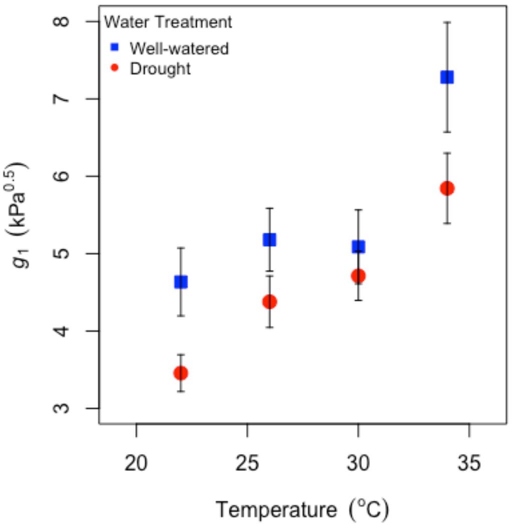


Figure 34**:** The fitted parameter g_1 or leaf diffusive behaviour obtained from the unified stomatal model, see Eq. (1). Points and bars represent means and 95% CI, respectively. Data are significantly different when 95% CIs do not overlap.

$$g_s = g_0 + 1.6\left(1 + \frac{g_1}{\sqrt{D_s}}\right)\frac{A}{C_a} \qquad (1)$$

Our results show that the slight reduction in E was affected by the stomatal closure. For example, the sharp reduction in g_s of cotton grown in well-watered and SWD started at 2.0 kPa and at 2.5 kPa, respectively, where E also started to decrease under both water treatments. This result indicated that there was a corresponding effect of g_s and E to increasing VPD and SWD. This finding also indicated that air temperature may have a stronger effect on E than RH as we observed the reduction in E at the higher temperature was greater than E at lower temperature at any given VPD.

Implications of the results

E and g_s responded to different climate factors but may not result in a significant change in carbon assimilation. We found that increasing VPD decreased g_s and that warmer temperature increased E. g_s reacted readily to increasing VPD suggested that g_s in this cotton variety may be restricted under drier climate, but the plant will continue to use water as its E will increase with warmer air temperatures (within the level of SWD that we tested). On the other hand, our results suggested that higher VPD decreased A and warmer temperature increased A which indicated that the difference in carbon assimilation under the combination of drier and warmer conditions may be minimal. Future study should address the growth, yield and the response of this cotton variety to longer exposure to the studied condition as we found in the source-sink experiment that A does not associate with the growth.

Ability to better model and predict the impact of future climate scenarios on water use and yield

To address this milestone, we have used a multi-faceted approach, using Cottassist to explore the climatic changes that have already occurred at key locations throughout the Australian cotton industry. We also adjusted the external parameters of the OZCOT model to better understand how the model responds to changing atmospheric CO_2 concentration and air temperature parameters. Key outcomes are outlined in Table 24.

Table 24: Key outcomes for better modelling and prediction of the impact of future climate scenarios on water use and yield.

Key outcomes: Ability to better model and predict the impact of future climate scenarios on water use and yield
CO_2 and temperature parameters were tested using the OZCOT model to simulate cotton yield and crop-level water use efficiency responses to warmer temperatures and elevated atmospheric CO_2. Our study demonstrated that the simulations are consistent with other experimental research, suggesting that yield and crop WUE efficiency may be increased with elevated atmospheric CO_2 concentrations, but that these positive effects may become far more variable and/or be negated combined with warmer air temperatures. We found that the OZCOT model (version 2014) can be used to simulate the effects that climate change may potentially have on the yield and water use efficiency of cotton; however, further study may be required to more comprehensively assess the coding to ensure that the algorithms underpinning these results are consistent with current physiological understanding. Our Cottassist analyses suggests that there has already been an increase in the number of day degrees, an increase in the number of hot days, and a decrease in the number of cold days throughout the cotton growing season for eight key locations spanning the Australian cotton growing areas. These findings may help define adaptation strategies by linking regions with similar past and present climatic conditions.

Testing the capacity of OZCOT to model yield and water use efficiency responses to climate change

The OZCOT model (version 2014) was used to explore how changing the temperature and CO_2 parameters altered yield and water use efficiency predictions of cotton grown at Narrabri, NSW. Mean cotton season air temperature using recorded silo data for Narrabri, NSW from 1960 to 2019 is shown in Figure 35. The trend line indicates that air temperatures have been warming over time. The implications of this are a warmer start to the cotton season, potentially resulting in faster emergence and crop development, as well as reduced susceptibility to some diseases (e.g. black root rot; *Thielaviopsis basicola*).

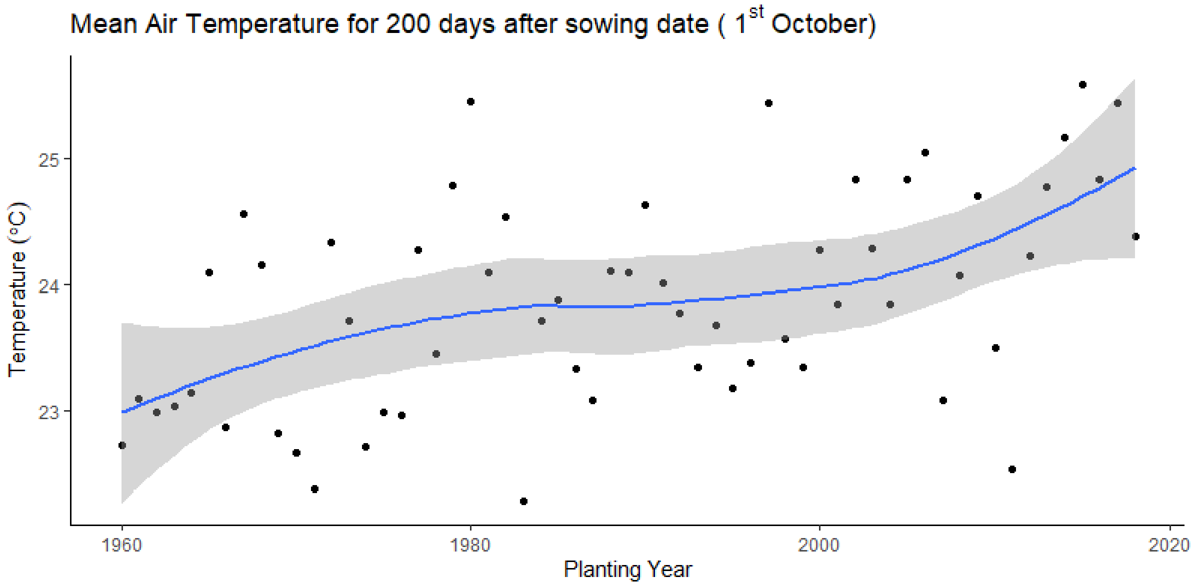


Figure 35: Mean air temperature from 1960 to 2019 at ACRI, Narrabri (-30.2083, 149.5967) for 200 days after sowing (1st October). Grey shading shows 95% confidence interval.

In addition to the trend shown in Figure 35, climate change projections suggest that air temperatures in Australian cotton growing regions are likely to continue to rise. Figure 36 shows how a 2°C rise in mean air temperature may influence cotton season temperatures in Narrabri over time.

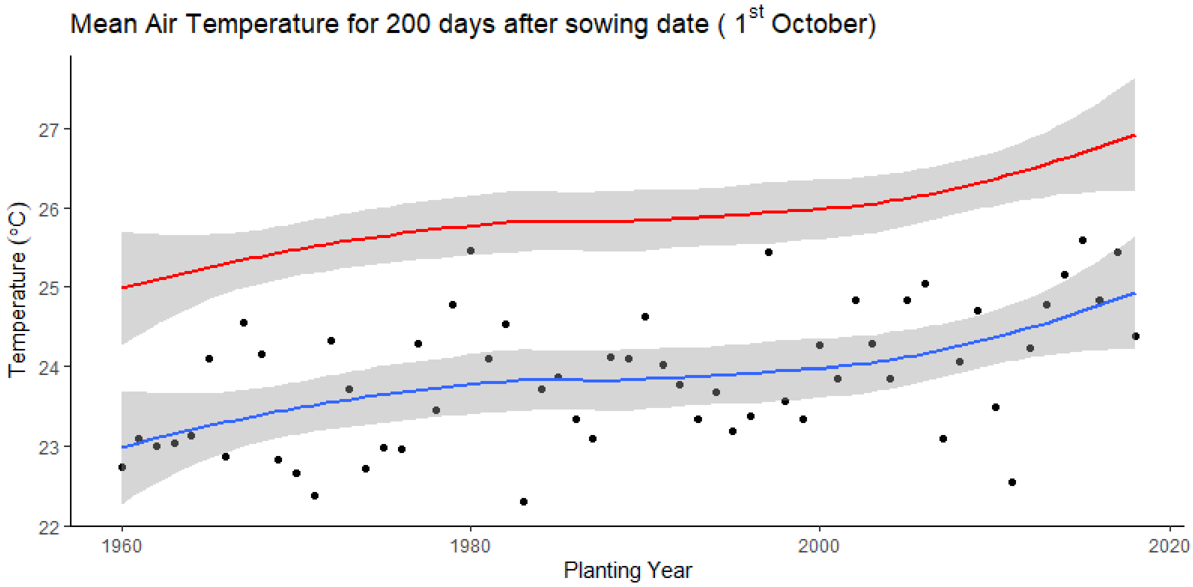


Figure 36: Mean air temperature using recorded silo data (blue) from 1960 to 2019 at ACRI, Narrabri (-30.2083, 149.5967) for 200 days after the sowing date (1st October). Red line shows the projected mean air temperatures for ACRI with a 2°C rise in temperature. Grey shading shows 95% confidence interval associated with each line.

There have been rapid increases in atmospheric CO_2 concentration over the past 200 years due to world-wide industrial activity from a pre-industrial concentration of about 280 ppm to 406 ppm in 2017, with projections for more rapid increases in the future. CO_2 levels were originally set at 330 ppm in the OZCOT model, but current atmospheric [CO_2] exceeds 400ppm. Simulated lint yield in response to eight past, present and future CO_2 levels (300, 350, 400, 450, 500, 550, 600 and 650 ppm) are shown in Figure 37. The OZCOT simulation shows that increased atmospheric CO_2 concentration will increase lint yield in any given year. The model also highlights the variability of increased yield, where the benefits of elevated CO_2 are much greater in some years (e.g. the year 2000) than other years (e.g. the year 2010). The photosynthetic rate is directly proportional to CO_2 concentration, but the model algorithm does not implicitly include interactions between CO_2 and temperature, despite the interaction between the two parameters being supported by our data. For example, the model may be missing interactions relating to reduced effects of elevated CO2 in warmer temperature conditions. Although the model showed that atmospheric CO_2 concentrations increased lint yield through increased photosynthetic rate built into the model, we are not sure that this function has been built with the appropriate photosynthetic limitations and thus further research may be required.

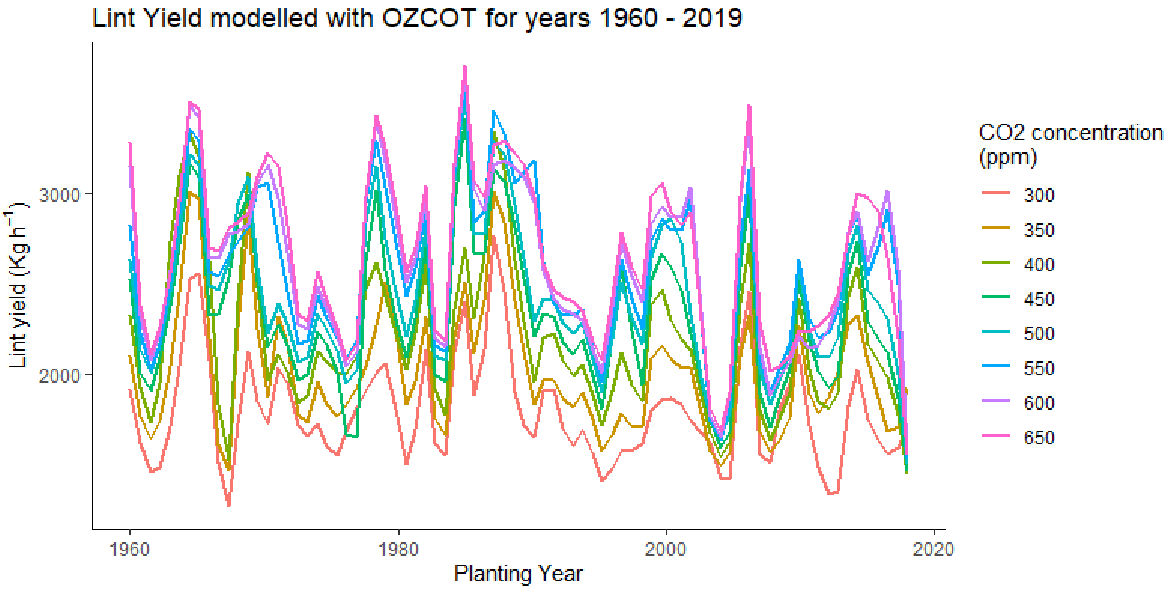


Figure 37: Lint yield (kg lint ha^{-1}) from 1960 to 2019 at ACRI, Narrabri for CO_2 concentrations ranging from 300 to 650 ppm.

With projected climatic changes, elevated atmospheric CO_2 concentrations are likely to be accompanied by warmer temperatures. We attempted to compare the OZCOT model with the chamber data, however, there were not enough seasons where final yield was obtained in the field experiments to confidently run this model using only field data. In addition, the OZCOT model only allows for standard temperature changes throughout the whole season, rather than changes in daily temperature, as we saw in the field (data not shown). Instead of using field chamber data, simulated lint yield responses to CO_2 concentration at both ambient (silo recorded) air temperatures and elevated temperatures (2°C warmer) are shown in Figure 38. At every CO_2 concentration assessed, the median lint yield of cotton was higher at ambient temperatures than at elevated temperatures. This suggests that warmer temperatures will reduce the yield benefits of rising atmospheric CO_2 concentration. Furthermore, the OZCOT simulation indicates that there is likely to be increased variability with integrated warmer temperatures and elevated CO_2. For example, the box for lint yield of cotton grown at warmer air temperatures and 400 ppm atmospheric CO_2 is much larger than the variation in lint yield at ambient temperature and 400 ppm CO_2. The simulated chances of obtaining increased lint yields are greater and less variable for cotton grown at silo recorded temperatures and high CO_2 than at elevated temperatures at each CO_2 concentration. Therefore, there is the potential for very high yields in the good years, but with warmer temperatures there is also the potential for very low yields.

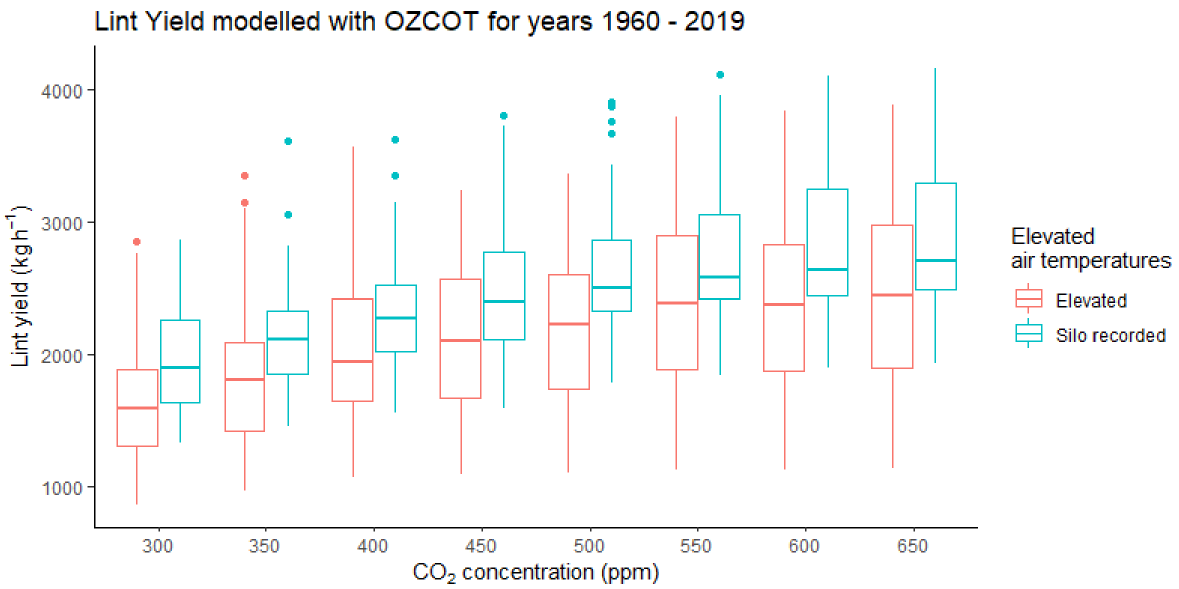


Figure 38: Lint yield (kg lint ha^{-1}) from 1960 to 2019 at ACRI, Narrabri across a range of CO_2 concentrations (ppm) modelled using silo recorded temperature data (blue) and 2°C elevated temperature data (red).

The simulated response of crop water use efficiency (WUE, kg lint mm^{-1}) with increasing atmospheric CO_2 and two temperature regimes are shown in Figure 39. The model suggests that crop WUE is increased with elevated CO_2, and warmer temperatures decreasing the potential for improved WUE at each CO_2 concentration. Elevated temperature greatly increases the variability in WUE at each CO_2 concentration, compared with the WUE of silo recorded temperatures.

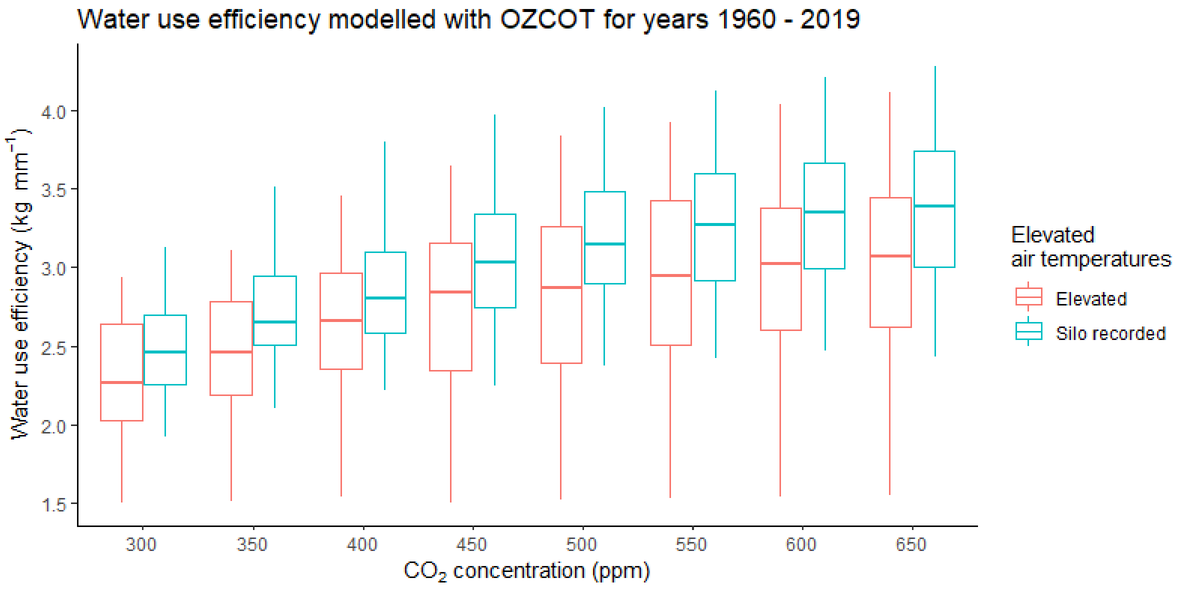


Figure 39: Crop water use efficiency (kg lint mm^{-1}) from 1960 to 2019 at ACRI, Narrabri across a range of CO_2 concentrations (ppm) using silo recorded temperature data (blue) and 2°C elevated air temperature data (red).

In conclusion, we used the OZCOT model to simulate cotton yield and crop-level water use efficiency responses to warmer temperatures and elevated atmospheric CO_2. The simulations are consistent with other research, suggesting that yield and crop WUE efficiency may be increased with elevated atmospheric CO_2 concentrations, but that these positive effects may become far more variable and/or be negated combined with warmer air temperatures. Therefore, the OZCOT model (version 2014) can be used to simulate the effects that climate change may potentially have on the yield and water use efficiency of cotton; however, further study may be required to more comprehensively assess the coding to ensure that the algorithms underpinning these results are consistent with current physiological understanding.

Understanding climatic changes that have already occurred throughout key Australian cotton growing regions

To complement our assessment of using OZCOT simulations to better model and predict the impact of future climate scenarios on water use and yield of cotton; our approach then expanded to understanding the degree of climatic change throughout key Australian cotton regions. Improved understanding of changes that have already occurred may provide an opportunity to apply existing knowledge from other regions that may be now experiencing similar climatic conditions that other regions have experienced in the past. This may help to define the adaptation strategies and develop an agenda for producing Australian cotton in a changing climate.

Climate data from eight locations spanning the current Australian cotton industry were assessed to explore the temperature trends over three periods; 1957 to 2017, 1957 to1996 and 1997 to 2017. We compared using the old day degree function (Equation 1) with using the new day degree functions (Equation 2 and Equation 3):

$$DDold = \frac{(Tmax-12)+(Tmin-12)}{2} \text{ (1) where } T_{min} > 12^{o}\text{C}$$

$$DDnew = \frac{(Tmax+Tmin)}{2} - 15.5 \text{ (2) where } T_{max} < 35^{o}\text{C}$$

$$DDnew = \frac{(35+Tmin)}{2} - 15.5 \text{ (3) where } T_{max} \geq 35^{o}\text{C}$$

Where DD is day degrees, and T_{max} and T_{min} are maximum and minimum air temperatures in ^{o}C each day. The new day degree functions better reflect optimum growing conditions for cotton, by accounting for reduced plant growth and function in very cold (<15.5^{o}C) and very hot (>35^{o}C) temperatures.

*Analysis using the old day degree function (*Figure 45*):* From 1957 to 1996, there was an increase in the number of degree days at Emerald, and during the period 1997 to 2017 there was an increase in the number of degree days at Griffith and Moree. Although the slopes of each regression were mostly positive, suggesting a possible increasing trend in day degree accumulation, the variation in the number of degree days between years was large over a relatively short timeframe. However, the significant increasing trend in the number of degree days from 1957 to 2018 for all eight locations indicates an increase in the number of hot days and warmer night-time (minimum) air temperatures.

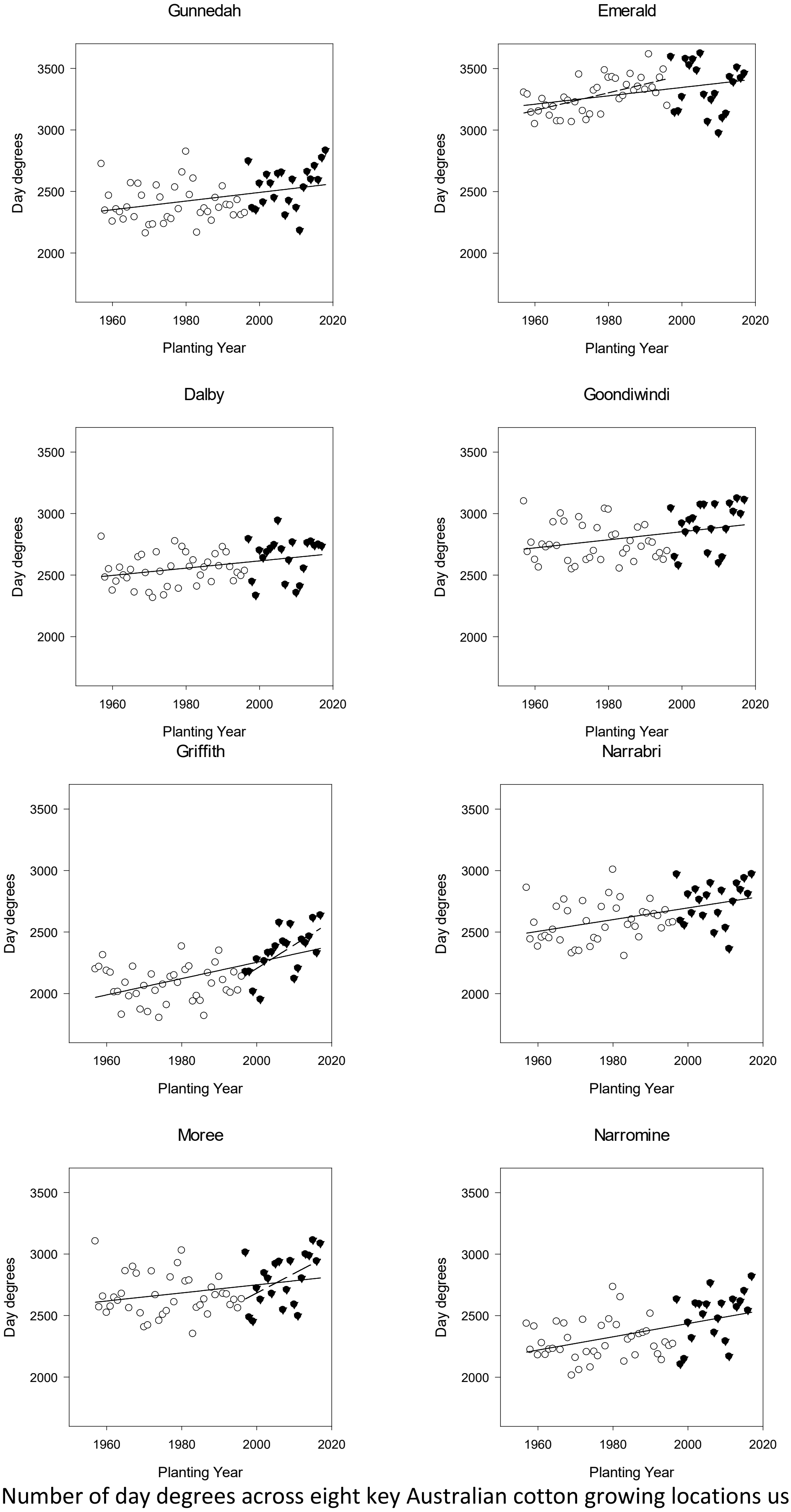
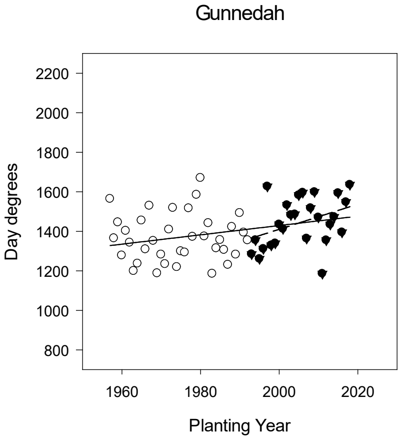
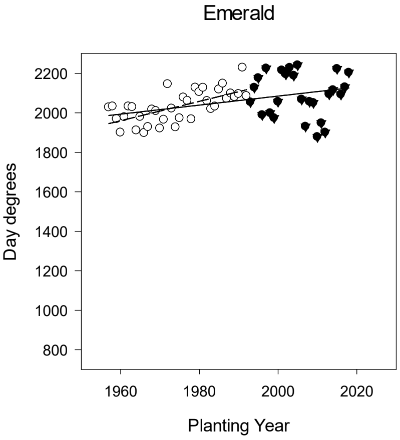
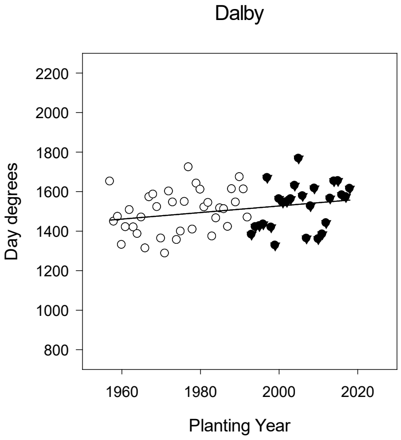


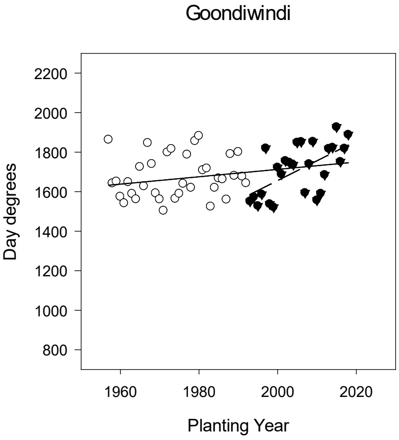
Figure 40: Number of day degrees across eight key Australian cotton growing locations using the old day degree function.

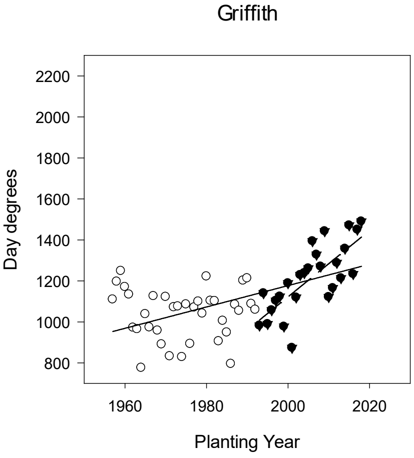
__Analysis using the new day degree function (Figure 41)__: From 1957 to 2018, there has been an increase in the number of degree days across all eight locations. From 1957 to 1996, there has been an increase in the number of degree days at Emerald, and during the period 1997 to 2018 there has been an increase in the number of degree days at Goondiwindi, Griffith, Gunnedah, Moree, Narrabri and Narromine.

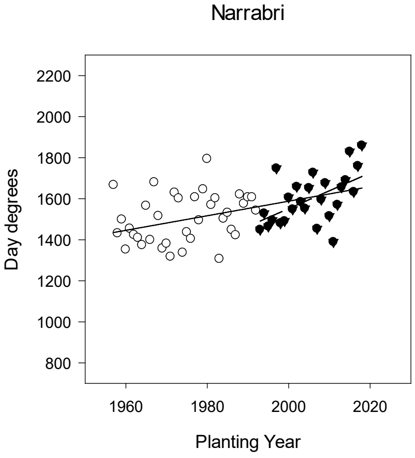


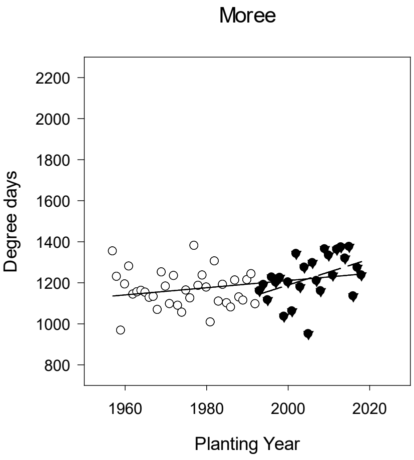












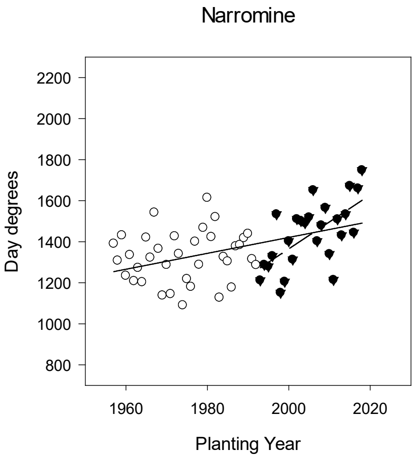


Figure 41: Number of day degrees across eight key Australian cotton growing locations using new day degree functions.

A comparison of the trends in seasonal day degree accumulation across the eight key locations using the old and new day degree functions is shown in Table 25. Overall, our data indicates that there has been an increase in seasonal heat accumulation in eight key cotton growing regions assessed, from the period 1957 to 2018, using both the old and new day degree functions. The increase in the number of day degrees from 1957 to 1996 at Emerald is consistent when assessing the trends using both old and new day degree functions. Over the period from 1997 to 2018, the new day degree function captures an increasing trend in seasonal heat accumulation at Goondiwindi, Gunnedah, Narrabri and Narromine, that was not captured using the old day degree function. The new day degree function highlights warmer seasons over the past 21 years (from 1997 to 2018) throughout many of Australia's cotton regions. It suggests that there are more optimum day degrees for growth, which are likely to have contributed to improved yields. However, it is also important to consider that increased yields would have been tempered with an increase in the number of days where growth was negatively affected by very high temperatures (i.e. above 35°C), and consequent higher atmospheric vapour pressure deficit, potentially leading to increased crop water use.

Table 25: Comparison of seasonal heat accumulation across eight key Australian cotton locations using the old and new day degree functions for cotton across three time periods. ↑ indicates that there has been a significant increase in the number of degree days, and 0 indicates there has been no significant trend at $P<0.05$.

Location	**1957-2018**		**1957-1996**		**1997-2018**	
	Old DD	**New DD**	**Old DD**	**New DD**	**Old DD**	**New DD**
Dalby	↑	↑	0	0	0	0
Emerald	↑	↑	↑	↑	0	0
Goondiwindi	↑	↑	0	0	0	↑
Griffith	↑	↑	0	0	↑	↑
Gunnedah	↑	↑	0	0	0	↑
Moree	↑	↑	0	0	↑	↑
Narrabri	↑	↑	0	0	0	↑
Narromine	↑	↑	0	0	0	↑

Our data shows not only that there has been an increase in the number of seasonal day degrees across many of our key cotton regions, but also demonstrates how regions have shifted over time. For example, the number of day degrees in Griffith prior to the year 2008 had been below 1400 day degrees, which had frequently occurred in Narrabri in the past (Figure 42). However, since 2008 there have been several years where the total number of day degrees in Griffith have exceeded 1400, with a significant trend suggesting further increases in the number of day degrees with projected climate change. In this example, we may be able to apply knowledge of cotton systems in Narrabri to the future management strategy of cotton grown in Griffith. Therefore, there may be the potential link similar past and present climatic conditions to adapt knowledge and understanding for the management of Australian cotton systems into the future.

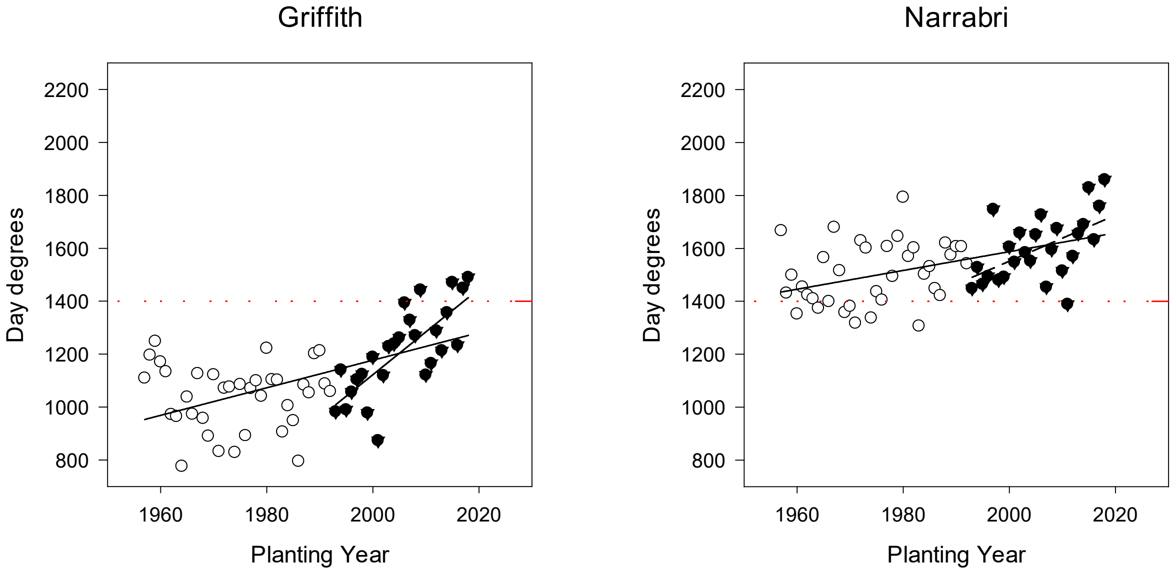


Figure 42: The number of day degrees (using the new day degree function) in Griffith and Narrabri. The red dotted line at 1400 day degrees shows that the number of day degrees in more recent planting years are similar to the number of day degrees that Narrabri has experienced in the past. Data is grouped by time periods (black circles= 1957-2018; white circles= 1957-1996; black triangles= 1997-2018). Regression lines indicate where the trend in the number of day degrees is significant at P<0.05.

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 while some aspects of cotton growth are improved by elevated CO_2, there are issues emerging on the distribution and efficient use of resources. Cotton yield in future climate scenarios may be significantly reduced compared with the current environment, resulting in large reductions in agronomic water use efficiency, and potentially the efficiency of other resources. This project explored possible management strategies, such as the application of mepiquat chloride and alternate cultivars, to reduce excessive vegetative growth and improve resource use efficiencies for cotton grown in future climate scenarios. Field experiments using the National Facility for Climate Change Research have been conducted over three seasons in Narrabri (despite severe hail damage in year 2 of the project), and several glasshouse experiments have been conducted at Western Sydney University in Richmond. Our research suggests that plant growth regulators, such as mepiquat chloride, may be a successful method of controlling excessive vegetative growth in future climates of warmer temperatures and elevated atmospheric CO_2 concentrations. Ensuring that Australian cotton can continue to be produced in resource efficient systems will be crucial to maintain sustainable cotton production systems into the future.

Additionally, Cottassist and OZCOT analyses have been undertaken to better understand both the changes that have occurred throughout Australia's cotton regions and the responsiveness of cotton models to projected environmental changes. Our data suggests that may be an opportunity to link similar past and present cotton systems to apply knowledge and understanding for management of cotton systems that have similar climates throughout time. Therefore, it will be necessary to consider area wide engagement across the different climatic zones to ensure effective communication over time. Furthermore, there is an opportunity to utilise OZCOT to simulate the response of cotton crops to altered climatic conditions into the future. Thus, on-going research requires a multi-faceted approach that incorporates model simulations, glasshouse and field studies to better our understanding of cotton system responses to projected environmental conditions for Australian cotton regions.

The objective of this project was to gain a better understanding of the canopy scale response to warmer temperatures and elevated CO_2, an understanding of the role and effectiveness of plant growth regulators in managing cotton in projected climatic scenarios, and the ability to better model and predict the impact of future climate scenarios on water use and yield. This research has enabled the collection of valuable data that has provided information on growth, physiology and water use of cotton grown in projected climatic conditions. The results from this project, and broader research on the effects of climate change on Australian cotton systems, has been summarised in our review to industry "An overview of recent research into the effects of climate change and extreme weather events on Australian cotton systems".

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?**

This project on water use efficiency in future climates (CSP1804) has contributed to a better understanding of the response of cotton growth, physiology, water use, and cotton production systems to the multiple environmental variables that are projected to alter with climate change. It has greatly increased our knowledge of climate effects on cotton growth and yield, which will assist cotton growers to remain viable under changing climatic conditions. Field experiments suggested that mepiquat chloride may be successfully used to reduce the excessive vegetative biomass of cotton grown in warmer temperatures and elevated atmospheric CO_2. However, our observations in the field also indicate that there may be emerging issues of poor defoliation potentially associated with more vegetative cotton crops in warmer temperature environments, which may be an important avenue for future research. Our research also found that there have been changes in seasonal climatic factors such an increased number of degree days throughout eight key locations spanning the Australian cotton industry. These findings may help define adaptation strategies by linking regions with similar past and present climatic conditions.

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.**

Future presentation and dissemination of the project outcomes is likely to be through publication of results in scientific journals. Where appropriate, further industry engagement with the outcomes of this research may occur.

At the end of this project the electronic equipment used within the field chambers was decommissioned and put into storage. The field chambers used in this study could continue to be used in field-based climate change research to develop a better understanding of the effects of climate change on defoliation efficacy. Substantial upgrades are required; however, this is an opportunity to incorporate new and improved technology.

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, titled "*Impacts of growth temperature, water deficit and heatwaves on carbon assimilation and growth of cotton plants (Gossypium hirsutum L.)*" has accepted by Environmental and Experimental Botany.

A manuscript, titled "*Effects of elevated CO_2 and warmer temperature on early-season field-grown cotton in high-input systems*" has been accepted by Crop Science.

Planned publications

"*Effect of vapour pressure deficit on gas exchange of field-grown cotton*".

"The effects of vapour pressure deficit and soil water deficit on leaf gas exchange parameters in cotton"

"The effects of growth regulators on cotton growth and physiology in elevated CO_2 and temperature"

"Source/sink dynamics of cotton grown in warmer temperatures and elevated [CO_2]"

"An exploration of how Australian cotton regions have changed over time"

"Field chambers for elevated atmospheric CO2 and temperature experimentation in Australian cotton systems"

Throughout this project we have presented our data at numerous conferences and seminars:

Conference presentations

CSIRO Agriculture and Food PhD and Postdoc Forum, Adelaide, July 2018

CSIRO Cotton Stream Seminar, Narrabri, 16th July 2018

Australian Cotton Conference, Gold Coast, August 2018

3rd Agriculture and Climate Change Conference, Budapest, Hungary, 24th-26th March 2019

Better Cotton Forum, Sydney, 22nd May 2019

Crop Consultants Australia Seminar, Narrabri, 20-21st June 2019

Cotton Collective, Griffith, 24th-25th July 2019

Australian Agronomy Conference, Wagga Wagga, 25th-29th August 2019

AACS Australian Cotton Research Conference, Armidale, 28th-30th October 2019

ICAC Conference, Brisbane, 2nd-5th December 2019

Cotton Open House, Sydney, 11th March 2020

Industry articles and presentations

CRDC Spotlight magazine, Spring 2018

The Australian Cottongrower, Oct-Nov 2018

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

An industry review paper "An overview of recent research into the effects of climate change and extreme weather events on Australian cotton systems" has been published online: https://www.cottoninfo.com.au/publications/climate-effects-climate-change-and-extreme-weather-australian-cotton. The intent of this document is that it can continue to be updated with information as it becomes available.

A Cotton Info climate change video was developed by Paul and Tonia Grundy, and Sharna Holman: https://youtu.be/OtG1BCqVxdU

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 internet. 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, warmer air temperatures, higher Vapour Pressure Deficit (VPD) and reduced water availability as a consequence of climate change is likely to affect cotton production. Previous field and glasshouse studies in Australian cotton systems have shown that warmer air temperatures may increase stomatal conductance and transpiration, resulting in reduced leaf-level water use efficiency. Increased photosynthesis and increased vegetative biomass under scenarios of projected climatic conditions will not necessarily equate to higher yields. Furthermore, elevated CO_2 may potentially exacerbate the negative effects of warmer temperature in cotton, leading to large reductions in water and resource use efficiencies. Therefore, further studies were 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.

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 concentration on cotton growth, physiology and water use. Overarching aims were to (1) investigate the interactive effects of atmospheric vapour pressure deficit (VPD) and soil water deficit on the physiology of cotton; (2) identify potential management practices that mitigate the interactive effects of climate change; (3) improve understanding of leaf to canopy level scaling; and (4) better model and predict the impact of future climate scenarios on water use and yield of cotton.

Glasshouse studies showed that increasing VPD stimulated stomatal closure across a range of temperatures. Soil water deficit reduced stomatal conductance of cotton at all temperatures measured, particularly at lower VPD compared with well-watered plants given the same VPD. Therefore, drier climatic conditions may reduce stomatal conductance, although transpiration increases with warmer temperatures, within the range of at 50% water deficit that was tested in our study.

Glasshouse studies showed that fruit loss may greatly increase the vegetative growth rate (VGR) of cotton; however, mepiquat chloride applications were also shown to reduce the VGR of cotton that had lost fruit in warmer temperature and elevated atmospheric CO_2 environments. Similarly, our field studies demonstrated that vegetative biomass was controlled by the application of mepiquat chloride in two out of the three seasons, although observations of increased difficulty in defoliating cotton plants grown at warmer temperatures and elevated CO_2 suggest that understanding timing of defoliants in future climate scenarios may require further research. Therefore, this research suggests that mepiquat chloride may be a successful method of controlling excessive vegetative growth in future climates of warmer temperatures and elevated atmospheric CO_2.

The climate chambers were used to measure the leaf and canopy level responses of cotton grown in warmer temperature and elevated CO_2 environments; however, further research may use modelling to further link these responses. We also used a multi-faceted approach to better model and predict the impact of future climate scenarios of water use and yield of cotton. Our research indicated that climatic changes, such as an increase in the number of seasonal day degrees, have already occurred across several key locations throughout the Australian cotton industry. These findings may help define adaptation strategies by linking regions with similar past and present climatic conditions. On-going research requires a multi-faceted approach that incorporates model simulations, glasshouse and field studies to better our understanding and knowledge of system responses to projected environmental conditions for Australian cotton regions. The outcomes of the broad research into the effects of climate change on Australian cotton systems, has been summarised in our review to industry, “An overview of recent research into the effects of climate change and extreme weather events on Australian cotton systems”, published by CottonInfo.

Part 4 – Summary for public release

This summary will be published on Inside Cotton, CRDC's digital repository, along with the full final report (if suitable for public release). It is designed to provide a short overview of the project for all interested parties. Please complete all fields, ensuring that this exceeds no more than two pages.

Project title:	***Improving water use efficiency in a changing climate***	
Project details:	CRDC project ID:	*CSP 1804*
	CRDC goal:	*1. Increase productivity and profitability on cotton farms*
	CRDC key focus area:	*1.1 Optimised farming systems*
	Principal researcher:	*Dr Katie Broughton, researcher*
	Organisation:	*CSIRO*
	Start date:	*01/07/2017*
	End date:	*30/06/2020*
Objectives	• *Please list the key objectives in bullet point format* • *Assess the use of plant growth regulators to manage excessive vegetative growth of cotton grown in environments of elevated CO2 and warmer temperatures in the field* • *Assess the use of plant growth regulators to manage excessive vegetative growth of cotton grown in elevated [CO2] and warmer temperatures in the glasshouse* • *Scaling leaf- to canopy-level responses* • *Ability to better model and predict the impact of future climate scenarios on water use and yield*	
Background	*Please insert details re the background issue or need that led to this project being undertaken* Research from field-based climate change studies conducted in Narrabri indicated that there were no significant differences in total plant biomass between three environmental treatments (outside unchambered control, 400 ppm CO_2 and +3°C air temperature, and 550 ppm CO_2 and +3°C air temperature), but that cotton grown at warmer temperatures in the chambers produced greater vegetative biomass and less fruit biomass than cotton grown in control plots. Thus, cotton yield in future climatic scenarios may be significantly reduced compared with the current environment, resulting in large reductions in agronomic water use efficiency. Therefore, management strategies need to be explored to reduce excessive vegetative growth and improve resource use efficiencies for cotton grown in future climatic scenarios. Furthermore, glasshouse and field research has shown that early-season growth benefits due to elevated atmospheric CO_2 may occur at ambient temperatures. However, elevated CO_2 may not mitigate negative effects, such as increased water use, on cotton growth and physiology in warmer temperatures expected in future environments. Similarly, the benefit of elevated CO_2 may be greatly reduced when cotton is grown in	

	soil water deficit conditions. These studies have clearly indicated that the magnitude of cotton response to elevated CO_2 is largely dependent upon air temperature and water availability, which therefore must be included as environmental factors in experimental designs to accurately predict cotton response to future climates. Our research has shown that cotton is strongly responsive to changes in leaf-to-air vapour pressure deficit (VPD), which accounts for a large proportion of environmental control on stomatal conductance, and hence photosynthetic rates. Similarly, soil water deficit strongly affects stomatal conductance and photosynthesis. However, very few studies have explicitly explored the independent and interactive effects of soil water deficit and atmospheric water deficit (VPD) on the physiological response of cotton in a changing climate, which is a crucial component of modelling cotton response to the environment. Glasshouse experiments as part of CRDC project CSP 1501 investigated the effects of increased day and/or night temperatures, soil water deficit and heatwave events on the physiology and growth of cotton. Increased day temperatures reduced cotton growth and physiological responses, but these negative effects were greater when night temperature was also higher. Heatwaves further exacerbated the negative effects, indicating that warmer average day/night temperatures will be particularly damaging to cotton during expected heatwave events and soil water deficit. Few studies address the independent effect of night-time warming or the additive effect with day-time warming, but both are crucial components of the general rise in air temperature, which in turn affects cotton response to subsequent heat wave events.
Research activities	*Please provide a high level summary of the research you undertook as part of this project, to address the research need and to meet the project objectives.* **Field experiments at the Australian Cotton Research Institute (ACRI), Narrabri** Field based climate change studies were conducted in Narrabri during the 2017/18, 2018/19, and 2019/20 cotton seasons. For the 2017/18 and 2019/20 cotton seasons, the experiments consisted of warmer temperature and ambient atmospheric CO_2, and ambient temperature and ambient atmospheric CO_2 treatments. For the 2018/19 cotton season, the experiment consisted of three environmental treatments: (a) warmer temperature and elevated atmospheric CO_2, (b) warmer temperature and ambient atmospheric CO_2, and (c) ambient temperature and ambient CO_2. The objectives of these experiments were to (1) assess the use of growth regulators on mitigating the effects of climate change; (2) identify optimal management strategies to improve resource use efficiency of cotton grown under future climate scenarios; and (3) improve understanding of scaling leaf- to canopy- level responses to determine water use efficiency dynamics in the field.

Glasshouse experiments at Western Sydney University (WSU), Richmond

Glasshouse experiments were conducted utilising the naturally-lit, [CO2] and temperature controlled glasshouses at Hawkesbury Institute for the Environment at Western Sydney University (WSU), Richmond.

1. SWC x VPD experiment to determine the relative impact of soil water deficit and atmospheric vapour pressure deficit (VPD) on the physiology of cotton. Leaf-level physiological responses to altered VPD was measured on well-watered and water-deficit cotton plants.

2. Source/Sink dynamics experiment to assess the use of plant growth regulators to manage excessive vegetative growth of cotton grown in elevated CO_2 and warmer air temperature. Several questions were addressed in conducting a single, very large glasshouse experiment at WSU to assess the use of cultivar and plant growth hormones to mitigate the effects of climate change on cotton growth and fruit losses. The objective of this experiment was to gain a more refined understanding of source and sink dynamics of cotton grown in future climatic conditions, particularly quantifying the relativity of the effects when all the factors (air temperature, atmospheric [CO_2], fruit retention and mepiquat chloride application) were considered together.

Improving the external parameters of models to better capture interactive effects of climate change on water use and yield using cotton simulation models

To improve our understanding of the external parameters of models to better capture interactive effects on water use and yield, temperature and CO_2 inputs into the OZCOT model were altered to explore the effects of changing CO_2 concentration in the model. To better understand how the climate in Australian cotton regions has already changed, Cottassist has been used to access BOM climate data to explore the climate trends in representative cotton regions since 1957. Eight locations spanning the current Australian cotton industry have been assessed to explore the temperature trends over three periods: (a) 1957 to 2018 (61 years), (b) 1957 to 1996 (39 years); and (c) 1997 to 2018 (21 years). Regression lines were fit to each time period of season-long weather data (1st September to 30th April) at each location, with a trend significant at $P<0.05$. The eight representative cotton locations selected were Dalby, Emerald, Goondiwindi, Gunnedah, Griffith, Moree, Narrabri, and Narromine. Climatic trends assessed included day degrees, the frequency of cold shocks, and the frequency of hot days (greater that 35°C, 40°C and 45°C). These assessments using the OZCOT model and the Cottassist analyses help inform potential adaptation strategies of cotton to a changing climate by identifying locations that may have had similar climates in the past.

Outputs	*Please detail the overarching outputs from this research projects: what did the project find/discover/create – be it new knowledge, technical advances etc.* *The overarching outputs from this research project are improved understanding of the integrated responses of cotton to warmer temperatures and elevated CO_2 environments. We conducted field experiments over three consecutive cotton seasons (2017 to 2020), glasshouse experiments, and model simulations to investigate the responses of cotton to integrated climatic changes.* *Glasshouse studies showed that increasing VPD stimulated stomatal closure across a range of temperatures. Soil water deficit reduced stomatal conductance of cotton at all temperatures measured, particularly at lower VPD compared with well-watered plants given the same VPD. Therefore, drier climatic conditions may reduce stomatal conductance, although transpiration increases with warmer temperatures, within the range of at 50% water deficit that was tested in our study.* *Glasshouse studies showed that fruit loss may greatly increase the vegetative growth rate (VGR) of cotton; however, mepiquat chloride applications were also shown to reduce the VGR of cotton that had lost fruit in warmer temperature and elevated atmospheric CO_2 environments. Similarly, our field studies demonstrated that vegetative biomass was controlled by the application of mepiquat chloride in two out of the three seasons, although observations of increased difficulty in defoliating cotton plants grown at warmer temperatures and elevated CO_2 suggest that understanding timing of defoliants in future climate scenarios may require further research.* *The climate chambers were used to measure the leaf and canopy level responses of cotton grown in warmer temperature and elevated CO_2 environments; however, further research may use modelling to further link these responses. We also used a multi-faceted approach to better model and predict the impact of future climate scenarios of water use and yield of cotton. Our research indicated that climatic changes, such as an increase in the number of seasonal day degrees, have already occurred across several key locations throughout the Australian cotton industry.* *Additional outputs from this research have been industry engagement through presentations, Spotlight, and Australian Cottongrower articles.*
Impacts	*Please detail the impact and implications that your research will have for the Australian cotton industry, including any best practice recommendations.* *Warmer temperatures and elevated atmospheric CO_2 are likely to affect the physiology, growth and water use efficiency of cotton. Previous research had shown that warmer temperatures and elevated CO_2 may exacerbate the negative effects of warmer*

	temperature, leading to excessive vegetative growth, resulting in large reductions in water and resource use efficiencies. This research suggests that mepiquat chloride may be a successful method of controlling excessive vegetative growth in future climates of warmer temperatures and elevated atmospheric CO_2*.* *Studies comparing climatic changes across key cotton regions indicated that there has been an overall increase in the number of degree days since 1957. These findings may help define adaptation strategies by linking regions with similar past and present climatic conditions.* *Overall, this research points to a need to use management strategies to balance vegetative and reproductive growth to optimise resource use, whilst maintaining yield and quality of cotton grown in variable climatic conditions. On-going research requires a multi-faceted approach that incorporates model simulations, glasshouse and field studies to better our understanding and knowledge of system responses to projected environmental conditions for Australian cotton regions. Better understanding of the challenges and opportunities for growth, productivity and resource use of cotton grown in warmer temperatures and higher* CO_2 *environments will allow growers to better manage their production systems effectively and sustainably into the future.*
Key publications	*Please detail any major publications resulting from your research.* *A manuscript, titled "Impacts of growth temperature, water deficit and heatwaves on carbon assimilation and growth of cotton plants (Gossypium hirsutum L.)" has accepted by Environmental and Experimental Botany.* *A manuscript, titled "Effects of elevated* CO_2 *and warmer temperature on early-season field-grown cotton in high-input systems" has been submitted to Crop Science.* *A manuscript, titled "Effect of vapour pressure deficit on gas exchange of field-grown cotton" has been submitted to Functional Plant Biology.* *An industry review paper "An overview of recent research into the effects of climate change and extreme weather events on Australian cotton systems" has been published online:* https://www.cottoninfo.com.au/publications/climate-effects-climate-change-and-extreme-weather-australian-cotton.