COPYRIGHT AND USE OF THIS THESIS

This thesis must be used in accordance with the provisions of the Copyright Act 1968.

Reproduction of material protected by copyright may be an infringement of copyright and copyright owners may be entitled to take legal action against persons who infringe their copyright.

Section 51 (2) of the Copyright Act permits an authorized officer of a university library or archives to provide a copy (by communication or otherwise) of an unpublished thesis kept in the library or archives, to a person who satisfies the authorized officer that he or she requires the reproduction for the purposes of research or study.

The Copyright Act grants the creator of a work a number of moral rights, specifically the right of attribution, the right against false attribution and the right of integrity.

You may infringe the author’s moral rights if you:

- fail to acknowledge the author of this thesis if you quote sections from the work
- attribute this thesis to another author
- subject this thesis to derogatory treatment which may prejudice the author’s reputation

For further information contact the University’s Copyright Service.

sydney.edu.au/copyright
The integrated effects of projected climate change on cotton growth and physiology

By
Katrina Jean Broughton
B.Sc.Agr (Hons)
The University of Sydney

A thesis submitted for the degree of Doctor of Philosophy
Faculty of Agriculture and Environment
The University of Sydney

Australia

September, 2015
Declaration of originality

This thesis reports the original work of the author, except as otherwise stated. It has not been submitted previously for a degree at this or any other university.

Katrina Broughton
Abstract

Changes in atmospheric [CO₂], temperature, precipitation and consequently atmospheric vapour pressure deficit (VPDₐ) under projected climate change scenarios present a challenge to crop production. This may have significant impacts on the physiology and yield of cotton and hence the profitability of the Australian cotton industry. Understanding the implications of integrated environmental impacts on cotton is critical for developing cotton systems that are resilient to stresses induced by climate change.

Elevated [CO₂] generally increases photosynthesis, reduces transpiration and improves leaf- and plant-level water use efficiency (WUE) of well-watered C₃ plants, but this effect may be altered by rising temperature and reduced water availability. Cotton responds to changes in vapour pressure deficit (VPD), yet there has been little research on the leaf-level physiological response to altered VPD in field-grown cotton. In addition, a number of studies have investigated the effect of elevated [CO₂] and temperature on physiology and growth of a range of cotton cultivars, yet there has not been a comparison between older and current varieties used in Australian production systems to identify if there has been inadvertent selection of beneficial traits for a changing climate. It is important to understand potential interactions as it is likely that multiple variables will be altered with future climatic changes.

This thesis aims to investigate the integrated effects of projected climate change (warmer temperature, elevated [CO₂], altered VPD and water stress) on physiology, growth and water use of cotton in high-yielding and high-input modern cotton systems in Australia. This will facilitate development of crop management strategies and improve cotton yield and water use efficiencies. This was achieved through a combination of glasshouse and field-based studies. Glasshouse experiments were conducted during 2010 and 2011 at the University of Western Sydney, Richmond, Australia. In these experiments, cotton was grown in sun-lit glasshouse bays in two [CO₂] (Cₐ: 400 µL L⁻¹ and Cₑ: 640 µL L⁻¹) and two temperature (Tₐ: 28/17 °C day/night and Tₑ: 32/21 °C day/night) treatments. Field experiments were conducted during the 2011/12 and 2012/13 cotton seasons at the Australian Cotton Research Institute, Narrabri, Australia.

The objective of glasshouse experiment I (Chapter 3) was to quantify the physiological and growth capacity of different cotton genotypes to current and future climate regimes. This experiment compared the early-season growth and physiology response of a past (DP 16) and a current (Sicot 71BRF) cotton cultivars grown in ambient and elevated atmospheric [CO₂] and two temperature treatments under well-watered conditions. This study demonstrated that elevated [CO₂] increased
biomass and photosynthetic rates compared with the ambient [CO₂] treatment, and that warmer air temperatures (32/21 °C, day/night) also increased plant biomass. Although limited by the comparison of only one older and one modern cultivar, this study indicated that current cultivars may have an advantage over older varieties in future, warmer environments due to smaller, more compact morphology of the modern cultivar. However, no interaction between elevated temperature (Tₑ) and elevated [CO₂] (Cₑ) indicated that substantial potential may exist to increase breeding selection of cotton varieties that are responsive to both Tₑ and Cₑ.

The aim of glasshouse experiment II (Chapter 4) was to assess the physiological and growth response of cotton to drought and drought-recovery phases of a production system in projected climates. This experiment investigated the interactive effects of elevated [CO₂], warmer temperatures and soil water deficit on biomass production, leaf-level physiology and whole plant water use and efficiency of cotton. Cₑ increased vegetative biomass, photosynthetic rates (A) and decreased stomatal conductance (gₛ–sat); however, warmer air temperatures (32/21 °C, day/night) negated the positive responses to Cₑ. Cotton grown at Tₐ were able to withstand soil water deficits for longer than plants grown at Tₑ, due to reduced leaf biomass and lower evaporative demand compared with plants grown in a warmer environment. This indicates that cotton may be more susceptible to long dry periods in projected warmer environments. Cₑ increased water use at Tₐ, although plant WUE was improved, whereas increased water consumption at Tₑ resulted in lower plant WUE regardless of atmospheric [CO₂]. Therefore growth and water use benefits of Cₑ may occur at Tₐ with the cost of increased water requirements which may have implications on future cotton production in Australia, but Cₑ will not mitigate the negative effects of rising temperature on cotton growth and physiology in future environments.

Field experiment I (Chapter 5) assessed the impact of altered VPD on leaf level physiology of field-grown cotton to improve current understanding of the plant x environment interaction, thereby contributing to validation and improvement of physiological and yield response models. Different VPD environments in the field were generated by planting cotton on three dates within the sowing window (early (S1) = 5th October 2011; mid (S2) = 9th November 2011; and late (S3) = 30th November 2011). Three irrigation treatments were (a) fully watered- “non-stressed” (NS); (b) limited water- “early stress” (ES); and (c) limited water- “late stress” (LS). VPD accounted for a proportion of the variation in both stomatal conductance and photosynthetic responses of cotton. Generally, smaller percentages of variation were also attributed to other factors such as the individual plant (Plant), leaf temperature-air differential (Tₕ-Tₛ), accumulated temperature stress hours (ASH) and leaf vapour pressure deficit (VPDₛ) x Tₙ-Tₛ, Plant x Tₙ-Tₛ and VPDₛ x ASH interactions; however, a proportion of variation was due to
something that we did not or cannot measure. This study highlights the importance of accounting for VPD in climate change research, given that stomata are highly responsive to changes in VPD. In addition, the $A_{\text{sat}}/E$ (ITE) model developed using cotton grown in the glasshouse was tested to determine if the model and associated parameters applied to cotton grown in the field. Using parameters estimated from (a) field and (b) glasshouse data, modelled $A_{\text{sat}}/E$ and measured $A_{\text{sat}}/E$ were compared. This indicated that the $A_{\text{sat}}/E$ model developed using cotton grown in the glasshouse can also be used to estimate $A_{\text{sat}}/E$ of cotton grown in field conditions. This experiment provides a basis for physiology and production models, particularly in terms of cotton response to projected climatic environments.

The objective of field experiment II (Chapter 6) was to investigate the impacts of increased atmospheric $[\text{CO}_2]$ on whole canopy physiology of field-grown cotton in high-input/high-yielding production systems. Canopy EvapoTranpiration and Assimilation (CETA) chambers were used to elevate atmospheric $[\text{CO}_2]$ in the field. CETA chambers were a successful method of increasing atmospheric $[\text{CO}_2]$ of field-grown cotton, despite limitations with increased temperature and altered humidity and VPD. $C_\text{e}$ increased early stage biomass by 67% of well-watered, field-grown cotton. Although there were increases in leaf-level photosynthesis ($A_{\text{sat}}$), a reduction in stomatal conductance ($g_{\text{sat}}$) and transpiration ($E$), and a corresponding increase in leaf-level photosynthetic efficiency ($A_{\text{sat}}/g_{\text{sat}}$), our data indicated there were no large changes in leaf-level biochemistry. In this study, we did not obtain a definitive answer to the integrated effects of $C_\text{e}$ on plant water use as there were no detectable differences in water use for early-stage cotton growth in the field, but $C_\text{e}$ increased plant water use in the glasshouse (Chapter 4). Given the large increases in biomass with $C_\text{e}$ and the disparities between glasshouse and field studies, further studies should be conducted to explore the integrated environmental effects of climate change on field-grown cotton in Australian production systems.

This project highlights the implications for interactive effects of elevated atmospheric $[\text{CO}_2]$ and warmer temperatures on early-stage cotton growth and physiology in high-input/high-yielding systems. Overall, these studies have shown that projected climate change is likely to affect cotton physiology, growth and water use, but the magnitude will depend on the combination factors including temperature, $[\text{CO}_2]$, VPD, plant water availability and cultivar selection. The glasshouse experiments have shown that although plant water use efficiency may be improved with elevated atmospheric $[\text{CO}_2]$ at ambient temperatures, total water use is likely to increase. Although plant biomass is also increased with elevated $[\text{CO}_2]$ in field studies, differences in plant water use and efficiencies are yet to be confirmed. This will facilitate development of crop management strategies
and could potentially lead to improvements in yield and water use efficiencies. Therefore, this data contributes to the understanding of how high-input/high-yielding cotton crops may respond to the integrated effects of projected climate change.
# Table of contents

Declaration of originality ........................................................................................................... i
Abstract ........................................................................................................................................ ii
Table of contents ........................................................................................................................ vi
List of figures ............................................................................................................................... xi
List of tables ............................................................................................................................... xvi
Abbreviations ............................................................................................................................. xvii
Acknowledgements .................................................................................................................... xix
Chapter 1: General introduction ............................................................................................... 1
  Central research question ........................................................................................................... 4
  Objectives ................................................................................................................................... 4
Chapter 2: Review of literature ................................................................................................. 6
  2.1. Introduction to projected climate change ...................................................................... 6
  2.2. Introduction to Australian cotton production ............................................................... 8
  2.3. A summary of the basics of photosynthesis ................................................................. 9
  2.4. Increased CO₂ .................................................................................................................... 10
    2.4.1. Photosynthesis and respiration ............................................................................... 10
      Models ................................................................................................................................. 11
      Photosynthetic acclimation of plants to long-term CO₂ exposure ................................... 11
      Effects of elevated [CO₂] on respiration ........................................................................... 12
      Net carbon assimilation ................................................................................................... 12
    2.4.2. Transpiration and water use ................................................................................... 13
      Effect of elevated [CO₂] on stomatal conductance (gₛ) ................................................... 13
      Effect of elevated [CO₂] on transpiration rates ............................................................... 14
      Effect of elevated [CO₂] on WUE .................................................................................... 15
    2.4.3. Growth, yield and quality ....................................................................................... 15
  2.5. Increased temperature ...................................................................................................... 16
    2.5.1. Photosynthesis and respiration ............................................................................... 16
      Respiration at warmer air temperatures ......................................................................... 18
    2.5.2. Transpiration and water use ................................................................................... 18
    2.5.3. Growth, yield and quality ....................................................................................... 19
Quality ........................................................................................................................................ 21
2.6. Vapour pressure deficit effects ................................................................................................. 21
  2.6.1. Plant response to the physical environment ......................................................................... 22
  2.6.2. Models to explain stomatal response to VPD ..................................................................... 24
2.7. Water availability and demand .................................................................................................. 26
  2.7.1. Water use in Australian cotton systems ............................................................................. 26
  2.7.2. Photosynthesis and respiration ......................................................................................... 28
  2.7.3. Transpiration and water use ............................................................................................. 29
    Water use efficiency ................................................................................................................. 29
  2.7.4. Growth, yield and quality ................................................................................................. 30
    Quality ...................................................................................................................................... 30
2.8. Combined effects of temperature, CO$_2$ and water availability .............................................. 31
  2.8.1. Combined temperature and CO$_2$ effect .......................................................................... 31
2.9. Combined CO$_2$ and water effects .......................................................................................... 33
2.10. Controlled environment facilities ........................................................................................... 34
2.11. Implications of climate change for Australian cotton ........................................................... 36
2.12. Conclusions ........................................................................................................................... 37
Chapter 3: The effect of elevated atmospheric [CO$_2$] and warmer temperatures on a past and a current cotton cultivar ............................................................................................................. 39
  3.1. Introduction ............................................................................................................................. 39
  3.2. Methods ................................................................................................................................... 42
    3.2.1. Plant materials and growing conditions ......................................................................... 42
    3.2.2. Leaf gas exchange measurements .................................................................................. 43
    3.2.3. Plant growth measurements .......................................................................................... 44
    3.2.4. Statistical analyses ....................................................................................................... 44
  3.3. Results ..................................................................................................................................... 44
    3.3.1. Vegetative biomass production ...................................................................................... 44
    3.3.2. Physiological response of two different cotton cultivars to elevated [CO$_2$] and warmer temperatures under well-watered conditions .................................................. 48
  3.4. Discussion ................................................................................................................................ 52
    3.4.1. Summary of hypotheses findings .................................................................................... 52
3.4.2. Impacts of $C_e$ and $T_e$ on cotton physiology and growth ........................................... 52
3.4.3. Differences in growth and physiology between the two cultivars ............................... 54
3.4.4. Conclusions ....................................................................................................................... 55

Chapter 4: Warming negates the positive impact of elevated $[CO_2]$ on cotton growth and physiology during soil water deficit ................................................................. 56
4.1. Introduction ......................................................................................................................... 56
4.2. Materials and Methods ...................................................................................................... 59
  4.2.1. Plant material and growing conditions ........................................................................ 59
  4.2.2. Drought treatments ...................................................................................................... 60
  4.2.3. Leaf gas exchange measurements .............................................................................. 61
  4.2.4. Plant growth measurements ....................................................................................... 62
  4.2.5. Statistical analyses ...................................................................................................... 62
4.3. Results ................................................................................................................................. 63
  4.3.1. Soil water deficit ........................................................................................................ 63
  4.3.2. Vegetative and reproductive biomass production ....................................................... 63
  4.3.3. Photosynthesis and stomatal conductance ................................................................. 68
  4.3.4. Whole plant water use ............................................................................................... 72
4.4. Discussion ............................................................................................................................ 73
  4.4.1. Elevated $[CO_2]$ may ameliorate moderate soil water deficit .................................. 74
  4.4.2. Elevated temperature increased water use and exacerbated moderate drought stress 75
  4.4.3. Interactive effects of elevated $[CO_2]$ and elevated temperature during soil water deficit 76
  4.4.4. Conclusions ................................................................................................................. 77

Chapter 5: Environmental effects on the relationship of leaf-level conductance and photosynthesis to VPD ................................................................................................................. 78
5.1. Introduction ......................................................................................................................... 78
5.2. Materials and Methods ...................................................................................................... 81
  5.2.1. Experimental design and plot management ................................................................. 81
  5.2.2. Leaf gas exchange ....................................................................................................... 83
  5.2.3. Leaf water potential .................................................................................................. 84
  5.2.4. Soil water .................................................................................................................... 84
  5.2.5. Canopy temperature ................................................................................................. 84
List of figures

Figure 2.1: Mean yearly atmospheric [CO$_2$] between the years 1959 to 2014 measured at Mauna Loa, Hawaii (Tans and Keeling, 2014). ................................................................. 6

Figure 2.2: Diurnal trends in canopy transpiration rates and photosynthetic photon flux density 62 DAE (Days After Emergence) for cotton canopies grown at various temperatures in 350 and 700 µL CO$_2$ L$^{-1}$ air (Reddy et al., 1995d). ........................................................................................................ 19

Figure 2.3: Transpiration rates for a number of C$_3$ (white symbols) and C$_4$ (shaded symbols) species at different VPD and an air temperature of 26 °C (Rawson et al., 1977). R values for linear regressions appear in brackets. Prior to the experiment, plants were well-watered and fertilised, and grown at 27/22 °C (day/night) in a naturally lit glasshouse. Leaves were stabilised in irradiated assimilation chambers for 4 h at 26 °C, and humidity was increased in steps until the dewpoint of the system was reached. An open gas exchange system recorded measurements at each level of humidity. ............ 23

Figure 2.4: Responses of transpiration (E; a and c) and stomatal conductance (g; b and d) to vapour pressure difference between leaf and air (VPD$_L$) in soybean (a and b) and cocklebur leaves (c and d) at two carbon dioxide partial pressures (3.5 Pa; circle and 35 Pa; triangle) where leaf temperature was maintained at 28 °C (Yong et al., 1997). ................................................................. 24

Figure 2.5: Daily water use of cotton at Narrabri, Australia (ITC International, 2014) .................. 27

Figure 2.6: Effects of CO$_2$ concentration (350- white circle; 450- black circle; and 700- square µL L$^{-1}$) and temperature (Day/Night: 26/18, 31/23, 36/28 °C) on (a) net photosynthetic rates and (b) stomatal conductance in cotton leaves. Measurements were made at regular intervals (30, 40, 50, 60 days after germination) on at least three different plants from each chamber. Values are average of five independent determinations (Reddy et al., 1998a). ............................................................................... 32

Figure 3.1: Effect of growth temperature, atmospheric [CO$_2$] on leaf (a and b), stem (c and d), root (e and f) and total dry biomass production (g and h; g plant$^{-1}$) of cotton cultivars DP16 (white) and 71BRF (shaded) until 38 DAP. Values represent the mean of 6 plants. Refer to Table 3.1 for a summary of significant main treatment effects and interactions. ................................................................. 46

Figure 3.2: Effect of growth temperature, atmospheric [CO$_2$] on number of nodes (a and b), number of leaves (c and d), leaf area (e and f) and plant height (g and h; g plant$^{-1}$) of cotton cultivars DP16 (white) and 71BRF (shaded) until 38 DAP. Values represent the mean of 6 plants. Refer to Table 3.1 for a summary of significant main treatment effects and interactions. .................................................. 47
Figure 3.3: Effect of [CO₂] and temperature on photosynthetic rate (A_{sat}; a and b) and stomatal conductance (g_{s-sat}; c and d) of DP16 (white) and 71BRF (shaded). Values represent the mean of 6 plants. Refer to Table 3.1 for a summary of significant main treatment effects and interactions. 48

Figure 3.4: Effect of [CO₂] and temperature on night respiration (a and b) of DP16 (white) and 71BRF (shaded). Values represent the mean of 6 plants, measured on 13th Dec 2010 (34 DAP). Refer to Table 3.1 for a summary of significant main treatment effects and interactions. 49

Figure 3.5: Effect of [CO₂] and temperature on the ratio of photosynthesis to stomatal conductance (A_{sat}/g_{s-sat}, a and b), photosynthesis to transpiration rate (A_{sat}/E, c and d), and intercellular to ambient [CO₂] ratio (Ci/Ca, e and f) of DP16 (white) and 71BRF (shaded). Values represent the mean of 6 plants. Refer to Table 3.1 for a summary of significant main treatment effects and interactions. 50

Figure 3.6: Effect of [CO₂] and temperature on V_{cmax} (a and b), J_{max} (c and d), and J_{max}/V_{cmax} (e and f) of DP16 (white) and 71BRF (shaded). Values represent the mean of 6 plants. Refer to Table 3.1 for a summary of significant main treatment effects and interactions. 51

Figure 4.1: Soil water content (SWC) expressed as a percentage field capacity of the soil for different temperature and [CO₂] treatments, during two drought cycles and two recovery phases. Circles used for 400 µL L⁻¹ [CO₂] (Cₐ: a and b), triangles used for 640 µL L⁻¹ [CO₂] (Cₑ: c and d). Ambient temperature (a and c) is shown in blue, elevated temperature (b and d) is shown in red. Values represent means ± SE of 5 plants (in the water-stressed (open symbols) treatment, in the well-watered (solid symbols) treatment TDR measurement was of one pot). All plants were well-watered during recovery phase (shaded). 61

Figure 4.2: Effect of growth temperature, atmospheric [CO₂] and water availability on leaf (a and b), stem & petiole (c and d), root (e and f), total vegetative (g and h) and total fruit (i and j) biomass production (g plant⁻¹) of cotton until 70 DAP. Ambient temperature (Tₐ) is shown in blue, elevated temperature (Tₑ) is shown in red, well watered (wet; shaded) and water-stressed (dry; white). Values represent the mean of 5 plants. Refer to Table 4.2 for a summary of significant main treatment effects and interactions. 64

Figure 4.3: Effect of growth temperature, atmospheric [CO₂] and water availability on leaf area (cm² plant⁻¹; a and b) of cotton until 70 DAP. Ambient temperature is shown in blue, elevated temperature is shown in red. Values represent the mean of 5 plants. Refer to Table 4.2 for a summary of significant main treatment effects and interactions. 65
Figure 4.4: Effect of growth temperature, atmospheric CO₂ and water availability on photosynthetic rates at 1800 μmol m⁻² s⁻¹ light and growth CO₂ (A_sat, a and b), photosynthetic rate at 1800 μmol m⁻² s⁻¹ light and saturating CO₂ of 1500 μL L⁻¹ CO₂ (A_max, c and d), stomatal conductance rates at 1800 μmol m⁻² s⁻¹ light (gₛ-sat, e and f), and stomatal conductance rate at 1800 μmol m⁻² s⁻¹ light and 1500 μL L⁻¹ CO₂ (gₛ-max, g and h) of cotton at the end of the second drought phase. Ambient temperature (T_A) is shown in blue, elevated temperature (T_E) is shown in red, well watered (wet; shaded) and water-stressed (dry; white). Values represent the mean of 5 leaves. Refer to Table 4.2 for a summary of significant main treatment effects and interactions.

Figure 4.5: Effect of growth temperature, atmospheric CO₂ and water availability on photosynthesis to stomatal conductance ratios (A_sat/gₛ-sat, a and b), photosynthesis to transpiration rate (A_sat/E, c and d), and intercellular to ambient CO₂ ratio (Cᵢ/Cₐ, e and f) of cotton at the end of the second drought phase. Ambient temperature (T_A) is shown in blue, elevated temperature (T_E) is shown in red, well watered (wet; shaded) and water-stressed (dry; white). Values represent the mean of 5 leaves. Refer to Table 4.2 for a summary of significant main treatment effects and interactions.

Figure 4.6: Photosynthesis at saturating light (A_sat, 1800 μmol m⁻² s⁻¹) for well-watered (closed symbol) and water-stressed (open symbol) plants grown at ambient (CA: circles) and elevated (CE: triangles) [CO₂], and ambient (TA: blue) and elevated (TE: red) temperatures. Values represent means ± SE of 5 leaves. All plants were well-watered during the recovery phase (shaded).

Figure 4.7: Stomatal conductance at saturating (1800 μmol) light (gₛ-sat, mol m⁻² s⁻¹) for well-watered and water-stressed plants grown at ambient and elevated [CO₂], and ambient and elevated temperatures. Circles used for CA: 400 μL L⁻¹ [CO₂], triangles used for CE: 640 μL L⁻¹ [CO₂]. Ambient temperature (TA) is shown in blue, elevated temperature (TE) is shown in red. Values represent means ± SE of 5 leaves. All plants were well-watered during the recovery phase (shaded).

Figure 4.8: (a and b) Whole plant water use (kg plant⁻¹), and (c and d) whole plant water use efficiency (g kg⁻¹) of well-watered (wet; shaded) and water-stressed (dry; white) cotton grown at ambient (CA: 400 μL L⁻¹) and elevated (CE: 640 μL L⁻¹) [CO₂] and ambient (TA: blue) and elevated (TE: red) temperatures until 70 DAP. Values represent the mean of 5 plants. Refer to Table 4.2 for a summary of significant main treatment effects and interactions.

Figure 5.1: Field layout for experiment depicting combination of sowing times and water treatments for each plot.

Figure 5.2: Daily minimum (°C, blue) and maximum (°C, red) air temperature and rainfall (mm, grey) at ACRI, Narrabri from 5 October 2011 to 23 May 2012.
Figure 5.3: (a) Stomatal conductance \( (g_{s-sat}) \) and (b) photosynthesis \( (A_{sat}) \) for VPD response curves for each sowing treatment of field-grown cotton. Sowing treatments are coloured red (S1), blue (S2) and green (S3). Lines represent each VPD curve and hence includes the complete dataset. ................................. 88

Figure 5.4: The predictions for water treatment (early stress (ES), late stress (LS) and non-stressed (NS)) and sowing time (S1, S2 and S3) on (a) stomatal conductance \( (g_{s-sat}) \) and (b) photosynthesis \( (A_{sat}) \) of field-grown cotton using Eq. (1). The points are predictions that account for other variables in the model that are not shown in the plot. ........................................... 90

Figure 5.5: The predictions of how sowing time (S1: red, S2: green, and S3: blue) and VPD, affects (a) stomatal conductance \( (g_{s-sat}) \) and (b) photosynthesis \( (A_{sat}) \) of field-grown cotton using Eq. (1). The points are predictions that account for other variables in the model that are not shown in the plot.91

Figure 5.6: \( A_{sat}/E \) response to VPD of “well-watered” field-grown cotton. Black solid line represents model fit using \( g_1 \) and \( k \) estimates from field data. Blue dashed line represents \( g_1 \) and \( k \) model prediction based on cotton grown in the glasshouse. ................................................................. 93

Figure 5.7: Comparison of modelled and measured \( A_{sat}/E \) using Eq. (2) where \( g_1 \) and \( k \) parameters are from (a) field data and (b) glasshouse data prediction from Duursma et al. (2013). Also shown are the 1:1 lines (black). (a) RMSE= 0.714; MAD= 0.546 and (b) RMSE does not apply; MAD= 0.551. .......... 93

Figure 6.1: CETA chambers used to generate CO\(_2\) treatments in the field during 2013 at ACRI Narrabri. ......................................................................................................................... 102

Figure 6.2: Average daily (a) air temperature, (b) relative humidity and (c) [CO\(_2\)] from 8 am – 6 pm (AEDT) for ambient CO\(_2\) (C\(_A\) circle), elevated CO\(_2\) (C\(_E\), triangle) and control (C\(_C\), square) for 43 - 72 DAP. Values represent mean ± SE of two chambers (sample size of one in the control treatment). Target [CO\(_2\)] was 650 μL L\(^{-1}\), data range between 300 - 800 μL L\(^{-1}\) with a gap in data at 54 DAP due to malfunction of dataloggers (panel c). Average daily [CO\(_2\)] for C\(_C\) was not monitored. ................................. 106

Figure 6.3: Average daily VPD\(_a\) from 8am - 6pm (AEDT) for ambient CO\(_2\) (C\(_A\): white circle), elevated CO\(_2\) (C\(_E\): black triangle) and control (C\(_C\): square) for 43 - 72 DAP. Values represent mean ± SE (sample size of one in the control treatment) ........................................................................................................... 107

Figure 6.4: Average daily (a) canopy temperature (°C) and (b) soil temperature (°C) between 8 am and 6 pm (AEDT) for ambient CO\(_2\) (C\(_A\): white circle), elevated CO\(_2\) (C\(_E\): black triangle) and no chamber (C\(_C\): white square) for 43 - 72 DAP. Values represent mean ± SE. Gap in data at 54 DAP due to malfunction of dataloggers. C\(_C\) data not available for soil temperature......................................................... 108
Figure 6.5: Change in volumetric soil water content (VSWC %) to a depth of 90 cm for (a) 43 - 51 DAP and (b) 53 - 73 DAP for Cc, Ca and Ce treatments measured using green-light-red-light (GLRL) sensors. Values represent mean. Horizontal bars for Ca and Ce treatments represent SE of two GLRL sensors (sample size of one in the control treatment). Plants were irrigated at 52 DAP. There were no significant differences between treatments across depths for either of the time periods.

Figure 6.6: Change in the sum of volumetric soil water content (VSWC) between (a) Cc and Ca (Adj R² = 0.712); (b) Cc and Ce (Adj R² = 0.704; and (c) Ca and Ce (Adj R² = 0.922). Data are for individual chambers. T-tests showed that the slopes of each were not significantly different from 1.0. Also shown are the 1:1 lines (dashed).

Figure 6.7: Effect of ambient CO₂ (Cc), and elevated CO₂ (Ce) on (a) leaf, (b) stem, (c) total vegetative and (d) total fruit dry biomass production (g plant⁻¹) of cotton at 72 DAP. Values represent the mean of plants in two chambers. Refer to Table 6.1 for significant differences.

Figure 6.8: Final (a) plant height (cm plant⁻¹) (b) number of nodes (plant⁻¹) and (c) leaf area (cm² plant⁻¹) of cotton grown at ambient CO₂ (Cc) and elevated CO₂ (Ce). Values represent the mean of plants in two chambers at 72 DAP. Refer to Table 6.1 for significant differences.

Figure 6.9: Effect of ambient CO₂ (Cc) and elevated CO₂ (Ce) on cotton (a) photosynthesis, A_sat; (b) stomatal conductance, g_sat; (c) transpiration, E; and (d) photosynthetic efficiency, A_sat/g_sat. Measurements were made at 70 and 71 DAP between 9 am and 3 pm (AEDT). Values represent the mean of 11 plants. Refer to Table 6.1 for significant differences.

Figure 6.10: (a) A_max and (b) g_sat-max of cotton grown with ambient CO₂ (Cc) and elevated CO₂ (Ce). Measurements were made at 70 and 71 DAP between 9 am and 3 pm (AEDT). Values represent the mean of 11 plants. Refer to Table 6.1 for significant differences.

Figure 6.11: Final (a) V_cmax, (b) J_max and (c) J_max/V_cmax of cotton grown with ambient CO₂ (Cc) and elevated CO₂ (Ce). Measurements were made at 70 and 71 DAP between 9 am and 3 pm (AEDT). Values represent the mean of 11 plants. Refer to Table 6.1 for significant differences.
List of tables

Table 3.1: Three-way ANOVA table for [CO$_2$], temperature (Temp) and Cultivar effects on growth and physiological parameters of cotton. Leaf gas exchange measurements were made 23, 26, 36 and 38 DAP, with the exception of night respiration measurements which were made 34 DAP. Biomass production was measured at 38 DAP. F-values in bold represent significant effects at a P< 0.05 level of significance. Least significant difference (lsd) at P< 0.05 are shown for significant ^main effects and ^two-way interactions. Measurements were made on 6 plants in each treatment.................45

Table 4.1: Three-way ANOVA table for [CO$_2$], temperature (Temp) and water effects on various parameters of cotton for vegetative biomass until 70 DAP, leaf gas exchange measured at the end of the second drought phase, and plant water use and water use efficiency until 70 DAP. ↑ shows main effect increase; ↓ shows main effect decrease; *, **, *** shows significant interactions at P ≤ 0.05, 0.01 and 0.001, respectively; and - shows no significant difference at P> 0.05...............................66

Table 4.2: Three-way ANOVA table for [CO$_2$], temperature (Temp) and water effects on various parameters of cotton for vegetative and reproductive biomass until 70 DAP, leaf gas exchange measured at the end of the second drought phase, and plant water use and water use efficiency until 70 DAP. Values in bold represent significance at P< 0.05. Least significant difference (lsd) at P< 0.05 are shown for significant ^main effects, ^two-way interactions, and ^three-way interactions...........67

Table 5.1: P-values for the treatment effects on stomatal conductance ($g_{sat}$) and photosynthesis ($A_{sat}$) of the complete (ANOVA) and ambient (REML) datasets; where VPD$_L$ is vapour pressure deficit of the leaf, TBlk is block temperature of the cuvette (°C), Sowing is the sowing treatment and WaterTrt is the water treatment. *, **, *** show significant differences at P ≤ 0.05, 0.01 and 0.001, respectively. Figures in bold represent significance at P< 0.05. ........................................................................89

Table 6.1: Statistical analyses for plant biomass, harvest and water count data. REML analysis was used to test for differences between CA and CE chamber treatments for biomass and harvest at 72 DAP. * represents significance at P< 0.05, ** represents significance at P< 0.01 and *** represents significance at P<0.001. Values in bold represent significance at P< 0.05. .................................................................111
<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Net carbon assimilation (µmol mol$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>ACi</td>
<td>Relationship between leaf photosynthesis (A; µmol mol$^{-2}$ s$^{-1}$) and intercellular [CO$_2$] (µL L$^{-1}$)</td>
</tr>
<tr>
<td>ACRI</td>
<td>Australian Cotton Research Institute</td>
</tr>
<tr>
<td>$A_{\text{max}}$</td>
<td>Carbon assimilation at saturating light and saturating CO$_2$ (µmol mol$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$A_{\text{sat}}$</td>
<td>Carbon assimilation at saturating light (µmol mol$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>ASH</td>
<td>Accumulated stress hours</td>
</tr>
<tr>
<td>BOM</td>
<td>Bureau of Meteorology</td>
</tr>
<tr>
<td>BRF</td>
<td>Bollgard Round-up Ready Flex</td>
</tr>
<tr>
<td>Bt</td>
<td><em>Bacillus thuringiensis</em></td>
</tr>
<tr>
<td>$C_A$</td>
<td>Ambient CO$_2$ concentration</td>
</tr>
<tr>
<td>$C_E$</td>
<td>Elevated CO$_2$ concentration</td>
</tr>
<tr>
<td>CETA</td>
<td>Canopy EvapoTranspiration and Assimilation</td>
</tr>
<tr>
<td>[CO$_2$]</td>
<td>Carbon dioxide concentration (µL L$^{-1}$)</td>
</tr>
<tr>
<td>CSIRO</td>
<td>Commonwealth Scientific and Industrial Research Organisation</td>
</tr>
<tr>
<td>DAP</td>
<td>Days After Planting</td>
</tr>
<tr>
<td>DP</td>
<td>Deltapine</td>
</tr>
<tr>
<td>$E$</td>
<td>Transpiration rate (mol m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>ET</td>
<td>Evapotranspiration</td>
</tr>
<tr>
<td>FACE</td>
<td>Free-Air Carbon Enrichment</td>
</tr>
<tr>
<td>$g_s$</td>
<td>Stomatal conductance (mol m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$g_{s\text{-max}}$</td>
<td>Stomatal conductance at saturating light and saturating CO$_2$ (mol m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$g_{s\text{-sat}}$</td>
<td>Stomatal conductance at saturating light (mol m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>IRGA</td>
<td>Infra-Red Gas Analyser, used in gas exchange measurements</td>
</tr>
<tr>
<td>IRT</td>
<td>Infra-Red Thermometer, used for measurements of canopy temperature</td>
</tr>
<tr>
<td>ITE</td>
<td>Instantaneous Transpiration Efficiency (µmol mmol$^{-1}$)</td>
</tr>
<tr>
<td>$J_{\text{max}}$</td>
<td>Maximum rate of electron transport (µmol m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>LA</td>
<td>Leaf Area (cm$^2$ plant$^{-1}$)</td>
</tr>
<tr>
<td>LAI</td>
<td>Leaf Area Index</td>
</tr>
<tr>
<td>NMM</td>
<td>Neutron Moisture Meter, used to measure soil moisture content</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales</td>
</tr>
<tr>
<td>OTC</td>
<td>Open Top Chamber</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetic Active Radiation</td>
</tr>
<tr>
<td>PS II</td>
<td>Photosystem II</td>
</tr>
<tr>
<td>QLD</td>
<td>Queensland</td>
</tr>
<tr>
<td>Rubisco</td>
<td>Ribulose-1,5-bisphosphate carboxylase-oxygenase</td>
</tr>
<tr>
<td>SPAR</td>
<td>Soil-Plant-Atmosphere Research</td>
</tr>
<tr>
<td>TA</td>
<td>Ambient Temperature (°C)</td>
</tr>
<tr>
<td>TE</td>
<td>Elevated Temperature (°C)</td>
</tr>
<tr>
<td>TC</td>
<td>Canopy Temperature (°C)</td>
</tr>
<tr>
<td>Ti</td>
<td>Leaf Temperature (°C)</td>
</tr>
<tr>
<td>Ti-Ta</td>
<td>Leaf to air temperature differential (°C)</td>
</tr>
<tr>
<td>TDM</td>
<td>Total Dry Matter (g plant⁻¹)</td>
</tr>
<tr>
<td>VPD</td>
<td>Vapour Pressure Deficit (kPa)</td>
</tr>
<tr>
<td>VPDₐ</td>
<td>Atmospheric Vapour Pressure Deficit (kPa)</td>
</tr>
<tr>
<td>VPDₗ</td>
<td>Leaf Vapour Pressure Deficit (kPa)</td>
</tr>
<tr>
<td>Vₜₘₐₓ</td>
<td>Maximum carboxylation efficiency (µmol m² s⁻¹)</td>
</tr>
<tr>
<td>WUEₗ</td>
<td>Leaf-level Water Use Efficiency</td>
</tr>
<tr>
<td>WUEₚ</td>
<td>Plant-level Water Use Efficiency (g kg⁻¹)</td>
</tr>
<tr>
<td>ψl</td>
<td>Leaf water potential (MPa)</td>
</tr>
</tbody>
</table>
Acknowledgements

I would like to thank and acknowledge the support and advice of my supervisors, A/Prof Daniel Tan, Dr Michael Bange, Prof David Tissue and Dr Paxton Payton for their guidance throughout this PhD. Thank you for sharing your wealth of knowledge, and I appreciate the enthusiasm, encouragement and patience.

The financial support of The University of Sydney, the Cotton Catchment Communities Cooperative Research Centre and the Cotton Research and Development Corporation is gratefully acknowledged. Without the 2012 Science and Innovation Award for Young People in Agriculture, Fisheries and Forestry (CRDC), I would not have had the opportunity to travel to the U.S. to learn construction and operation techniques for the whole-canopy chambers. The provision of field and glasshouse resources by CSIRO Plant Industry (CSIRO Agriculture), The University of Western Sydney-HIE, USDA-ARS Lubbock, Texas, and The University of Sydney is also gratefully appreciated.

I really appreciate the assistance of Dr Jeff Baker and Charles Yates, who both have been extremely patient in their response to my seemingly endless questions about the CETA chambers. I also gratefully acknowledge the advice and feedback provided by Dr Nicola Cottle, Dr Warren Conaty, Dr Rose Brodrick, Dr Michael Braunack, Mr Tony Nadelko, and Dr Remko Duursma.

Sincere thanks to the staff of ACRI, Narrabri, especially Jo Price, Alan Thompson, Darin Hodgson, Jane Caton, Loretta Clancy, Max Barnes, Scott McCarron, Dominic Cross, Stephanie Jamison, Marty Tann, Nathan Burley, James Fitt, Kate McMaster, Yvonne Chang, Kellie Gordon, and Tony Pfeiffer. I would also like to thank Renee Smith at UWS and Marie Syapin in Lubbock, for technical support and advice throughout my candidature. I would like to thank James and Carla for welcoming me into their home, during my visit to Lubbock, Texas, and similarly, Yui and Mitch for my enjoyable stay in Richmond.

I appreciate each of my wonderful friends, in Narrabri and beyond, who have been fantastic mentors, and have provided support and encouragement at each step of the way. Importantly, for the incredible love and support of my family, especially Mum, Dad, Elise and Nanny, without which I would not have had the courage to undertake and complete this PhD. Lastly, for John, whose memory has given me the strength to persevere.
Chapter 1: General introduction

Current climate projections indicate that Australia can expect more heatwaves, changes in rainfall distribution, an increase in the intensity of droughts, and small decreases in relative humidity (Whetton and Power, 2007). Current climate trends for Australia show a rise in air temperature and a decline in rainfall over the eastern states. Since the 1950s, each decade has been warmer than the previous decade (CSIRO and Bureau of Meteorology, 2012). Compared with the climate of 1980 to 1999, Australian average air temperatures are projected to rise 0.6 to 1.5 °C by 2030 and 1.0 to 5.0 °C by 2070, depending on the range of global greenhouse gas emissions (CSIRO and Bureau of Meteorology, 2012). Maximum and minimum air temperatures have warmed. Since 1910, daytime maximum temperatures have warmed by 0.8 °C and overnight minimum temperatures have warmed by 1.1 °C (CSIRO and Bureau of Meteorology, 2014). More hot days and nights are expected, with a substantial increase in the number and intensity of heatwaves. A study by Luo et al. (2014) has indicated that key Australian cotton production regions are likely to experience fewer cold temperatures and a longer growing season, which are beneficial for cotton production, but also increased incidence of heat stress and faster crop development. Warmer air temperatures and higher incidence of heat stress may increase water requirements of cotton plants, and thus access to water resources for irrigation purposes may be critical.

Atmospheric carbon dioxide concentration ([CO₂]) has increased in the past 200 years from a pre-industrial concentration of about 280 µL L⁻¹ to 400 µL L⁻¹ in 2013 (IPCC, 2013), with projections for further increases in the future. In general, elevated [CO₂] stimulates photosynthesis in C₃ plants, which may lead to increased crop growth and yield (Ainsworth and Long, 2005; Mauney et al., 1994; Reddy et al., 1995b). Studies in cotton have shown elevated atmospheric [CO₂] to increase biomass production, yield and plant water use efficiency (Ephrath et al., 2011; Mauney et al., 1994; Radin et al., 1987), increased photosynthetic rates (A) and decreased transpiration rates (E) (Reddy et al., 1998a; Reddy et al., 1995d). The reduction in conductance of CO₂ and water vapour through the stomata with elevated [CO₂] can improve leaf level water-use efficiency (WUEₑ), potentially benefiting crop production in water-limited environments (Ainsworth and McGrath, 2010). However, these benefits may be counter-balanced by higher plant water use as a consequence of greater leaf surface area for transpiration (Samarakoon and Gifford, 1996).

Temperature plays an important role in plant photosynthesis and respiration. For this reason, increases in both day and night air temperatures are important. Optimal leaf temperature for growth and metabolism of cotton is around 28 °C (Conaty et al., 2012) and the thermal kinetic window of
cotton is 23.5 - 32 °C (Burke and Upchurch, 1989). Prolonged exposure to high air temperatures (> 40 °C) can result in irreversible damage to the photosynthetic apparatus (Cottee et al., 2012; Cottee et al., 2010). Well-watered plants open their stomata at high temperatures, using evaporative cooling to reduce the temperature of the leaves. The efficiency of leaf cooling by evapotranspiration decreases with increasing vapour pressure deficit (VPD), or when transpiration is reduced due to water deficit (Salvucci and Crafts-Brandner, 2004). Cotton growth and development are sensitive to temperature at all stages of development (Reddy et al., 1999). In testing a range of air temperatures (20/12 °C to 40/32 °C; day/night), Reddy et al. (1992b) reported optimum temperature for stem elongation, leaf area expansion, and biomass accumulation to be 30/22 °C, for cotton grown to 56 DAE. However, developmental rates (depicted by the number of mainstem nodes, number of fruiting branches, and fruiting branch nodes) were not as sensitive to temperatures above 30/22 °C as were growth rates (Reddy et al., 1992b). However, further studies in cotton showed that boll growth increased with warmer temperatures up to 25 °C, but then declined at higher temperatures (32 °C) (Reddy et al., 1999). Therefore, an increase in the frequency of days and nights with very high temperatures may have a negative impact on both growth and development (Stockton and Walhood, 1960). Reddy et al. (1992a) found that the number of bolls produced and boll retention were reduced with increased exposure to high temperature (40 °C) each day. High night temperatures (> 25 °C) have been shown to increase respiration rate and reduce carbohydrates, increase abscission and lower yield (Arevalo et al., 2004; Oosterhuis and Snider, 2011).

Vapour pressure is determined by air temperature and humidity. VPD is the difference between the amount of moisture the air can hold when it is saturated and current moisture in the air (Bureau of Meteorology, 2011). Therefore, changes in temperature and humidity will affect VPD. Rawson et al. (1977) and Slatyer and Bierhuizen (1964) have shown that increasing VPD increases transpiration rates over a range of species, thereby potentially increasing water use by cotton in future production systems, but stomata respond to increased VPD by reducing aperture to partially control the increase in transpiration and to maintain turgor pressure (Knox et al., 2005). Therefore, it is important to understand the leaf-level responses to altered VPD, although plant and crop level water use is also largely determined by other factors such as leaf area index (Hearn, 1980; Krieg, 2000; Turner et al., 1986).

Water availability is of major concern throughout Australian agricultural regions and water is one of the most limiting factors in Australian cotton production (Tennakoon and Hulugalle, 2006). With projected climate change, water availability may become more variable and limited in Australia’s cotton production regions (CSIRO and Bureau of Meteorology, 2012). Cotton production is negatively
affected by soil water deficit (Pettigrew, 2004b). At a leaf level, stomatal closure minimises water loss through transpiration, but also lowers intercellular CO$_2$ concentration (Ci), thereby limiting photosynthesis (Carmo-Silva et al., 2012). Prolonged water stress reduces growth and productivity through reduced biomass, loss of fruit and decreased lint yield and quality (Hearn, 1980). Plant water use and efficiency of cotton may change with combined warmer temperatures and elevated [CO$_2$] (Reddy et al., 1995d). However, it is currently difficult to predict water use of cotton when both temperature and atmospheric [CO$_2$] are increased, and thus is important to understand the interactive effects of elevated temperature, [CO$_2$] and water stress on cotton production. Given positive growth responses to warmer temperatures and elevated [CO$_2$], it is likely that cotton will benefit in these environments in the absence of water stress. However, it is possible that larger plants will have increased water requirements. Conversely, cotton grown in these future environments may also be more susceptible to water stress due to greater biomass and larger leaf area, linking back to greater water requirements.

Australia’s modern irrigated cotton industry developed in the 1960s in northern New South Wales and southern Queensland (Constable et al., 2001; Hearn and Fitt, 1992). The expansion of the modern industry was first based on varieties from the USA, however domestic breeding efforts have led to the development of varieties more suited to the Australian environment (Constable et al., 2001; Liu et al., 2013). Modern varieties have improved yield, fibre properties, and disease and insect resistance compared with the original varieties imported from the USA (Constable et al., 2001; Liu et al., 2013). There may be genotypic differences within a species that affects adaptation to projected environmental conditions, such as elevated atmospheric [CO$_2$] (Ziska et al., 2012). For example, intraspecific crop comparisons in rice (Oryza sativa L.) (Baker, 2004; Moya et al., 1998; Ziska et al., 1996), wheat (Triticum aestivum L.) (Manderscheid and Weigel, 1997; Ziska et al., 2004), soybean (Glycine max L.) (Ziska et al., 2001) and cowpea (Vigna unguiculata L.) (Ahmed et al., 1993) have suggested that there may be intraspecific variations in the yield response to elevated [CO$_2$], yet there has been little research comparing cotton genotypes. Although a number of studies have investigated the effect of [CO$_2$] and temperature on growth and physiology of a variety of cotton cultivars (Reddy et al., 1998a; Reddy et al., 1995a; Reddy et al., 1995c; Reddy et al., 1995d; Yoon et al., 2009), there has not been a comparison between the response of older and current cultivars in Australian production systems. Given that there have been changes in plant morphology and physiology (Constable et al., 2001), it is important to examine current cultivars when determining responses to temperature and elevated [CO$_2$], and the integrated effects of both simultaneously.
It is currently difficult to predict water use of cotton when both air temperature and \([\text{CO}_2]\) are increased, and thus experiments are required to explore the interactive effects of elevated temperature, \([\text{CO}_2]\) and water availability on cotton growth and physiology. In addition, the majority of climate studies in cotton have used controlled environments such as soil-plant-atmosphere-research (SPAR) or free air carbon enrichment (FACE) facilities, and have not been conducted in high input systems, such as those common in Australian cotton production. Benefits and limitations of each of these methods are discussed in the literature review (Chapter 2). Given the impact that warmer air temperatures, elevated atmospheric \([\text{CO}_2]\) and altered VPD may have on cotton physiology, growth and water use, it is necessary to improve our understanding of the integrated effect that these environmental changes may have on cotton production in future environments.

**Central research question**

How do the integrated effects of projected climate change (warmer air temperatures, higher VPD, elevated atmospheric \([\text{CO}_2]\), and water stress) affect physiology, growth and water use of cotton in high yielding/high input cotton systems in Australia?

**Objectives**

The broad aim of this study was to investigate the effect of projected future environments on physiology and growth of cotton to facilitate development of crop management strategies to improve cotton yield and water use efficiencies. The key environmental factors are elevated \([\text{CO}_2]\), warmer air temperatures, altered vapour pressure deficit and soil water deficit, and their impact on glasshouse- and field-grown cotton. Two glasshouse and two field experiments were conducted to evaluate the impact that projected climatic changes will have on cotton production in Australia.

The specific objectives of this project were to:

Quantify the physiological and growth capacity of different genotypes to current and future climate regimes. I conducted a glasshouse experiment to compare early-season growth and physiology of past and current cotton varieties grown in ambient and elevated atmospheric \([\text{CO}_2]\) and temperature treatments under well-watered conditions (Chapter 3);

Assess the physiological and growth response of cotton to drought and drought-recovery phases of a production system in future climatic environments. I conducted a glasshouse experiment to
investigate the interactive effects of elevated [CO₂], warmer air temperature and soil water deficit on biomass production, leaf-level physiology and whole plant water use and efficiency of cotton (Chapter 4);

Assess the impact of altered VPD on leaf level physiology of field-grown cotton to improve current understanding of the plant x environment interactions, thereby contributing to validation and improvement of physiological response models (Chapter 5); and

Evaluate the impact of elevated [CO₂] on field-grown cotton in high input systems. I used Canopy EvapoTranspiration and Assimilation (CETA) chambers to elevate atmospheric [CO₂] of cotton grown in the field, to improve our understanding of the implications of projected environmental conditions on cotton production regions in Australia (Chapter 6).
Chapter 2: Review of literature

2.1. Introduction to projected climate change

There have been substantial increases in atmospheric CO\(_2\) concentration ([CO\(_2\)]) since the beginning of the industrial-age. The natural atmospheric [CO\(_2\)] during the past 800 000 years ranged between 170 to 300 µL L\(^{-1}\) (CSIRO and Bureau of Meteorology, 2012). Atmospheric [CO\(_2\)] has increased in the past 200 years from a pre-industrial concentration of about 280 µL L\(^{-1}\) to 400 µL L\(^{-1}\) in 2013 (IPCC, 2013), with projections for further increases in the future. The rate at which atmospheric [CO\(_2\)] is rising is also increasing (Figure 2.1), and global atmospheric [CO\(_2\)] increased from 2009 to 2011 at a rate of 2 µL L\(^{-1}\) per year (CSIRO and Bureau of Meteorology, 2012). Atmospheric [CO\(_2\)] may reach 450 - 1000 µL L\(^{-1}\) by 2100 (Boote et al., 2011).

---

Figure 2.1: Mean yearly atmospheric [CO\(_2\)] between the years 1959 to 2014 measured at Mauna Loa, Hawaii (Tans and Keeling, 2014).
Current climate change projections indicate that Australia can expect more heatwaves, changes in rainfall distribution, an increase in the intensity of droughts, and small decreases in relative humidity (Whetton and Power, 2007). Current climate trends for Australia show a rise in air temperatures and a decline in rainfall over the eastern states. Since the 1950s, each decade has been warmer than the previous decade. There has been an increase in the frequency of warm weather and decrease in the frequency of cold weather. Since 1910, Australian annual average overnight minimum temperatures have warmed by 1.1 °C and daily maximum temperatures have increased by 0.75 °C indicating that minimum temperatures have warmed more rapidly than daytime maximum temperatures (CSIRO and Bureau of Meteorology, 2012). The frequency of very hot (> 40 °C) daytime temperatures have been increasing since the 1990s (CSIRO and Bureau of Meteorology, 2012). Furthermore, Australian average air temperatures are projected to rise by 0.6 to 1.5°C by 2030 when compared with the climate between 1980 and 1999 (CSIRO and Bureau of Meteorology, 2014). Compared with 1980 to 1999, Australian air temperatures are projected to be in the range of 1.0 to 5.0 °C warmer by 2070 if global greenhouse gas emissions are within the range of projected future emission scenarios considered by the Intergovernmental Panel on Climate Change (IPCC) (CSIRO and Bureau of Meteorology, 2014). Models suggest that the occurrence of warmer air temperatures across the key cotton production areas in Australia may extend the growing season (by reducing the incidence of cold shocks or days with air temperatures less than 11 °C), and the higher incidence of hot days may also impact growth and increase the rate of crop development (Luo et al., 2014).

Australia’s rainfall is already highly variable (CSIRO and Bureau of Meteorology, 2012; CSIRO and Bureau of Meteorology, 2014). Seasonal shifts are expected in some regions. With future climate change droughts are expected to become more frequent and severe in southern Australia with further decreases in average rainfall, and up to a 30% decrease in rainfall by 2070 compared with the 1980 to 1999 climate (CSIRO and Bureau of Meteorology, 2014). An increase in the number and intensity of extreme rainfall events is projected for most regions (CSIRO and Bureau of Meteorology, 2014). Rainfall projections for northern Australia range from a 30% decrease to 20% increase by 2070, compared with the 1980 to 1999 climate. South-East Australia may experience decreased precipitation in spring and increases in autumn (CSIRO and Bureau of Meteorology, 2014), potentially leading to reduced water availability over the Australian cotton season, and therefore reduced in-crop rainfall necessary for dryland cotton production. Although the majority of Australian cotton is produced in high-input irrigated systems, rainfall patterns throughout production areas are relevant for all growers as these affect management strategies in all systems. The most direct result of redistributed precipitation is altered soil water content (SWC), though specific patterns will depend on differences in soil characteristics (Zeppel et al., 2014). Changes in SWC are likely to be exacerbated
by predicted rising temperatures and intense heat waves increasing evaporation and transpiration (Zeppel et al., 2014).

2.2. Introduction to Australian cotton production

Cotton production in Australia extends from central Queensland to southern New South Wales (NSW) but the majority of cotton is grown in the inland regions of northern New South Wales (NSW) and southern Queensland (DAFF, 2011).

The expansion of the modern industry was initially based on varieties from the USA; however, domestic breeding efforts led to the development of superior cultivars, specifically suited to the Australian environment and management systems (Constable et al., 2001). Modern cotton cultivars exhibit improved yield, fibre properties, and disease and insect resistance compared with the original USA varieties (Constable et al., 2001; Liu et al., 2013). In addition, introduction of transgenic cotton varieties with insect resistance have helped the Australian cotton industry and reduced pesticide use. Bt cotton, which contains genes from *Bacillus thuringiensis* (Bt) expressing the insecticidal proteins Cry1Ac and Cry2Ab, has reduced pesticide use for the control of major Lepidopteran pests (particularly *Helicoverpa* spp. in Australia) (Bange et al., 2008). Herbicide tolerant and Bt cotton constitute > 90% of Australia’s cotton crop (Smith, 2011). Thus, there have been significant changes to cotton genotypes used in Australian production systems and these have all had significant contribution to the development of the modern Australian cotton industry.

Australian cotton production is highly mechanised, with significant inputs of water, fertiliser and pesticides (Braunack, 2013; Hearn and Fitt, 1992). The majority of Australian cotton is grown using furrow-irrigation (Roth et al., 2013; Tennakoon and Milroy, 2003), using Australian bred cotton cultivars suited to the climate and the soil (Liu et al., 2013). The use of fertilisers on Australian cotton farms reflects the demand for nutrients from cotton plants. The nutrients N, P, K, S, Ca and Mg are required in large amounts for the production of both cotton lint and seed (Rochester et al., 2012). Current cotton cultivars tend to take up more N, and other nutrients, than has been historically measured, a result of improved higher-yielding cultivars and improved soil fertility (Rochester, 2011). Improvements in varieties and management have meant that over the past 25 years lint yields have steadily increased, to the point at which the average Australian cotton yield (2320 kg lint/ha) was 3 times the world average (776 kg lint/ha) in 2013 (The Australian Cottongrower, 2013), representing some of the highest lint yields in the world.
This review examines current literature on the effects of projected climate change on cotton physiology and growth. This review summarises the effects of elevated atmospheric \([\text{CO}_2]\), warmer temperatures, vapour pressure deficits (VPD) and water availability and demand on photosynthesis and respiration, transpiration and water use efficiency and growth, yield and quality. This review also examines the effects of combined environmental interactions (temperature, \(\text{CO}_2\) and water) on cotton physiology and growth. In addition, different controlled environment facilities are investigated and the implications of climate change for Australian cotton production is assessed.

2.3. A summary of the basics of photosynthesis

Intercellular \([\text{CO}_2]\) (\(C_i\)) and leaf temperature (\(T_l\)) are two important factors that affect the rate of leaf photosynthesis. Thus, changes in atmospheric \([\text{CO}_2]\) and air temperature in scenarios of future climatic change may affect photosynthesis of cotton. Photosynthesis is an oxidation-reduction process that takes place in two key steps. In light reactions, light energy drives the synthesis of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) by energising the two photosystems located in the thylakoid membranes of the chloroplast. In dark reactions (Calvin-Benson Cycle), \(\text{CO}_2\) is reduced to carbohydrates by utilising ATP and NADPH produced by the light reaction (Ashraf and Harris, 2013). The enzyme ribulose-1, 5-bisphosphate (RuBP) carboxylase-oxygenase (Rubisco) combines RuBP with \(\text{CO}_2\) to form two molecules of 3-phosphoglycerate. However, Rubisco can also catalyse a reaction where oxygen (\(O_2\)) is the substrate and results in less net carbon fixation and leads to the production of \(\text{CO}_2\) in a process known as photorespiration.

The proportion of the time that Rubisco catalyses \(\text{CO}_2\) versus \(O_2\) is dependent on the \([\text{CO}_2]/[O_2]\) ratio. Thereby, the role of \(\text{CO}_2\) as a substrate is important in these reactions, with higher \(C_i\) favouring increased rates of carbon assimilation (von Caemmerer and Farquhar, 1981). The reaction is also temperature dependent, with oxygenase activity increasing with warmer temperature (Carmo-Silva and Salvucci, 2012; Ehleringer and Cerling, 2002). The dependence of Rubisco on the \([\text{CO}_2]/[O_2]\) ratio establishes a firm link between current atmospheric conditions and photosynthetic activity. As a consequence of Rubisco sensitivity to \(O_2\), the efficiency of the \(C_3\) pathway decreases at lower atmospheric \([\text{CO}_2]\) but efficiency is increased at elevated \(\text{CO}_2\) environments because photorespiration is reduced (Ehleringer and Cerling, 2002). Therefore, as the photosynthetic process is influenced by both temperature and atmospheric \([\text{CO}_2]\), there may be changes in photosynthesis of cotton plants grown in future warmer, higher-\(\text{CO}_2\) environments.
Both light and dark reactions take place in the chloroplast. However, the chloroplast is highly sensitive to stressful environments such as salinity, drought, extremes of temperature, flooding, varying light intensity, and UV radiation.

2.4. Increased CO₂

Projected increases in atmospheric [CO₂] may directly affect physiological processes and growth rates of plants (Reddy et al., 1995b). The increase in crop dry weight depends primarily on the balance between photosynthesis and respiration (Hearn and Constable, 1984). Elevated atmospheric [CO₂] stimulates photosynthesis in C₃ crops which can lead to increases in crop growth and yield. Furthermore, elevated [CO₂] can decrease conductance of CO₂ and water vapour through the stomata in both C₃ and C₄ plants by reduced stomatal aperture, which can improve leaf-level water-use efficiency potentially benefitting crop production in water-limited environments (Ainsworth and McGrath, 2010). Specific physiological and growth responses to elevated atmospheric [CO₂] will now be discussed.

2.4.1. Photosynthesis and respiration

*Increased atmospheric [CO₂] and Rubisco*

Plants sense and respond to changes in atmospheric [CO₂] through leaf gas exchange. In C₃ plants, mesophyll cells containing Rubisco are in direct contact with the intercellular air space that is connected to the atmosphere via stomatal pores in the epidermis. Rubisco has a low affinity for CO₂ on carboxylation, and this reaction is not saturated at current atmospheric [CO₂] (approximately 400 µL L⁻¹). For C₃ crops, rising atmospheric [CO₂] may increase net CO₂ assimilation through the saturation of Rubisco because Rubisco is not CO₂-saturated in the current atmosphere (~ 400 µL L⁻¹), and because CO₂ inhibits the competing oxygenation reaction leading to photorespiration (Drake et al., 1997; Long et al., 2006). There are two ways that plants benefit from photorespiration: the production of amino acids, glycine and serine, which can be used for protein synthesis; and as an alternative electron sink when CO₂ is not available, e.g. during water stress (Sharkey, 2001). However, the overall effect of photorespiration is deleterious (Sharkey, 2001).

The biochemical processes of a plant may be partially evaluated by the relationship between A and Ci. At low Ci, assimilation rate depends on RuBP carboxylase activity (carboxylation efficiency; V_{cmax}). As Ci increases, assimilation rate is limited by the rate of electron transport through photosystem II (PS II) and RuBP regeneration (J_{max}). Changes in the net rate of CO₂ assimilation reflect changes in both stomatal conductance and mesophyll capacity for photosynthesis (also referred to as stomatal and
non-stomatal limitations). In turn, the mesophyll capacity depends on the activity of ribulose bisphosphate (RuP2) carboxylase-oxygenase and on the capacity for photosynthetic electron transport to regenerate RuP2 (Farquhar et al., 1980; Sharkey et al., 2007; von Caemmerer and Farquhar, 1981). Elevated [CO₂] favours carboxylation efficiency and the limitation is shifted toward RuBP regeneration (Bernacchi et al., 2005; Sage et al., 1989). Therefore, elevated [CO₂] increases photosynthesis by increasing the carboxylation rate of Rubisco and competitively inhibiting the oxygenation of RuBP, thereby favouring the carboxylation reaction (Ainsworth and Long, 2005; Tissue et al., 1993).

**Models**

Models can be used to predict plant responses to changing conditions. The Farquhar et al. (1980) model was based on the kinetics of Rubisco, and it has been widely used for predicting the response of photosynthetic CO₂ fixation, and thus plant biomass production, to environmental change (Crafts-Brandner and Salvucci, 2004). The activation state of Rubisco is the primary metabolic limitation to photosynthetic CO₂ fixation under conditions of high temperature and high atmospheric [CO₂] (Crafts-Brandner and Salvucci, 2004).

**Photosynthetic acclimation of plants to long-term CO₂ exposure**

Many C₃ species often exhibit photosynthetic acclimation/down-regulation when exposed to long-term elevated [CO₂], thus reducing photosynthetic potential (Arp, 1991; Singh et al., 2013a). Prolonged exposure to elevated [CO₂] has also been shown to lead to a decrease in the levels of transcripts for proteins involved in photosynthesis (Stitt and Krapp, 1999). The initial stimulation of photosynthesis in elevated [CO₂] is often followed by a decline of photosynthesis, that is typically accompanied by a decrease in Rubisco content (hence Vₐₐₚₚₚ and Jₐₐₚₚₚ) (Ainsworth and McGrath, 2010; Singh et al., 2013a). The reduction of photosynthetic capacity found when plants are exposed to elevated CO₂ for extended periods is a function of the balance between supply and demand of carbohydrates (Arp, 1991). High CO₂ generally increases the supply of carbohydrates, but this may not affect photosynthetic capacity if sink size is sufficient (Arp, 1991). If the capacity of the sink for carbohydrates is reduced by low nitrogen, low temperature or restricted root growth (in pot experiments), then the increased supply of carbohydrates in elevated CO₂ results in feedback inhibition and a decrease in photosynthetic capacity (Arp, 1991). In addition, Thomas & Strain (1991) found that reduced photosynthetic capacity of plants grown at elevated levels of CO₂ was associated with inadequate rooting volume. They suggested that a possible mechanism for regulating net photosynthesis of plants grown in elevated CO₂ is through sink-limited feedback inhibition (Thomas and Strain, 1991). Therefore, stresses such as
root restrictions, which are an important consideration for studies conducted in non-field conditions, may negate plant physiological and acclimation responses to elevated [CO₂].

**Effects of elevated [CO₂] on respiration**

Dark respiration (R₀) of a cotton leaf varies with age. However, there has been much uncertainty surrounding the effects of elevated [CO₂] on leaf respiration. At ambient [CO₂], R₀ peaks during rapid growth of the leaf, to about 5 ng CO₂ cm⁻² s⁻¹ (1.1 µmol CO₂ m⁻² s⁻¹), then declines to about 1.7 ng CO₂ cm⁻² s⁻¹ (0.3 µmol CO₂ m⁻² s⁻¹) for a 50 day old leaf (Hearn and Constable, 1984). Elevated [CO₂] has previously been suggested to inhibit R₀ (Amthor, 1997; Drake et al., 1997; Reuveni and Gale, 1985); however, re-evaluation of methods used to measure dark respiration of plants grown under elevated [CO₂] suggest that short-term exposure to elevated [CO₂] does not affect respiration (Ainsworth and Long, 2005; Amthor, 2000; Davey et al., 1999; Jahnke et al., 2001). Until recently, the most common approach has been to use open gas exchange systems, designed for assessing photosynthesis. Measurement of O₂ uptake or CO₂ efflux in such a way as to avoid leaks and diffusion between the chamber and the atmosphere through gaskets or leaves, indicate that there is little or no instantaneous effect of [CO₂] (Leakey et al., 2009a).

However, long-term exposure to elevated [CO₂] may alter rates of respiration in response to the stimulation of photosynthesis and biomass production. For instance, Reddy et al. (1995d) found that increased canopy respiration rates in cotton plants grown at 700 µL L⁻¹ [CO₂] for 70 days could be attributed to greater accumulation of biomass and faster growth rate. Leakey et al. (2009b) reported that for soybean, greater respiratory quotient and leaf carbohydrate status at 550 µL L⁻¹ [CO₂] indicated that stimulated rates of leaf-level respiration were supported through the use of the additional photoassimilate from enhanced photosynthesis at elevated [CO₂]. Therefore, elevated [CO₂] may increase respiration rates (on a ground area basis) of cotton grown in future, higher-[CO₂] environments as a result of enhanced anabolic processes that consume respiratory ATP (Watanabe et al., 2014). However, elevated [CO₂] may reduce respiration on a dry weight basis if elevated [CO₂] increases plant mass without changes in leaf-level respiration.

**Net carbon assimilation**

Net carbon assimilation is a combination of photosynthesis and respiration, and therefore will be affected by the response of each of these factors in future, higher CO₂ environments. The potential rate of net photosynthesis of an individual cotton leaf at ambient CO₂ levels is approximately 30 µmol CO₂ m⁻² s⁻¹ for a recently fully expanded leaf (13-15 days after leaf unfolding, when the leaf was 75 - 90% of maximum area), well-watered and fertilised, about 30 °C leaf temperature and saturating light
Plant factors, such as leaf age (photosynthesis begins to decline at approx. 12 days after unfurling), and environmental effects alter the rate of photosynthesis. A 50 day old leaf has about half the maximum rate of photosynthesis (Hearn and Constable, 1984). However, long-term elevated [CO\textsubscript{2}] of 550 µL L\textsuperscript{-1} increased midday net photosynthetic rates of leaves and canopies of cotton by 19 - 41% compared with 370 µL L\textsuperscript{-1} [CO\textsubscript{2}], with the greatest CO\textsubscript{2} effect on canopy and leaf photosynthesis occurring mid-season (in June and July, respectively) (Hileman et al., 1994). This indicates that carbon assimilation rates respond to elevated [CO\textsubscript{2}], but also change throughout the season.

### 2.4.2. Transpiration and water use

**Effect of elevated [CO\textsubscript{2}] on stomatal conductance (g\textsubscript{s})**

Changes in atmospheric [CO\textsubscript{2}] are sensed by the plasma membrane of the guard cells (Knox et al., 2005) and are thought to respond to the intercellular [CO\textsubscript{2}] (Ci) rather than [CO\textsubscript{2}] at the leaf surface and in the stomatal pore (Mott, 1988). Electrophysiological studies showed that elevated [CO\textsubscript{2}] alters the activity of K\textsuperscript{+} channels which are involved in ion and organic solute concentrations that mediate the turgor pressure in the guard cells (Brearley et al., 1997; Hanstein and Felle, 2002). These changes depolarise the membrane potential of the guard cells and cause water to move out of the guard cells, thus resulting in stomatal closure (Assmann, 1993; Hanstein and Felle, 2002). Therefore, greater depolarisation at elevated [CO\textsubscript{2}] will result in a reduced stomatal aperture (Ainsworth and Rogers, 2007; Assmann, 1999; Macrobbie, 1983; Travis and Mansfield, 1979). It is expected that guard cell signalling is organised as a network, although the signal transduction pathways that function upstream of the ion channel activities are not well known. Short term exposure to elevated [CO\textsubscript{2}] generally decreases stomatal aperture. In the long term, decreases in g\textsubscript{s} can be caused by changes in stomatal density or stomatal index (the percentage of epidermal cells that are guard cells), as well as stomatal aperture (Ainsworth and Rogers, 2007).

One of the most consistent responses of plants to elevated [CO\textsubscript{2}] is a decrease in g\textsubscript{s}. Averaged across all plant species grown at elevated [CO\textsubscript{2}] in free-air CO\textsubscript{2} enrichment (FACE) experiments, g\textsubscript{s} was reduced by 22% (Ainsworth and Rogers, 2007), although the response of different types of plants (e.g. trees, shrubs, C\textsubscript{3} and C\textsubscript{4}) varied. However, in FACE experiments, the decrease in g\textsubscript{s} at elevated [CO\textsubscript{2}] did not appear to be caused by a significant change in stomatal density (Estiarte et al., 1994; Reid et al., 2003). Therefore, it is likely that changes in stomatal aperture, rather than density, determine the response of g\textsubscript{s} to elevated [CO\textsubscript{2}] (Ainsworth and Rogers, 2007). While the sensitivity of guard cells to environmental factors does not appear to acclimate with growth at elevated [CO\textsubscript{2}], the magnitude of
the effect of higher \([\text{CO}_2]\) on \(g_s\) varies considerably with environmental factors. There is generally a smaller effect of elevated \([\text{CO}_2]\) on \(g_s\) during dry periods (Ainsworth and Rogers, 2007) as stomates close in response to water deficits.

**Effect of elevated \([\text{CO}_2]\) on transpiration rates**

Responses of transpiration to elevated \([\text{CO}_2]\) are varied, particularly between SPAR and FACE experiments. In a SPAR study, Reddy et al. (1995b) showed that whole canopies of cotton plants grown in high (700-900 \(\mu\text{L L}^{-1}\)) \(\text{CO}_2\) environments transpired less than plants grown in ambient (350 \(\mu\text{L L}^{-1}\)) \(\text{CO}_2\) conditions, under optimal conditions (in the absence of water deficit). Similarly, transpiration per unit leaf area was lower at elevated \([\text{CO}_2]\) (710 \(\mu\text{L L}^{-1}\)) compared with cotton grown at low \([\text{CO}_2]\) (352 \(\mu\text{L L}^{-1}\)) for plants grown in a phytotron (Samarakoon and Gifford, 1996). In addition, SPAR studies in rice (\textit{Oryza sativa L.}) have shown that elevated \([\text{CO}_2]\) (700 \(\mu\text{L L}^{-1}\)) reduced canopy transpiration by approximately 10% (Baker and Allen, 2005).

In FACE studies, Bhattacharya et al. (1994) demonstrated that \(\text{CO}_2\) enrichment decreased stomatal conductance and single-leaf transpiration of cotton only towards the end of the season. However, Hileman et al. (1994) found that canopy transpiration generally was not affected by \(\text{CO}_2\) enrichment, except late in the season, as the decrease in leaf stomatal conductance was negated by an increase in canopy size. This suggests that cotton crops grown in future, higher \(\text{CO}_2\) environments may have increased photosynthetic rates and greater yields, but will require the same amount of water as crops grown under current conditions, although this study did not account for changes in air temperatures. Similarly, other FACE experiments found that elevated \([\text{CO}_2]\) (550 \(\mu\text{L L}^{-1}\)) did not significantly change cotton crop transpiration (Dugas et al., 1994; Hunsaker et al., 1994; Kimball et al., 1994). Studies using water balance evaporation method (Hunsaker et al., 1994), sap flow (Dugas et al., 1994) and energy balance methods (Kimball et al., 1994) for measuring canopy evapotranspiration of cotton found that there was no significant difference in canopy evapotranspiration of cotton grown in at elevated \([\text{CO}_2]\) (550 \(\mu\text{L L}^{-1}\)) compared with ambient \([\text{CO}_2]\). This suggests that elevated \([\text{CO}_2]\) may decrease transpiration at the leaf level, but increased overall plant size and leaf area may not equate to reduced water use at the plant and crop level. However, altered plant response to high frequency \(\text{CO}_2\) pulses in FACE studies (Bunce, 2012) may be a factor for the differences in canopy transpiration rates of plants grown with elevated \([\text{CO}_2]\) given fluctuating \([\text{CO}_2]\) could under-estimate plant growth at projected future atmospheric \([\text{CO}_2]\). Therefore, there is still much uncertainty regarding the effect of elevated \([\text{CO}_2]\) on canopy transpiration rates, and thus plant water use, in cotton.
**Effect of elevated [CO₂] on WUE**

Leaf-level or instantaneous water use efficiency (WUEᵢ) is defined as the ratio of photosynthesis to transpiration (Hileman et al., 1994) and plant-level water use efficiency (WUEₚ) is measured by kg of biomass accumulated per kg of water used. Water use efficiency of a crop may be increased either by an increase in the biomass produced or a reduction in water use. Thereby, factors such as plant size, planting density and configuration (e.g. skip-rows) will also affect crop-level WUE. It has been suggested that CO₂-enrichment increased WUE due to partial stomatal closure and reduced transpiration coupled with increased biomass (Kimball and Idso, 1983; Mauney et al., 1994). Increasing [CO₂] can improve WUE of single, sunlit leaves (Hileman et al., 1994). An increase in [CO₂] from 400 µL L⁻¹ to 600 µL L⁻¹ increased WUEᵢ by 30 - 40% (Ko and Piccinni, 2009). However, in FACE experiments in cotton, elevated [CO₂] increased biomass (Mauney et al., 1994) without significantly changing transpiration (Dugas et al., 1994; Hunsaker et al., 1994; Kimball et al., 1994), thereby increasing WUEₚ by an increase in biomass rather than a reduction in water use. Additionally, despite greater growth responses to elevated [CO₂] in well-watered conditions, limited water treatments in the Arizona FACE experiments in 1990 and 1991 also showed there were some growth benefits with elevated [CO₂]. Therefore, there may also be benefits for Australian cotton production if growers can access similar quantities of water in the future.

**2.4.3. Growth, yield and quality**

Growth, yield and leaf photosynthetic rates of cotton all respond strongly to CO₂ enrichment (Hileman et al., 1994). The increase in crop dry weight depends primarily on the balance between photosynthesis and respiration (Hearn and Constable, 1984). Cotton responds positively to CO₂ enrichment by increasing biomass to a greater extent than plants grown in ambient [CO₂]. Elevated [CO₂] stimulates early biomass production, extends the period of rapid growth and eliminates late-season slow down (Bhattacharya et al., 1994). Increasing atmospheric [CO₂] increased non-structural carbohydrate levels in leaves, stems and roots of cotton (Hendrix et al., 1994; Zhao et al., 2004). Cotton leaves export carbon to the rest of the plant as sucrose (Tarczynski et al., 1992). If metabolic demands upon the leaf sucrose pool are met, excess photosynthate produced during the day is stored within leaf chloroplasts as starch. Stored carbohydrates can be converted to sucrose and used during periods of high metabolic demand, such as heavy fruit set or root growth, to allow such plants to resist metabolically stressful periods better than plants grown at 370 µL L⁻¹ [CO₂] (Hendrix et al., 1994).

Prior et al. (1994) indicated that in a FACE experiment, increases in atmospheric [CO₂] will enhance plant root growth, with taproots of CO₂ enriched cotton displaying greater volume, dry weight, length,
and tissue density than those grown at ambient \([\text{CO}_2]\) (370 \(\mu\text{L L}^{-1}\)). Cotton grown at doubled atmospheric (720 \(\mu\text{L L}^{-1}\)) \([\text{CO}_2]\) produced 40% more leaf, stem and root mass than plants grown at 360 \(\mu\text{L L}^{-1}\) \([\text{CO}_2]\) (Reddy et al., 1997). Similarly, Mauney et al. (1994) found that increasing \([\text{CO}_2]\) to 550 \(\mu\text{L L}^{-1}\) for 144 days increased biomass by 37%, indicating similar results in both SPAR and FACE experiments. In addition, Kimball and Mauney (1993) found that increasing \([\text{CO}_2]\) to 650 \(\mu\text{L L}^{-1}\) increased yield by 60% and biomass by 62%, for cotton grown in open top chambers. Plants grown in high \(\text{CO}_2\) produced more vegetative branches and more secondary fruiting branches than plants grown in ambient \(\text{CO}_2\) environments (Reddy et al., 1995b). Cotton grown at 720 \(\mu\text{L L}^{-1}\) atmospheric \([\text{CO}_2]\) had about 40% more squares and bolls than 360 \(\mu\text{L L}^{-1}\) \([\text{CO}_2]\) (Reddy et al., 1999). The developmental events of cotton plants, such as floral initiation, days to first flower, and the rate of mainstem node production (Reddy et al., 1995b; Reddy et al., 1997) are relatively insensitive to \(\text{CO}_2\). Boll maturation period was also not affected by atmospheric \([\text{CO}_2]\) (Reddy et al., 1999). Harvestable yield was increased by 43% by a 48% increase in \([\text{CO}_2]\) (Mauney et al., 1994), with increased biomass and yield attributed to increased early leaf area, more profuse flowering and a longer period of fruit retention. In a SPAR study, elevated \([\text{CO}_2]\) (720 \(\mu\text{L L}^{-1}\)) did not affect any fibre parameters of cotton (Reddy et al., 1999), thereby indicating that fibre quality may not be directly affected by elevated \([\text{CO}_2]\), however, should be tested in the field given the potential of pot effects affecting the response of plants to elevated \([\text{CO}_2]\) (Arp, 1991).

### 2.5. Increased temperature

Temperature is an important factor in controlling the rate of plant growth, developmental events such as organ initiation (leaf, flower, node), and the time interval between anthesis and fruit maturation. Temperature also affects the rate of biochemical reactions through an effect on the kinetic energy of reacting molecules, and an effect on tertiary structure of enzymes and membranes (Knox et al., 2005). As such, physiological processes dependent on enzymatic function, such as photosynthesis and respiration are sensitive to high temperature stress thus reducing growth, development and ultimately yield.

### 2.5.1. Photosynthesis and respiration

Temperature plays an important role in plant photosynthesis and respiration. Temperature affects all biological activity because it determines the rates of chemical reactions and the activity of enzymes (Knox et al., 2005), including Rubisco, an important enzyme involved in photosynthesis. The activation
state of Rubisco is the primary metabolic limitation to photosynthetic CO\textsubscript{2} fixation under conditions of high temperature (Crafts-Brandner and Salvucci, 2004).

Optimal leaf temperature of cotton is around 28 °C (Conaty et al., 2012) and the thermal kinetic window of cotton is 23.5 - 32 °C (Burke and Upchurch, 1989). Net photosynthesis of cotton begins to decline above 35 °C (El-Sharkawy and Hesketh, 1964), however the response is greatly affected by acclimation to air temperature and water vapour pressure deficit (Hearn and Constable, 1984) as leaves were exposed to altered environmental conditions for a period of 20 minutes (El-Sharkawy and Hesketh, 1964). A study by Reddy et al. (1991a) has shown that grown at slightly above optimum temperatures (30 and 35 °C day temperature), cotton canopies fixed twice as much CO\textsubscript{2} than plants growing at 20/10 °C (day/night) and 40/30 °C, during the fruiting period and a similar trend during the boll-filling period. This was attributed to boll development, thus providing greater sink strength than vegetative structures. Similarly, Cottee et al. (2010) showed a small genotype specific drop in leaf photosynthesis and electron transport rate of cotton exposed to higher than ambient air temperatures under tents in the field indicating a negative photosynthetic response to very high temperatures.

Prolonged exposure to high air temperatures (T\textsubscript{a}) (> 40 °C) generally results in irreversible damage to photosynthetic pathways due to the disruption in thylakoid membranes and damage to PSII. Heat stress causes disruption at the thylakoid membrane, thereby reducing the activities of membrane-associated electron carriers, which ultimately results in a reduced rate of photosynthesis (Ashraf and Harris, 2013). Crafts-Brandner and Salvucci (2000) reported that Rubisco activity was inhibited in both cotton and tobacco when leaf temperatures exceeded 35 °C, which may be genotype specific (Cottee et al., 2012). Changes in temperatures also affect the specificity of Rubisco for CO\textsubscript{2}/O\textsubscript{2} and the solubility of CO\textsubscript{2} and O\textsubscript{2} in the atmosphere. Higher temperatures decrease the relative specificity of Rubisco for CO\textsubscript{2} compared with O\textsubscript{2} due to decreased solubility of CO\textsubscript{2} relative to O\textsubscript{2} and decreased affinity of Rubisco for CO\textsubscript{2} relative to O\textsubscript{2} (Drake et al., 1997; Salvucci and Crafts-Brandner, 2004). The solubility ratio describes the relative levels of O\textsubscript{2} and CO\textsubscript{2} in the intercellular spaces of leaves. The solubility ratio O\textsubscript{2}/CO\textsubscript{2} increases with increasing leaf temperature (Ku and Edwards, 1977). Temperature has a major influence in the CO\textsubscript{2}/O\textsubscript{2} ratio due to the differential solubility of each gas with increasing temperature (Perry et al., 1983). The activities of enzymes involved in C\textsubscript{3} and C\textsubscript{4} photosynthetic pathways are altered under stressful environments; however, it depends on the type of species, stomatal and non-stomatal factors, as well as their interaction, how far the changes in activities of these enzymes affect photosynthetic capacity (Ashraf and Harris, 2013). Given cotton is a C\textsubscript{3} plant, this is likely to influence photosynthesis and respiration rates of cotton plants grown in warmer environments.
Respiration at warmer air temperatures

Increases in temperature increase the rates of both photorespiration and dark respiration in cotton (Harley et al., 1992). This can ultimately result in lower translocation rates to developing sinks. High night temperature (above 25 °C) increase respiration rates, decrease soluble carbohydrate concentrations in source leaves, and increase abscission which result in significantly lower yield (Arevalo et al., 2004; Oosterhuis and Snider, 2011). Under high night temperatures, more carbohydrates are utilised by the high respiratory rates at the expense of plant growth (Arevalo et al., 2004). A study by Reddy et al. (1991a) demonstrated that the respiration rates of cotton are higher during the first hour after sunset. This suggests that respiration rates are strongly influenced by carbohydrate supply and that carbohydrate is more readily available immediately after sunset than later in the night. In this study, Reddy et al. (1991a) also showed that plants grown at higher temperatures (both day and night) had higher respiration values, except in the very high temperature treatment (40/30 °C, day/night) where growth was severely limited. However, responses of respiration to environmental variables usually depend on how respiration is expressed (i.e. on a dry weight or ground area basis).

2.5.2. Transpiration and water use

During the day, incoming radiant energy intercepted by the crop must be dissipated by transpiration to avoid a rise in leaf temperature; therefore, higher transpiration rates are likely to occur at high air temperatures (Burke and Upchurch, 1989). However, this may change with other environmental factors such as atmospheric [CO₂] or VPD. Field and glasshouse studies have shown that cotton grown at temperatures between 33 - 40 °C during the day and 20 - 40 °C during the night is able to maintain leaf temperatures between 27 and 32 °C when there was adequate water available for transpirational cooling at high temperatures (Burke and Upchurch, 1989). Thus, higher temperatures are likely to have increased atmospheric VPD in this study, although VPD was not reported (Burke and Upchurch, 1989). A study by Reddy et al. (1995d) demonstrated that elevated temperature increase the transpiration rate of cotton, and that transpiration rate increase to a peak around mid-day then begin to decline and thus varies throughout the day (Figure 2.2). This study also showed that although warmer temperature increases the rate of transpiration, CO₂ enrichment reduced transpiration at all temperatures (Reddy et al., 1995d). However, the efficiency of leaf cooling by evapotranspiration decreases with increasing VPD, or when transpiration slows because of water deficit (Salvucci and Crafts-Brandner, 2004). However, changes in stomatal aperture may also impact Ci of the leaf (Radin et al., 1987), which may change photosynthesis and respiration rates as a result of CO₂ available to the Rubisco enzyme.
Figure 2.2: Diurnal trends in canopy transpiration rates and photosynthetic photon flux density 62 DAE (Days After Emergence) for cotton canopies grown at various temperatures in 350 and 700 µL CO₂ L⁻¹ air (Reddy et al., 1995d).

2.5.3. Growth, yield and quality

Air temperature regulates the rate of phenological development and biomass accumulation in cotton. It determines the start and end of the growing season (Baker et al., 1972; Hearn and Constable, 1984). Increased average daily temperatures at the beginning and end of the season may have a positive effect on yield by extending the window for cotton growth and boll development (Bange, 2007), by reaching day degree (thermal time) requirements for temperature sensitive stages of development (Hearn and Constable, 1984; Tian et al., 2014). However, an increase in the frequency of days and nights with very high air temperatures may have a negative impact on both growth and development (Stockton and Walhood, 1960). The ideal air temperature range for cotton growth and development is 20 °C to 30 °C (Reddy et al., 1991b), although cotton is successfully grown at temperatures exceeding 40 °C (Loka et al., 2011). However, the impact that very high air temperatures have on cotton will also depend on the length of time of exposure, the time of occurrence within the growth cycle, other environmental conditions such as water available to the plant, and heat-tolerance characteristics of the variety. Once air temperatures reach approximately 35 °C, growth begins to decrease, which may be associated with reduced photosynthesis and increased respiration (Reddy et al., 1991a). Three-
week old plants exposed to high air temperatures for four days had reduced biomass production, associated with an inhibition of net photosynthesis (Crafts-Brandner and Salvucci, 2004). In the field, however, it is often difficult to distinguish the effects of air temperature from water stress. In addition, high vapour pressure deficits are also often associated with high temperatures (Hearn and Constable, 1984).

Although adverse air temperatures can affect cotton at all stages of development (Reddy et al., 1999), the crop seems to be particularly sensitive to adverse temperatures during reproductive development (Loka et al., 2011). In temperature controlled growth chambers, the majority of squares and bolls of cotton were aborted at air temperatures above 30/20 °C (day/night) (Reddy et al., 1991b). In addition, developmental events such as floral initiation, days to first flower, and the rate of mainstem node production are very temperature dependent (Reddy et al., 1995b) with the sequence of reproductive development hastened as temperatures increase (Reddy et al., 1996).

In a study by Reddy et al. (1992a), an increase in (day/night) air temperature from 30/22 °C to 40/32 °C increased the number of fruiting sites per plant by 50%. However, an increase in the number of fruiting sites does not always translate into greater yields. Cotton grown at 32 °C produced large numbers of squares, with most producing flowers but a higher proportion of fruit were abscised 3-5 days after anthesis (Reddy et al., 1999). Boll retention decreased significantly under high temperature and is the most sensitive yield component of cotton (Reddy et al., 1999; Reddy et al., 1991b; Zhao et al., 2005). Young bolls abscise when exposed to average daily temperatures above 28 °C and the longer the exposure to greater than optimum temperatures, the higher the abscission frequency. Yield reduction under high air temperatures in the field was positively associated with level of fruit abscission, which was negatively associated with boll number (Cottee et al., 2010). The number of bolls and squares retained per plant was essentially the same between 30/22 °C and 35/27 °C but dropped to nearly zero at 40/32 °C (Reddy et al., 1999). Therefore, both glasshouse and field experiments have demonstrated the negative correlation between high air temperatures and fruit retention for cotton and thus fruit retention may be negatively affected by warmer average temperatures and high temperature extremes in a future climate.

The rate of boll filling increases with temperature up to 25 °C and then declines at 32 °C (Reddy et al., 1999). However, Baker et al. (1972) found that boll growth was twice as fast at 32/23 °C as at 23/20 °C. The differences between these may be explained by different periods of exposure to the temperature treatments, and use of different cultivars. Boll weight was highest at 30/20 °C and was reduced at both higher and lower temperatures. Maximum boll size occurred at lower temperatures (17 - 18 °C). Temperatures above 28 °C were detrimental to mid- to late- season boll retention and
growth (Reddy et al., 1997). Heat stress resulted in substantial alterations in the carbohydrate balance of reproductive tissues, causing poor reproductive success under high temperature. For example, Zhao et al. (2005) reported that high temperature conditions (36/28 °C, day/night) resulted in lower levels of non-structural carbohydrates in one day old cotton bolls and significantly higher abscission rates of young bolls; abscission rates were negatively correlated with the non-structural carbohydrate content of the young boll.

Boll maturation period (the time from anthesis to mature open boll) declines dramatically with increased temperature (Reddy et al., 1999). Faster maturation periods do not equate to improved fibre properties, and Reddy et al. (1999) reported an increased percentage of short-fibre mote at higher temperatures and may thus impact fibre quality.

**Quality**

Changes in air temperature affect fibre properties. Fibre quality attributes such as fibre length, strength, maturity and micronaire are affected by temperature impacting photosynthesis and consequently carbohydrate supply to developing bolls (Bange et al., 2010; Gipson and Joham, 1968; Gipson and Joham, 1969; Reddy et al., 1999). At temperatures less than 25°C during boll growth, fibres are longer (Reddy et al., 1999). As air temperatures increase, fibre length becomes more uniform, but the percentage of short-mote fibre may also be increased (Reddy et al., 1999). High micronaire (> 4.5) may indicate coarse fibre, resulting in low fibre count yarn and thus reduced strength (Bange et al., 2010). As photosynthesis increases with temperature (in the absence of water stress) more resources are available to mature the fibres, thus increasing micronaire. Therefore, high micronaire is more likely to occur in cotton grown in seasons with warmer temperatures during boll filling (Bange, 2007). Fibre fineness and maturity increase with increasing temperature up to 26 ºC, but decrease above 32 ºC (Reddy et al., 1999). Therefore, obtaining maximum fibre quality is determined through a balance of optimum temperature for length, strength and maturity characteristics.

### 2.6. Vapour pressure deficit effects

Vapour pressure is determined by air temperature and humidity. The capacity of air to hold water vapour increases rapidly with an increase in temperature, and thus the same relative humidity at different temperatures indicates very different atmospheric moisture conditions (Anderson, 1936). Atmospheric vapour pressure deficit (VPDₐ) is the difference between moisture in the air and the amount of moisture the air can hold when it is saturated (Bureau of Meteorology, 2011). Therefore, changes in temperature and humidity will affect VPDₐ.
VPD is likely to increase with higher air temperatures provided there is not a marked asymmetry between the increases in night-time and daytime temperatures (Stokes and Howden, 2010). However, historically, minimum overnight temperatures have warmed more rapidly than daytime maximum temperatures (CSIRO and Bureau of Meteorology, 2012), indicating that an asymmetric response is expected. An additional effect on plants may arise from the influence of elevated [CO₂] in reducing stomatal aperture, which may reduce transpiration and hence evaporative cooling of the leaf, and thereby increase the temperature differential between the leaf boundary layer and the atmospheric air and thus, increase effective VPD (Stokes and Howden, 2010). Therefore, there is a need to understand how crops respond to projected changes in VPD.

### 2.6.1. Plant response to the physical environment

Plants respond to environmental stimuli, and crop physiology and yield are greatly influenced by environmental conditions that a plant is exposed to in the field (Pettigrew et al., 1990). Stomatal opening and closing, and thus plant gas exchange, are affected by light, intercellular [CO₂], air humidity, VPD<sub>a</sub>, and plant and soil water deficits (Grantz, 1990; Xue et al., 2004). If air flowing over a leaf changes from high humidity to low humidity, the potential diffusion rate of water in the leaf will increase. However, guard cells will lose turgor and stomatal aperture will decrease (Knox et al., 2005; Lange et al., 1971), thus reducing transpiration and conserving water. Changes in humidity alter transpiration, energy balance and tissue temperature. These in turn, may affect ion uptake, carbon assimilation, water transport and other processes, each of which has further physiological consequences that obscure direct responses to relative humidity (Grantz, 1990). Reducing stomatal aperture restricts water loss from the leaf by avoiding high transpiration rates that would otherwise be caused by high VPD (Oren et al., 1999).

VPD is recognised as one of the most important sources of variation in stomatal conductance, but the mechanism of the response is unknown (Bunce, 1997; Grantz, 1990). Stomata respond to factors that affect the rate of transpiration, such as leaf to air vapour pressure difference (VPD<sub>L</sub>) (Farquhar and Sharkey, 1982; Yong et al., 1997). It is generally agreed that stomatal conductance usually decreases as VPD increases, due to plants closing stomata (Baker et al., 2007; Farquhar and Sharkey, 1982). However, a number of studies have demonstrated an increase in transpiration rates at high VPD<sub>L</sub> for a wide range of plant species (Duursma et al., 2013; Rawson et al., 1977; Ray et al., 2002; Slatyer and Bierhuizen, 1964; Yong et al., 1997). The transpiration response of a number of different species to VPD ranging between 0.8 – 2.7 kPa is shown in Figure 2.3 (Rawson et al., 1977). Therefore at high VPD (at constant temperature), plant water requirements may be greater due to higher transpiration rates, despite a reduction in stomatal conductance (Figure 2.4). In addition, there is evidence that high VPD
may inhibit photosynthesis unrelated to stomatal closure (Morison and Gifford, 1983; Pettigrew et al., 1990) and in response to stomatal closure (Gilbert et al., 2011), although Duursma et al. (2013) found that photosynthesis of glasshouse-grown cotton was relatively insensitive to VPD as it decreased on average only 13% from the maximum photosynthetic rate over the range of VPD (1 – 4 kPa). Rawson et al. (1977) also found that photosynthesis and diffusion resistances were not affected by VPD over the range of 0.8 - 2.7 kPa for a number of species, including wheat, soybean, sunflower and sorghum. Differences between plant responses are evident between species (Rawson et al., 1977), but may also be due to use of different genotypes within a species (Sadok and Sinclair, 2009a; Sadok and Sinclair, 2009b), or differences in experimental methods such as the length of time and range of VPD that plants were exposed to. Although there may not be any direct photosynthetic response to increased VPD, reductions may occur as a consequence of reduced stomatal conductance and increased transpiration rates. Therefore, if VPD does increase, higher water demand and lower water use efficiency may be two compounding negative effects.

Figure 2.3: Transpiration rates for a number of $C_3$ (white symbols) and $C_4$ (shaded symbols) species at different VPD and an air temperature of 26 °C (Rawson et al., 1977). R values for linear regressions appear in brackets. Prior to the experiment, plants were well-watered and fertilised, and grown at 27/22 °C (day/night) in a naturally
lit glasshouse. Leaves were stabilised in irradiated assimilation chambers for 4 h at 26 °C, and humidity was increased in steps until the dewpoint of the system was reached. An open gas exchange system recorded measurements at each level of humidity.

Figure 2.4: Responses of transpiration (E; a and c) and stomatal conductance (g; b and d) to vapour pressure difference between leaf and air (VPD) in soybean (a and b) and cocklebur leaves (c and d) at two carbon dioxide partial pressures (3.5 Pa; circle and 35 Pa; triangle) where leaf temperature was maintained at 28 °C (Yong et al., 1997).

2.6.2. Models to explain stomatal response to VPD

Responses of stomatal conductance to increasing VPD generally follow an exponential decrease described by several empirical functions (Oren et al., 1999). Two long-standing theories of modelling stomatal conductance are the empirical approach (Ball et al., 1987) and the optimal approach (Cowan and Farquhar, 1977). Stomatal conductance is generally modelled using an empirical representation of stomatal conductance; however, the parameters of such models have no fundamental biophysical meaning. As they have been developed from experimental observations rather than mechanistic understanding or theory of stomatal behaviour, limitations occur when applying the model to
circumstances such as elevated atmospheric [CO₂]. Consequently, there is limited understanding of how the parameters vary with species and do not account for responses to climate change, so many of these models assume that the parameters are constant for all C₃ species (Medlyn et al., 2011). The theory of optimal stomatal behaviour (Cowan and Farquhar, 1977) is based on the idea that stomata should act to maximise carbon gain (photosynthesis, A) while minimising water loss (transpiration, E). Medlyn et al. (2011) combined the two models: Cowan and Farquhar (1977) theory of optimal stomatal behaviour and Farquhar et al. (1980) model of photosynthesis, to produce a unified model that explains the responses of stomatal conductance to changing atmospheric [CO₂]. Medlyn et al. (2011) showed that stomatal conductance approximately follows:

\[ g_s = g_0 + 1.6 \left( 1 + \frac{g_1}{\sqrt{VPD}} \right) \frac{A}{C_a} \]

where, \( g_0 \) is a residual stomatal conductance (mol m\(^{-2}\) s\(^{-1}\)), \( g_1 \) is the ‘slope parameter’ which is related to the marginal cost of water (i.e. water use for the assimilation of each additional unit of carbon), \( A \) is the net CO₂ assimilation rate (µmol m\(^{-2}\) s\(^{-1}\)), \( C_a \) is the atmospheric CO₂ concentration (µmol mol\(^{-1}\)), and VPD is the leaf-to-air vapour pressure deficit (kPa).

Glasshouse experiments show that cotton is very responsive to VPD, where the model adapted by Duursma et al. (2013) describes an equation for stomatal conductance response to atmospheric [CO₂] and leaf-to-air vapour pressure deficits. The model used by Duursma et al. (2013) is a simplified form of the optimal \( g_s \) model, which illustrates the correlation between \( g_s \) and a combination of \( C_a \), VPD and \( A \):

\[ g_s = g_0 + g_1 \frac{A}{C_a f(VPD)} \]

where, \( g_0 \) is a residual conductance (mol m\(^{-2}\) s\(^{-1}\)), \( g_1 \) is the ‘slope parameter’ which is related to the marginal cost of water, \( A \) is the net CO₂ assimilation rate (µmol m\(^{-2}\) s\(^{-1}\)), \( C_a \) is the atmospheric CO₂ concentration (µmol mol\(^{-1}\)), and VPD is the leaf-to-air vapour pressure deficit (kPa). This model was a better fit than the Ball-Berry model for cotton grown in the glasshouse (Duursma et al., 2013). Duursma et al. (2013) also re-arranged the model to obtain an equation for instantaneous transpiration efficiency (ITE; equivalent to \( A_{sat}/E \) and WUE\(_L\)):

\[ ITE = \frac{C_a P_a}{1.6 (g_1 D_s^k + D_s)} \]
However, this model has not yet been validated using field-grown cotton. Testing this model using cotton grown in the field will improve understanding of leaf-level physiological responses to changes in environmental variables and ensure valid links between glasshouse and field-based studies.

2.7. Water availability and demand

2.7.1. Water use in Australian cotton systems

Cotton is often grown in environments where water stress commonly occurs (Krieg and Sung, 1991). Increases in water demand have placed stress on supply capacity for irrigation, cities, industry and environmental flows (IPCC, 2007). Globally, 80 - 90% of all freshwater used by humans is used in agriculture, mostly for crop production (Morison et al., 2008). Farmers in many countries are now faced with legislative restrictions on use of water, which are being imposed to try and secure safe and adequate water supplies for domestic users (Morison et al., 2008) and maintain healthy environmental systems such as the Murray Darling Basin (Ritchie et al., 2004). Water availability is of major concern throughout Australian agricultural regions and water is one of the most limiting factors in Australian cotton production. Recent droughts caused a reduction in the area sown to cotton, as water allocations for cotton irrigators were reduced (Tennakoon and Hulugalle, 2006).

Ninety-five percent of irrigated cotton grown in Eastern Australia is irrigated by surface/furrow irrigation (Tennakoon and Hulugalle, 2006), however, these proportions change depending on region, year and season (The Australian Cottongrower, 2014). For instance, during the 2013/14 Australian cotton season, 92% of the cotton produced in the Lower Namoi was in irrigated systems, compared with 69% in the Upper Namoi (The Australian Cottongrower, 2014). When the volume of available water is limited, it is crucial to maximise water use efficiency. Irrigation scheduling is the decision of when and how much water to apply to an irrigated crop to maximise crop productivity.
The generalised water use pattern of irrigated cotton in Australia is illustrated in Figure 2.5. The graph is a bell shaped curve with daily water use increasing to a peak of approximately 8 mm/day in early February during flowering and boll fill. Irrigation water required to maximise cotton yield and thus profit varies by location, and depends on stored soil water, in-season rainfall and evapotranspiration (Cammarano et al., 2012). In a survey across six Australian cotton production areas during the late 1990s, the quantity of irrigation water accessed from rivers and bores each season varied between 2.2 ML/ha and 13.1 ML/ha, with an overall seasonal average of 7 ML/ha (Tennakoon and Milroy, 2003). On average, cotton production in the Darling Downs region of Australia requires 4500 m³/ha/year (4.5 ML/ha/year) of applied irrigation water to meet cotton crop demand (Maraseni et al., 2010). Millyard (2014) reports that during the 2013/14 season, cotton grown in Southern NSW used between seven and 12 ML/ha, with higher yielding crops using 12 ML/ha. Therefore, water use within the cotton industry depends on region and seasonal factors. Plant response to water deficits is
dependent on timing, rate of development, intensity and duration of stress (Krieg and Sung, 1991). The impact of water deficits on photosynthesis and respiration, transpiration and water use, and growth, yield and quality will be discussed.

2.7.2. Photosynthesis and respiration

Plant water availability impacts photosynthesis and respiration rates, as a fraction of total water usage is used in the chemical equation. Water is required for photosynthesis, where light energy is used to oxidise H\textsubscript{2}O to produce O\textsubscript{2}, NADPH and ATP (Knox et al., 2005). Therefore, water stress affects the efficiency with which absorbed radiation is used to carry out carbon fixation at the leaf level. However, there is debate as to the mechanisms by which water deficits limits photosynthesis, through stomatal closure or by metabolic impairment (Flexas and Medrano, 2002; Medrano et al., 2002). In addition, there may also be interspecific differences in the response of stomatal conductance and photosynthesis to leaf water potential (Medrano et al., 2002). The period of time and extent of water deficit a plant is exposed to may also effect photosynthesis and respiration responses.

Reduced photosynthetic rates of cotton with water deficits were associated with decreases in leaf stomatal conductance (Turner et al., 1986). Plants respond to mild and moderate water stress by closing their stomata (Arriaga et al., 2009; Ko and Piccinni, 2009), restricting gas exchange and resulting in decreased Ci (Ennahli and Earl, 2005; Massacci et al., 2008). A decrease in Ci results in a decrease in [CO\textsubscript{2}]/[O\textsubscript{2}] of Rubisco and, therefore leads to a higher rate of photorespiration (Flexas and Medrano, 2002; Massacci et al., 2008).

The onset of water stress in cotton promotes photosynthetic electron transport due to a higher efficiency of the open PSII reaction centres, which prevents an over-reduction of the photosynthetic apparatus (Massacci et al., 2008). Therefore, additional energy is used to increase the rate of photorespiration while photosynthesis is kept constant or slightly decreases (Massacci et al., 2008), leading to higher rates of respiration of water-stressed plants. Similarly, under severe water stress, inhibition or down-regulation of metabolic processes at the level of the chloroplast can lead to decreased RuBP regeneration and inhibition of photosynthesis (Baker et al., 2007; Ennahli and Earl, 2005; Flexas and Medrano, 2002). Chloroplast-level effects are typically observed only under very severe stress where net photosynthetic assimilation is reduced by more than 80% (Ennahli and Earl, 2005). Re-watering of severely stressed cotton plants completely reversed the diffusive limitation (C\textsubscript{c} returned to control levels), but A\textsubscript{w}/C\textsubscript{c} (net photosynthetic carbon assimilation/CO\textsubscript{2} concentration in the chloroplast) did not recover completely and net carbon assimilation continued to be reduced relative to control plants because of a lasting chloroplast-level inhibition (Ennahli and Earl, 2005).
Therefore, severe water stress can permanently impact plant photosynthetic carbon assimilation at the individual leaf-level, and thus canopy-level recovery happens through the growth of new leaves.

2.7.3. Transpiration and water use

Stomatal conductance and consequently transpiration, are affected by a complex interaction of factors internal and external to the plant leaf, including soil water availability (Ko and Piccinni, 2009). Continued transpiration results in a depletion of soil moisture, which triggers root-leaf signalling within the plant. The chemical signal, abscisic acid (ABA) is synthesised in the roots, reaches the leaves through the transpiration stream and induces stomatal closure (Medrano et al., 2002). As plant available water drops below 60%, evapotranspiration is reduced as a result of slower hydraulic conductance of water to roots, reduced transpiration from stomatal closure, and parahelionastic leaf movements (Hearn and Constable, 1984).

Water use efficiency

Water-use efficiency (WUE) has been used interchangeably to refer to observations ranging from gas exchange by individual leaves for a few minutes, to yield response to irrigation treatments through an entire season (Sinclair et al., 1984). For this reason, WUE can be defined as a ratio of biomass accumulation, expressed as carbon dioxide assimilation, total crop biomass, or crop yield, to water consumed, expressed as transpiration, evapotranspiration, or total water input to the system. The ratio of biological or agronomic yield to seasonal evapotranspiration gives biological WUE or agronomic WUE (Hearn, 1994). A survey of over 100 commercial Australian cotton producers between the years 1996-1999 indicated crop WUE across all Australian cotton regions averaged 2.5 kg/ha/mm, however, there was considerable variability across regions (Tennakoon and Milroy, 2003). The time-scale for defining water-use efficiency can be instantaneous, daily or seasonal (Sinclair et al., 1984). It is the daily or instantaneous value that describes gas exchange of a leaf or canopy, and is the physiological basis of WUE (Hearn, 1994). In addition, WUE as affected by water availability needs to take into account the timing of the water stress relative to the growth stage and the severity of the water stress (Hsiao, 1993). Therefore, WUE at the leaf (WUE$_L$) and whole plant (WUE$_P$) levels are important measures for assessing physiological and growth responses of cotton to projected climate change, such as the impact of environmental variables such as elevated [CO$_2$], warmer temperatures and altered VPD.
2.7.4. Growth, yield and quality

Cotton production is negatively impacted by stress caused by moisture deficits (Pettigrew, 2004a), and limited soil water availability reduces crop growth and development more than all other environmental factors combined (Baker et al., 2007). Soil water deficit decreases water potential, photosynthesis and stomatal aperture (Arriga et al., 2009). Stresses involving water deficiencies will adversely affect cell turgidity, resulting in reduced crop production. Cell expansion, cell-wall synthesis and protein synthesis in fast growing tissues are sensitive to water deficits (Sadras and Milroy, 1996). Cotton responds to water deficits by a reduction in leaf expansion and stem elongation (Sadras and Milroy, 1996; Turner et al., 1986), thus reducing leaf area (and therefore reduced intercepted solar radiation) and plant height. In an experiment by Pettigrew (2004b), dryland cotton plants produced 35% less leaf area and 32% less overall vegetative growth than irrigated plants.

The agronomic effects of water stress in cotton include reduced biomass, loss of fruit and thus lint yield, and decreased lint quality. Typically, reproductive growth is more sensitive to plant water deficit than vegetative growth (Baker, 1965). Pettigrew (2004a) showed that irrigation altered the distribution of bolls both vertically and horizontally on the plants. Irrigated cotton set more bolls at higher plant nodes and further out on the sympodial branches than dryland cotton (Pettigrew, 2004a). A water-stressed plant tends to compensate for lack of moisture by shedding young fruit (Ramey, 1991), thereby reducing the number of bolls per plant. Infrequently irrigated watering regimes restricted vegetative growth and flowering so severely that the numbers of bolls set was reduced in spite of flower retention (Stockton et al., 1961). Large soil moisture deficits have been shown to reduce lint yield primarily by reducing the number of bolls, but also occasionally through a reduction in the amount of lint produced per seed (Pettigrew, 2004a).

Quality

Two important developmental stages for cotton fibre include cell elongation and thickening of the secondary wall (secondary wall deposition). Cell expansion occurs from the day of anthesis to approximately 21 to 26 days post anthesis (DPA) (Kim and Triplett, 2001). Cell expansion during growth is strongly driven by turgor (the pressure of fluid in the plant cell) (Dhindsa et al., 1975), so plant water relations in the period immediately following flowering may affect fibre elongation, with a deviation from optimum moisture causing shorter fibres (Ramey, 1991). Secondary wall deposition occurs after approximately two weeks of lengthening, where successive layers of cellulose are deposited until the wall is 3 to 4 µm thick (Kim and Triplett, 2001), but under water deficit cell walls may be less developed (Ramey, 1991). Therefore, water status and irrigation strongly influences fibre growth and ultimately final fibre length (Grimes et al., 1969), but fibre elongation is also affected by other factors such as
temperature and carbohydrate limitations. Saranga et al. (1998) also showed that drought conditions caused more motes (cotton ovules that fail to ripen into mature seeds) to be produced. A study by Pettigrew (2004a) found that cotton fibre length was generally shortened with soil moisture deficits and any irrigation effect on fibre length uniformity was too inconsistent to be definitively assessed. Therefore, water deficits during critical stages of fibre development are likely to have a negative effect on lint quality. Thus, managing water resources in future climates may be necessary to maintain standards of high quality from Australian production systems.

2.8. Combined effects of temperature, CO₂ and water availability

2.8.1. Combined temperature and CO₂ effect

Global climate models project that rising atmospheric [CO₂] may cause global average temperatures to increase and an increase in temperature extremes (IPCC, 2014). Interactions between elevated [CO₂] and temperature are complex.

Reddy et al. (1998a) showed that photosynthetic rates increased with elevated [CO₂] and warmer temperatures (Figure 2.6a). However, the response of stomata to increased [CO₂] and warmer temperatures is variable. An experiment conducted on cotton grown in controlled environment chambers showed that elevated [CO₂] reduced stomatal conductance at all three temperature (26/18 °C, 31/23 °C and 36/28 °C) treatments, but the magnitude of the reduction depended on growth temperature, with the greatest reduction of stomatal conductance with elevated [CO₂] occurring at 31/23 °C (Figure 2.6b) (Reddy et al., 1998a). In experiments conducted by Reddy et al. (1995d), transpiration rates of plants in high [CO₂] (700 µL L⁻¹) were lower than in ambient CO₂ (350 µL L⁻¹) in all temperatures grown (20/12 °C, 25/17 °C, 30/22 °C, 36/27 °C). However, transpiration increased with increasing temperature, despite the reduction in transpiration at elevated CO₂ (Reddy et al., 1995d). The higher photosynthetic rates and the slightly lower transpiration rates of plants grown in high CO₂ resulted in greater leaf-level water use efficiency. Hence, we cannot assume that the responsiveness of plant growth to elevated [CO₂] will become greater with global warming (Stokes and Howden, 2010).
Figure 2.6: Effects of CO$_2$ concentration (350- white circle; 450- black circle; and 700- square µL L$^{-1}$) and temperature (Day/Night: 26/18, 31/23, 36/28 °C) on (a) net photosynthetic rates and (b) stomatal conductance in cotton leaves. Measurements were made at regular intervals (30, 40, 50, 60 days after germination) on at least three different plants from each chamber. Values are average of five independent determinations (Reddy et al., 1998a).

Reddy et al. (1999) reported that cotton grown in high atmospheric [CO$_2$] produced more squares and bolls, because additional vegetative growth was associated with greater photosynthesis. This is based on higher photosynthetic rates and greater leaf area leading to the higher production of assimilates used in metabolic sinks, such as reproductive structures. Over a wide range of temperatures, increased [CO$_2$] increased the number of fruiting organs and the retention of bolls (Reddy et al., 1999); however,
Reddy et al. (1998b) found that although more fruiting sites were produced at 700 µL L⁻¹ [CO₂] for all temperatures, fruit retention at 32 °C and 36 °C were lower than at 27 °C thereby suggesting that it is unlikely that elevated [CO₂] will ameliorate the effect of high temperatures on flower abortion. Therefore, it is still unclear as to what the net outcome of warmer temperatures and elevated [CO₂] is for fruit retention and ultimately yield for cotton grown in projected future environments.

Although the effects of temperature x atmospheric [CO₂] interactions on cotton physiology, growth and development have been examined in the United States, there have been few studies examining the impact of these on Australian cotton varieties or in Australian production systems. This is important given Australian production differs in terms of soil type, climate and length of season (Constable et al., 2001; Hearn and Fitt, 1992). As the Australian production system is considered high-input (Braunack, 2013), responses to climatic changes may be greater in terms of fewer limitations of water and nutrient availability. In addition, considering that there are genotypic differences in relative heat tolerance of cotton (Cottee et al., 2012; Cottee et al., 2010), it is necessary to consider the implications of interactive temperature and [CO₂] effects for Australian cotton systems. Therefore, exploring the interactive effects of warmer temperatures and elevated [CO₂] on current cultivars within an Australian production system will improve our understanding of the implications of projected climate change for the Australian cotton industry.

2.9. Combined CO₂ and water effects

Changes in atmospheric [CO₂] may alter water use and water use efficiencies in plants, but conversely the effect of elevated [CO₂] on plant physiology and growth is likely to be influenced by plant water availability. In a SPAR experiment, Ephrath et al. (2011) showed that cotton grown in an elevated CO₂ treatment (700 µL L⁻¹) used less water than cotton grown in ambient CO₂ treatments (350 µL L⁻¹) in both well-watered and water-stressed conditions. This is attributed to lower stomatal conductance of cotton grown at elevated [CO₂] compared with plants grown at ambient [CO₂], resulting in lower transpiration rates (Ephrath et al., 2011; Reddy et al., 1998a). In contrast, glasshouse experiments have demonstrated that cotton grown at elevated [CO₂] (710 µL L⁻¹) had higher plant water use than ambient (352 µL L⁻¹), attributed to increased leaf area, and thus more rapid depletion of soil moisture as the canopy developed (Samarakoon and Gifford, 1995; Samarakoon and Gifford, 1996). However, FACE experiments in Arizona during 1990 and 1991 showed that there were no differences in evapotranspiration of cotton grown at elevated [CO₂] (550 µL L⁻¹) compared with ambient [CO₂], using three different methods (Dugas et al., 1994; Hunsaker et al., 1994; Kimball et al., 1994). These reported differences may also be due to pot and soil bin effects, variety and other environmental
effects and thus these concepts should be tested in both glasshouse and field environments using Australian cultivars and environmental conditions.

2.10. Controlled environment facilities

A wide range of experimental systems have been developed to artificially expose plants to elevated atmospheric [CO₂], including growth chambers, glasshouses, open-top chambers (OTC), field and laboratory-based mesocosms, and field-based free-air CO₂ enrichment (FACE) facilities (Barton et al., 2010). Advantages of using controlled environments include precise control of precipitation, humidity, and light. Air temperatures can be also controlled in these experimental systems. In addition OTC, horizontal flow through field chambers, whole tree chambers and naturally lit Soil-Plant-Atmosphere-Research (SPAR) facilities are capable of measuring whole canopy gas exchange at different [CO₂] (Baker et al., 2014b).

To compare and contrast the attributes of the various controlled environment facilities, a summary of chamber based systems (such as SPAR and OTC) and FACE facilities are as follows:

SPAR units have been built using naturally-lit plant growth chambers as a model. SPAR units are located outdoors, with each consisting of a steel soil bin and a plexiglass chamber to accommodate aerial plant parts, a heating and cooling system, and an environmental monitoring and control system (Reddy et al., 1996). Therefore, SPAR units can accurately control temperature and [CO₂] at predetermined set points for plant-growth studies in natural solar radiation regimes. Thus, within a range, control of VPD is possible. Canopy Evapotranspiration and Assimilation (CETA) chambers are open systems that have been used to measure canopy gas exchange of pot-grown and field-grown cotton plants in the US (Baker et al., 2014a; Baker et al., 2009). Although CETA chambers have not been extensively used to elevate [CO₂], they can be adapted to enrich [CO₂] to within ±12 μmol mol⁻¹ of the desired set-point (Baker et al., 2014b). In the field, plants can also be grown in OTCs. OTC walls are typically made of transparent plastic, which allow light penetration and create a wind barrier allowing easier control of atmospheric [CO₂] inside the chamber. Air temperatures observed inside OTCs are typically 0.5 to 2.5 °C warmer than outside. The degree of temperature rise of the foliage depends on transpiration rates, which strongly depend on environmental variables such as air vapour pressure. These are able to maintain natural edaphic conditions of the field setting, as plants are rooted in the ground and exposed to natural light and precipitation through the top of the chamber. However, all chambers alter air flow and intercept rainfall. In contrast, large scale FACE facilities have no walls, which allow plants to be grown in an elevated CO₂ environment under natural and fully open-air conditions. FACE technology uses a circular set array of vertical vent pipes to release either CO₂-
enriched air or pure CO$_2$ gas, and rely on natural wind and diffusion to disperse CO$_2$ across the experimental area (Ainsworth and Long, 2005; Hendrey and Kimball, 1994). The FACE technique is used internationally at more than 30 sites, for experiments investigating a range of ecosystems including cropping systems, pastures and forests (Primary Industries Climate Challenge Centre, 2014).

The primary advantages of facilities such as SPAR are the ability to measure and control environmental variables to minimise many of the confounding factors that occur in the field (Reddy et al., 2001). In addition, some chamber systems restrict infestations of pests and diseases. Advantages of CETA are that whole canopy net assimilation is more highly correlated with crop growth and final yield than leaf-level measurements (Baker et al., 2009). OTCs can also have ample ventilation and low vapour pressure, which can cool foliage temperatures inside these systems. Cotton and wheat experiments have shown that relative growth responses to elevated [CO$_2$] were not significantly different between OTCs and FACE, but the absolute growth of cotton was 30% greater inside OTCs (Kimball et al., 1997). Thus, for many studies the FACE approach is preferred because both absolute and relative responses to elevated [CO$_2$] can be obtained reliably.

However, there are also disadvantages associated with each system. Field bins and pots may restrict root growth, which can negatively influence photosynthetic capacity, shoot growth and harvestable yield potential, and thus reduce the response to CO$_2$ stimulation (Ainsworth et al., 2002; Ainsworth and McGrath, 2010; Arp, 1991; Thomas and Strain, 1991). Growth in pots can also alter nutrient availability, thereby changing the CO$_2$ response. The walls of chambers alter the air movement and consequently, they are notably warmer and more humid, light is attenuated, and wind speed is unrealistically low and constant (Hendrey and Kimball, 1994; Kimball and Mauney, 1993). Therefore, placing a chamber over a crop canopy changes a number of environmental variables that potentially alter leaf and canopy gas exchange via changes in the boundary layer resistance and mechanical stresses (Baker et al., 2014a; Kimball et al., 1997), and therefore are limitations of all chamber systems.

These different environments inside chambers compared with outside make it difficult to utilise data for validation of plant growth models. In addition, the size of chambers may also limit the capacity to allow researchers to follow crops to maturity (McLeod and Long, 1999). Typically agronomic trials use buffer rows, with a width approximately twice the height of the crop. Using chambers, most of the crop is within the buffer zone, which may cause edge effects and exaggerate the response to elevated [CO$_2$] (Ainsworth and McGrath, 2010). As a result, the effect of the chamber on plants may be greater than that of elevated [CO$_2$]. In addition, higher humidity and more shelter for pests and disease may accentuate epidemics inside chambers. However, in comparison, there are also a number of disadvantages associated with FACE facilities. As FACE systems rely on natural wind and diffusion to
disperse the CO$_2$ across the experimental area (Ainsworth and Long, 2005; Hendrey and Kimball, 1994), these systems encounter problems with CO$_2$-enrichment when wind speeds are low. In addition, FACE facilities are large and expensive to operate, suited to large and/or numerous simultaneous experiments with many researchers. Although some FACE experiments enrich [CO$_2$] continuously, many only enrich [CO$_2$] during the daytime due to inability to control [CO$_2$] at low wind speed in some systems and the cost of [CO$_2$]. However, a FACE study has shown that elevated [CO$_2$] at night increased plant growth and yield of common bean (*Phaseolus vulgaris*) (Bunce, 2014). There is also evidence to suggest that cyclically varying or surging [CO$_2$], which occur in FACE studies, may under-estimate the response of plants to long-term constant CO$_2$ exposure with the same mean [CO$_2$] (Bunce, 2012). Responses to pulses of CO$_2$ were related to both the extent of the change and the duration of CO$_2$-enrichment (Evans and Hendrey, 1992), but Holtum and Winder (2003) found lower mean rates of net photosynthesis when [CO$_2$] varied compared with constantly elevated [CO$_2$].

Despite the limitations of controlled environment experiments, data are valuable for validation of models being developed to predict the effects of increasing atmospheric [CO$_2$] and changing climate variables on plants, ecosystems, agricultural productivity and water resources (Hendrey and Kimball, 1994). Therefore, controlled environment facilities are useful systems to elevate atmospheric [CO$_2$] for studies effects of climate change on crops, including cotton.

2.11. Implications of climate change for Australian cotton

Australian agricultural systems are sensitive to climatic variability, including intra-annual variation such as timing of rainfall and heat shocks, year-to-year climate variability, and long-term climatic conditions. Projected elevated atmospheric [CO$_2$], warmer temperatures, and altered rainfall and VPD will require development of adaptation responses to both risks and opportunities associated with the projected changes. Australian agricultural regions are susceptible to extremely high temperatures and long periods of drought conditions. Since 1950, average annual minimum temperatures have increased by 0.9 °C and maximum temperatures have increased by 0.6 °C throughout cotton growing regions (International Trade Centre, 2011). The difference between day and night temperatures has also decreased, particularly in Queensland and parts of New South Wales. Projections indicate that most of Australia will warm by 0.4 °C to 2.0 °C by 2030, and by 1 °C to 6 °C by 2070. Warming is expected to be greater inland. The rate of warming will be higher in spring and summer. Climate change impacts will be complex and will vary greatly across different cropping and pasture regions (International Trade Centre, 2011), including across key Australian cotton production regions (Luo et al., 2014). Consequently, our agricultural systems will have to adapt to future climatic conditions.
Some impacts of projected climatic changes may be positive, such as the capacity of plants to use water more efficiently, as a result of higher atmospheric [CO₂]. However, this positive effect may be offset by the effects of increased temperatures and changes in water availability (Hatfield et al., 2011). With projected climate change, water availability may become more variable and limited in Australia’s cotton production regions (CSIRO and Bureau of Meteorology, 2012). Many cotton growing areas in Australia already experience extremely high temperatures during the growing season, particularly during flowering and boll development. Climate change may increase the frequency of these high temperatures. Excessively high temperatures (> 35 °C) during the day can reduce photosynthesis, while warm nights (> 25 °C) mean that leaf temperature and plant respiration remain high. In short-term experiments, maintenance respiration can double for every 10 °C rise in temperature (Bange, 2007; Stokes and Howden, 2010). These reduce the amounts of assimilates available for growth and ultimately yield.

Currently, Australia produces around 400 000 ha of irrigated cotton depending on water availability. About 80% of the cotton farms are irrigated. The area of dryland cotton varies considerably from year to year depending on commodity prices, soil moisture levels and rain. The area of dryland crop can vary from 5000 to 120 000 ha (Stokes and Howden, 2010). Globally, Australia produces about 2% of the world’s cotton. Over the past ten years, average cotton yields have been increasing, due to varieties and improvements in technology (such as GM) and crop management (International Trade Centre, 2011; Liu et al., 2013). Although Australia’s contribution to global cotton production is small, it is an important export commodity for Australia and thus it is necessary to understand the impact that projected climate change will have on Australian cotton production. Although some research has focused on the physiological and growth response of cotton to environmental variables such as warmer temperatures and elevated atmospheric [CO₂], there has been very little research on how these factors may affect Australian cotton varieties and cotton grown in high input and high yielding Australian production systems.

2.12. Conclusions

The major opportunities for research into cotton physiology and growth that emerge from this literature review are listed below. They provide a framework for evaluating the impact that projected climatic changes are likely to have on cotton production in Australia.

There is little information in the literature comparing how different genotypes within a species respond to CO₂ enrichment and changing temperature. Although a number of studies have investigated the effect of elevated [CO₂] and temperature of growth and physiology of a range of
cotton cultivars (Reddy et al., 1998a; Reddy et al., 1999; Reddy et al., 1998b; Reddy et al., 1995c; Reddy et al., 1995d), there has not been a comparison between the response of older and current cultivars used in Australian production systems. It is important to ensure that responses to temperature and elevated [CO$_2$] are representative of current cultivars, and to explore the responses of older cultivars. Findings from this research may aid physiological trait selection in the development of cultivars for future environments.

Although there have been some studies on the effect of elevated [CO$_2$], warmer temperatures and water deficits on cotton growth and physiology, there is little information on the response of cotton to the integrated projected climatic conditions. As cotton naturally experiences cycles of water deficits and recovery from drought conditions, it is important to understand potential interactions as it is likely that multiple variables will be altered with future climatic changes (IPCC, 2014). It is possible that elevated [CO$_2$] may reduce the negative effects of warmer temperatures (Dias de Oliveira et al., 2013), especially under conditions of soil water deficit. Improved understanding of the response of cotton to projected climatic changes may assist in the development of crop management strategies to maintain cotton yield and efficiencies in resource use, such as water.

Cotton responds to changes in vapour pressure deficit (VPD), yet there has been little research on the leaf-level physiological response to altered VPD in field-grown cotton. Given that projected climatic changes indicate that cotton may be exposed to altered VPD environments, there is an opportunity to undertake field studies to examine leaf physiological responses over a range of VPD environments.

High yielding and high input cotton in non-resource limited production systems, as in Australian production systems, may be affected by climatic changes. However, there are limited facilities to explore the physiological and growth response of field-grown cotton to elevated [CO$_2$] in Australian cotton production systems. Approaches need to be developed, tested and used to determine the impact projected climatic changes will have on cotton physiology, growth and water use in these non-resource limited production systems.
Chapter 3: The effect of elevated atmospheric [CO$_2$] and warmer temperatures on a past and a current cotton cultivar

3.1. Introduction

Australian average daily air temperatures have increased 0.9 °C from 1910 to 2011, and mean air temperatures are projected to rise by 0.6 to 1.5 °C by 2030 compared with the 1980 to 1999 climate (CSIRO and Bureau of Meteorology, 2012). Given these on-going and projected changes in climate, it is important to understand the impact of rising [CO$_2$] and air temperature on Australian cotton plant growth and physiology.

Increasing atmospheric CO$_2$ concentration ([CO$_2$]) and rising air temperatures may have significant impacts on physiology and growth of cotton. The global atmospheric [CO$_2$] has increased from a pre-industrial value of about 280 to 400 µL L$^{-1}$ in 2013 (IPCC, 2013). Elevated [CO$_2$] generally increases photosynthesis and biomass production in cotton. Canopy photosynthesis of cotton was increased 30-34% for plants grown at 500-900 µL L$^{-1}$ [CO$_2$], compared with plants grown at ambient [CO$_2$] (Idso et al., 1994; Reddy et al., 1995d), providing more carbon to support higher growth rates and dry matter accumulation. Biomass of well-watered cotton was increased up to 40% when grown at 550 µL L$^{-1}$ [CO$_2$] (Mauney et al., 1994; Reddy et al., 1997), and the number of squares was increased 31% when grown at 600 µL L$^{-1}$ (Yoon et al., 2009).

Temperature is an important factor in determining the rate of morphological development (Hearn and Constable, 1984). Optimal leaf temperature for photosynthesis in cotton is 28 °C and the sustained upper limit for cotton boll survival is 32 °C (Reddy et al., 1999). Plants growing at extremely high temperatures (> 35 °C) during the day assimilate less carbon than plants grown at 30 °C. Subsequently, this leads to 50% reductions in biomass (Reddy et al., 1991a; Reddy et al., 1991b) and lowered fruit retention (and therefore yield) to nearly 0 (Reddy et al., 1992a).

Previous studies have explored the interactive effects of [CO$_2$] and temperature on cotton growth and physiology (Reddy et al., 1995a; Reddy et al., 1995b), and other crop species such as soybean (Ruiz-Vera et al., 2013) and wheat (Dias de Oliveira et al., 2013). Cotton grown at elevated [CO$_2$] and warmer air temperatures grew taller and had a greater number of mainstem nodes (Reddy et al., 1995c). Similarly, Yoon et al. (2009) showed increased biomass and boll weight with elevated [CO$_2$] and
warmer temperatures (within the optimum range). Canopy photosynthesis of cotton was higher with elevated [CO$_2$] over a range of air temperatures (Reddy et al., 1995d). With increased photosynthetic rates, elevated [CO$_2$] and warmer temperatures generally increase plant biomass accumulation, but very high temperatures negate the positive effects of elevated [CO$_2$] on plant biomass. Boll production and retention is the most temperature sensitive aspect of cotton development and is reduced at very warm temperatures (Reddy et al., 1992a). Higher atmospheric [CO$_2$] has not been shown to ameliorate the adverse effects of high temperature on reproductive growth (boll size or abscission) or fibre quality of cotton (Reddy et al., 1997; Reddy et al., 2005). Similarly, elevated [CO$_2$] increased wheat biomass and grain yield at slightly warmer temperatures, but did not enhance biomass and yield at 4 °C or 6 °C above ambient temperature (Dias de Oliveira et al., 2013). Likewise, Ruiz-Vera et al. (2013) showed that the interactive effects of elevated [CO$_2$] and warmer temperature will likely not benefit soybean physiology, growth and development, due to variation of the impact of temperature over different growing seasons. Therefore, the benefits of elevated [CO$_2$] may not be as high as expected due to the interactive effect of high temperatures.

Australia’s modern irrigated cotton industry developed in the 1960s in northern NSW and southern Queensland (Constable et al., 2001; Hearn and Fitt, 1992). The expansion of the modern industry was initially based on varieties from the USA; however, domestic breeding efforts led to the development of varieties more suited to the Australian environment (Constable et al., 2001; Liu et al., 2013). Modern varieties exhibit improved yield, fibre properties, and disease and insect resistance compared with the USA varieties originally used (Constable et al., 2001; Liu et al., 2013). In addition, introduction of transgenic cotton varieties with insect and herbicide resistance have helped the Australian cotton industry and reduced pesticide use. Transgenic cotton, which contains genes from *Bacillus thuringiensis* (Bt) expressing the insecticidal proteins Cry1Ac and Cry2Ab, has reduced pesticide use for the control of major Lepidopteran pests (particularly *Heliothis* spp. in Australia) (Bange et al., 2008). Herbicide tolerant and Bt cotton constitute > 90% of Australia’s cotton crop (Smith, 2011). The CSIRO cotton breeding programme has improved lint yield 1.17% year$^{-1}$ (on average) with new cultivar releases over the past 30 years (Liu et al., 2013). In that same study, using linear regression, Liu et al. (2013) determined that gains in net yield were attributed 48% to cultivar, 28% to management and 24% to the cultivar x management interaction.

Alterations in agronomy, pests, diseases and climate may improve new cultivar response to modern conditions compared with older cultivars (Liu et al., 2013). Deltapine 16 (DP16) was a widely grown commercial variety bred by Delta & Pine Land Co (Scott, MS, USA) during the 1970s, but is not currently in production. DP16 plants are large, produce extensive branching with long internodes, possess an
indeterminate fruiting habit, and set fruit throughout the growing season. Sicot 71BRF is a current commercial variety, bred by CSIRO and released in 2008. Sicot 71BRF is a full season variety with compact growth habit suited to most Australian production areas, has high yield potential and good disease resistance (Stiller, 2008).

Yield is also dependent on crop phenotype, in which the interaction between genotype and environment is crucial (Miflin, 2000). A study examining yield improvement in the Australian industry showed that there have been changes in terms of the degree of genotypic and genotype x environment interactions that have contributed to increased yields in Australian cotton production systems (Liu et al., 2013). In that study, Liu et al. (2013) suggested that rising CO$_2$ might have contributed to yield increases over time. During the 1970s, mean atmospheric [CO$_2$] ranged from 326 to 337 µL L$^{-1}$, compared with the mean atmospheric [CO$_2$] for 2013 at 396 µL L$^{-1}$ (Tans and Keeling, 2014). Therefore, it is important to determine the relative contributions of genetics (cultivar) and environment (elevated CO$_2$ and temperature) to changes in yield when grown in past and future climate conditions.

There may be genotypic differences within a species that affect adaptation to elevated [CO$_2$] (Ziska et al., 2012). An evaluation of 17 cultivated rice lines at 373 and 664 µL L$^{-1}$ [CO$_2$], the largest intraspecific crop comparison made to date, indicated that there was considerable genetic variation among yield in responses to atmospheric CO$_2$ (Ziska et al., 1996). A study of four spring wheat cultivars, released in 1903, 1921, 1965 and 1996, showed that the response to recent increases in atmospheric [CO$_2$] was greater for older cultivars (Ziska et al., 2004), suggesting that traditional breeding did not select for [CO$_2$] responsiveness in newer cultivars (Ainsworth et al., 2008b). However, this greater yield sensitivity to [CO$_2$] in older cultivars was associated with whole-plant characteristics such as increased tillering and panicle formation (Ziska et al., 2004). Additional studies have confirmed that there is significant intraspecific variation in the yield response to elevated CO$_2$ among cowpea (Vigna unguiculata L.) (Ahmed et al., 1993), common bean (Phaseolus vulgaris L.) (Ziska et al., 1996), rice (Oryza sativa L.) (Baker, 2004; Moya et al., 1998), wheat (Triticum aestivum L.) (Manderscheid and Weigel, 1997; Ziska et al., 2004), and soybean (Glycine max L.) (Ziska et al., 2001), yet there has been little research comparing cotton genotypes.

A comparison of photosynthetic performance in four Pima cotton (Gossypium barbadense L.) cultivars found that, in general, modern Pima cotton cultivars were better adapted to high air temperature and achieved higher yields relative to older cultivars (Carmo-Silva et al., 2012). Although studies have investigated the effect of [CO$_2$] and temperature on growth and physiology of a range of cotton cultivars (Reddy et al., 1998a; Reddy et al., 1995a; Reddy et al., 1995c; Reddy et al., 1995d; Yoon et
older and current cultivars have not been specifically compared, especially those used in Australian production systems. Given that there have been significant changes in Australian cotton cultivars, management and yields, it is important to ensure that beneficial traits for CO₂ and temperature have not been omitted, which could contribute to maximising responses of cotton cultivars in future environments.

We tested the hypotheses that (1) cotton grown at 640 µL L⁻¹ [CO₂] will have increased plant biomass, increased photosynthesis rates, and decreased stomatal conductance compared with plants grown at 400 µL L⁻¹ [CO₂]; (2) plants grown at warmer temperatures (32 °C) will have higher rates of development and growth than plants at ambient temperature (28 °C); and (3) there will be cultivar x environment interactions, where the newer cultivar (Sicot 71BRF) will have higher photosynthetic rates, lower stomatal conductance and reduced biomass production compared with an older cultivar (DP16) when grown in conditions of warmer temperature (32 °C) and elevated [CO₂] (640 µL L⁻¹), representing future environments projected for current cotton regions.

3.2. Methods

3.2.1. Plant materials and growing conditions

Two cultivars of cotton, Deltapine 16 (DP16) and Sicot 71BRF [Bollgard II® Roundup Ready Flex®] were grown in a naturally-lit, [CO₂] and temperature controlled glasshouse at the Hawkesbury Institute for the Environment at the University of Western Sydney, Richmond, Australia. Seeds were sown on 9th November 2010 into 8 L pots (250 mm x 235 mm) containing natural Vertosol clay (Typic Haplustert) from Narrabri, NSW. Soil was collected from the top 20 cm. This soil has been characterised as very dark greyish brown (10YR3/2, 10YR3/2 dry) medium heavy clay, alkaline soils (pH ranging from 7.5 to 8.5) (Ward et al., 1999).

Plants were well-fertilised with Multigro® fertiliser (10.1% N, 3.5% P, 5.5% K, 16.3% S, 7.8% Ca) (Incitec Pivot Ltd, Melbourne) and Aquasol® (1.6 g/L) (23% N, 4% P, 18% K, 0.05% Zn, 0.06 % Cu, 0.0013% Mo, 0.15% Mn, 0.06% Fe, 0.011% B) (Hortico, Victoria) prior to sowing. Plants were watered daily using a hose, thus ensuring that plants were well-watered throughout the experimental period. Two glasshouse compartments were set to simulate average temperature (28/17 °C mid-day/night; Tₐ: “ambient temperature” treatment) and two compartments were set at a daily temperature cycle that was 4°C higher than the ambient temperature regime (32/21 °C mid-day/night; Tₑ: “high temperature” treatment). Air temperature was changed 5 times over 24 h to simulate natural field conditions (Appendix 1). Relative humidity was measured continuously in each glasshouse bay using Tinytag®
data loggers (TinyView, Gemini Data Loggers Ltd., Chichester, UK), and averaged 57 - 66% across the four rooms. As a result, air vapour pressure deficit during the day was higher in the elevated temperature treatment compared with the ambient temperature treatment. The glasshouse structure attenuated approximately 15% of direct sunlight (Duursma et al., 2013).

For each of the temperature treatments, there were two CO\textsubscript{2} treatments (target [CO\textsubscript{2}] (C\textsubscript{A}: 400 µL L\textsuperscript{-1} and C\textsubscript{E}: 640 µL L\textsuperscript{-1})). [CO\textsubscript{2}] treatments were achieved by the controlled input of CO\textsubscript{2} gas from pressurised cylinders. Detailed set-up for the glasshouse operation is described in Ghannoum et al. (2010a). Elevated [CO\textsubscript{2}] was achieved by injecting CO\textsubscript{2} gas (Food grade, AirLiquide, Australia) from pressurised cylinders through solenoid valves connected to a CO\textsubscript{2} monitor/controller (Lambda T, ADC BioScientific Ltd., Hoddesdon, Hertz, UK). CO\textsubscript{2} was initially passed through a Purafil® column to eliminate possible ethylene contamination. [CO\textsubscript{2}] was continually monitored by logging the voltage output of the CO\textsubscript{2} monitors/controllers using a data logger (DL2e, Delta-T Devices Ltd, Cambridge, UK) (Ghannoum et al., 2010a).

3.2.2. Leaf gas exchange measurements

The response of photosynthesis (A) to intercellular [CO\textsubscript{2}] (Ci) (A/Ci) was measured to calculate maximum Rubisco activity (V\textsubscript{cmax}), and maximum rate of electron transport used in the regeneration of RuBP (J\textsubscript{max}), as these parameters can be used to describe plant photosynthetic capacity at the leaf level. Similarly, J\textsubscript{max}/V\textsubscript{cmax} is used to determine carboxylation efficiency. A/Ci curves were obtained using an automatic programme with the portable open gas exchange system (LI-6400 XT, LI-COR, Lincoln, USA) on: 2 December 2010 (23 DAP), 5 December 2010 (26 DAP), 15 December 2010 (36 DAP) and 17 December 2010 (38 DAP). Measurements were taken on recently expanded leaves at saturating light (photosynthetic photon flux density of 1800 µmol m\textsuperscript{-2} s\textsuperscript{-1}) and with the cuvette temperature set to the mid-day growth temperature (28 or 32 °C). Leaf vapour pressure deficit (VPD\textsubscript{L}) in the leaf cuvette was maintained within the range 1.0 - 2.5 kPa using the Licor 6400 desiccant scrub function. Leaves were allowed to equilibrate prior to taking measurements. Net photosynthesis at saturating light (A\textsubscript{sat}), stomatal conductance (g\textsubscript{s-sat}), transpiration (E) and the ratio of intercellular to ambient [CO\textsubscript{2}] (Ci/Ca) were derived from the A/Ci data at saturating light (photosynthetic photon flux density of 1800 µmol m\textsuperscript{-2} s\textsuperscript{-1}), mid-day growth temperature (28 or 32 °C) and [CO\textsubscript{2}] (400 or 640 µL L\textsuperscript{-1}). A\textsubscript{sat}/g\textsubscript{s-sat} and A\textsubscript{sat}/E were calculated to describe leaf-level water use efficiency. Night-time dark respiration was measured on 13 December 2010 (34 DAP). Measurements were made 2 h after sunset at night-time temperatures (17 or 21 °C) and growth [CO\textsubscript{2}] (400 or 640 µL L\textsuperscript{-1}). VPD\textsubscript{L} in the leaf cuvette was maintained within the range 0.7 - 1.2 kPa using the Licor 6400 desiccant scrub function.
3.2.3. Plant growth measurements

A total of 48 plants were harvested on the 17th December 2010 (38 DAP) following the last measurement of gas exchange. Harvested plants were separated into vegetative (root, stem, leaf) organs. Leaf area (LA) of each plant was measured using a portable leaf area meter (LI-3100A, LI-COR, Lincoln, NE, USA). Harvested samples were oven-dried at 80 °C for a minimum of 48 h, and weighed to determine dry biomass production.

3.2.4. Statistical analyses

For the leaf gas exchange measurements, each leaf was considered a replicate. Similarly, for the plant growth measurements, each plant was considered a replicate. For both leaf gas exchange and plant growth measurements, six plants in each treatment were measured. Data were analysed by analysis of variance (ANOVA) using Genstat version 14, with growth temperature, growth [CO₂] and cultivar as independent factors. The assumptions of normality and homogeneity of variances were met for all variables and no transformations were necessary. Means of treatments were compared using least significant differences (lsd) at a 5% level of probability.

3.3. Results

3.3.1. Vegetative biomass production

Cₖ increased biomass of leaves (26%), stems (15%), roots (24%) and total biomass (22%) averaged over temperatures and varieties (Figure 3.1; Table 3.1). Cₖ also increased the number of nodes by 8% and the number of leaves by 10% (Figure 3.2a-d; Table 3.1). Biomass of leaves (91%), stems (98%), roots (73%) and total biomass (89%) were also increased by Tₑ. Tₑ increased the number of nodes by 37% and number of leaves by 68% (Figure 3.2a-d; Table 3.1). Growth differences between the two cultivars were evident. DP16 consistently had greater leaf (47%), stem (24%), root (36%) and total (36%) biomass compared with Sicot 71BRF (Figure 3.1; Table 3.1). DP16 also had higher number of nodes, number of leaves, total leaf area (LA) and were taller plants than 71BRF (Figure 3.2; Table 3.1).

Cₖ increased total LA only at Tₐ (Figure 3.2e, f; Table 3.1). In the CₖTₐ treatment, total LA was increased by an average of 48% across both varieties compared with CₐTₐ plants. Tₑ increased total LA of both cultivars of cotton in both CO₂ treatments. In addition, Cₖ increased plant height only at Tₐ (Figure 3.2g, h; Table 3.1). In the CₖTₐ treatment, plant height was increased by 15% compared with CₐTₐ plants. Warmer temperatures had a greater effect on plants grown at Cₖ, increasing plant height by 46%, compared with plants grown at Cₑ where a rise in temperature increased plant height by 26%.
Table 3.1: Three-way ANOVA table for [CO$_2$], temperature (Temp) and Cultivar effects on growth and physiological parameters of cotton. Leaf gas exchange measurements were made 23, 26, 36 and 38 DAP, with the exception of night respiration measurements which were made 34 DAP. Biomass production was measured at 38 DAP. F-values in bold represent significant effects at a P< 0.05 level of significance. Least significant difference (lsd) at P< 0.05 are shown for significant main effects and two-way interactions. Measurements were made on 6 plants in each treatment.

<table>
<thead>
<tr>
<th>Vegetative mass and plant characteristics</th>
<th>[CO$_2$]</th>
<th>Temp</th>
<th>Cultivar</th>
<th>lsd$^A$</th>
<th>[CO$_2$] x Temp</th>
<th>[CO$_2$] x Cultivar</th>
<th>Temp x Cultivar</th>
<th>lsd$^B$</th>
<th>[CO$_2$] x Temp x Cultivar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.307</td>
<td>0.304</td>
<td>0.531</td>
<td>0.173</td>
<td></td>
<td>0.423</td>
</tr>
<tr>
<td>Stem/petiole</td>
<td>0.047</td>
<td>0.001</td>
<td>0.004</td>
<td>0.236</td>
<td>0.197</td>
<td>0.191</td>
<td>0.385</td>
<td></td>
<td>0.910</td>
</tr>
<tr>
<td>Root</td>
<td>0.002</td>
<td>0.001</td>
<td>0.001</td>
<td>0.091</td>
<td>0.451</td>
<td>0.135</td>
<td>0.126</td>
<td></td>
<td>0.322</td>
</tr>
<tr>
<td>Total dry mass</td>
<td>0.002</td>
<td>0.001</td>
<td>0.001</td>
<td>0.608</td>
<td>0.414</td>
<td>0.281</td>
<td>0.259</td>
<td></td>
<td>0.535</td>
</tr>
<tr>
<td># Nodes</td>
<td>0.011</td>
<td>0.001</td>
<td>0.001</td>
<td>0.327</td>
<td>0.156</td>
<td>0.263</td>
<td>0.359</td>
<td></td>
<td>0.918</td>
</tr>
<tr>
<td># Leaves</td>
<td>0.049</td>
<td>0.001</td>
<td>0.001</td>
<td>0.722</td>
<td>0.446</td>
<td>0.280</td>
<td>0.096</td>
<td></td>
<td>0.871</td>
</tr>
<tr>
<td>Total leaf area</td>
<td>0.022</td>
<td>0.001</td>
<td>0.001</td>
<td>0.022</td>
<td>0.568</td>
<td>0.837</td>
<td>84.200</td>
<td>0.258</td>
<td></td>
</tr>
<tr>
<td>Plant height</td>
<td>0.018</td>
<td>0.001</td>
<td>0.001</td>
<td>0.010</td>
<td>0.108</td>
<td>0.641</td>
<td>2.031</td>
<td>0.825</td>
<td></td>
</tr>
</tbody>
</table>

Leaf gas exchange

| $A_{sat}$                                  | 0.001   | 0.084| 0.001    | 0.868  | 0.009          | 0.618               | 2.153          | 0.513  |                          |
| $g_{s-sat}$                                | 0.448   | 0.258| 0.001    | 0.831  | 0.611          | 0.009               | 0.091          |        | 0.432                    |
| $E$                                        | 0.810   | 0.001| 0.001    | 0.583  | 0.271          | 0.920               | 0.309          |        | 0.696                    |
| Night respiration                          | 0.200   | 0.001| 0.406    | 0.001  | 0.009          | 0.668               | 0.188          |        | 0.277                    |
| $A_{sat}/g_{s-sat}$                        | 0.001   | 0.688| 0.361    | 0.953  | 0.682          | 0.015               | 9.230          | 0.739  |                          |
| $A_{sat}/E$                                | 0.001   | 0.001| 0.817    | 0.004  | 0.137          | 0.579               | 0.459          |        | 0.748                    |
| Ci/Ca                                      | 0.021   | 0.201| 0.392    | 0.758  | 0.479          | 0.009               | 0.033          |        | 0.749                    |
| $V_{cmax}$                                 | 0.148   | 0.001| 0.365    | 14.690 | 0.130          | 0.427               | 0.833          |        | 0.886                    |
| $J_{max}$                                  | 0.003   | 0.479| 0.770    | 32.430 | 0.648          | 0.583               | 0.746          |        | 0.843                    |
| $J_{max}/V_{cmax}$                         | 0.001   | 0.001| 0.813    | 0.368  | 0.652          | 0.747               | 0.546          |        | 0.567                    |
Figure 3.1: Effect of growth temperature, atmospheric [CO$_2$] on leaf (a and b), stem (c and d), root (e and f) and total dry biomass production (g and h; g plant$^{-1}$) of cotton cultivars DP16 (white) and 71BRF (shaded) until 38 DAP. Values represent the mean of 6 plants. Refer to Table 3.1 for a summary of significant main treatment effects and interactions.
Figure 3.2: Effect of growth temperature, atmospheric [CO₂] on number of nodes (a and b), number of leaves (c and d), leaf area (e and f) and plant height (g and h) of cotton cultivars DP16 (white) and 71BRF (shaded) until 38 DAP. Values represent the mean of 6 plants. Refer to Table 3.1 for a summary of significant main treatment effects and interactions.
3.3.2. Physiological response of two different cotton cultivars to elevated [CO₂] and warmer temperatures under well-watered conditions

Aₛₐₜ was consistently higher for 71BRF cotton than DP16 cotton in each treatment (Figure 3.3a and b; Table 3.1). Aₛₐₜ of both cultivars responded positively to Cₑ with a significant CO₂ x cultivar interaction occurring because 71BRF responded more strongly to Cₑ (Figure 3.3a and b; Table 3.1). Aₛₐₜ of 71BRF was increased by 43%, and Aₛₐₜ of DP16 was increased by 28%. Tₑ did not alter Aₛₐₜ.

Figure 3.3: Effect of [CO₂] and temperature on photosynthetic rate (Aₛₐₜ; a and b) and stomatal conductance (gₛ-sat; c and d) of DP16 (white) and 71BRF (shaded). Values represent the mean of 6 plants. Refer to Table 3.1 for a summary of significant main treatment effects and interactions.

On average, Cₑ did not affect gₛ-sat, nor was there a significant CO₂ x cultivar interaction. However, gₛ-sat of the two cultivars responded differently to elevated temperature (Figure 3.3c and d; Table 3.1). Tₑ increased gₛ-sat of 71BRF by 23% (Figure 3.3a and b; Table 3.1). Stomatal conductance of DP16 did not respond to Tₑ.
\( T_E \) also increased night respiration across both CO\(_2\) treatments (Figure 3.4a and b; Table 3.1). \( T_E \) increased respiration of plants by 22\% when grown at \( C_a \), and by 84\% when grown at \( C_e \). \( C_e \) increased night respiration of plants grown at \( T_E \) by 27\%, but \( C_e \) did not increase night respiration of plants grown at \( T_a \). \( C_e \) increased night respiration of DP16, but not of 71BRF. At \( C_a \), night respiration of 71BRF was significantly higher than DP16.

Figure 3.4: Effect of [CO\(_2\)] and temperature on night respiration (a and b) of DP16 (white) and 71BRF (shaded). Values represent the mean of 6 plants, measured on 13\(^{th}\) Dec 2010 (34 DAP). Refer to Table 3.1 for a summary of significant main treatment effects and interactions.

\( C_e \) increased \( A_{sat}/g_{s-sat} \) by 41\% across all treatments (Figure 3.5a and b; Table 3.1). \( A_{sat}/g_{s-sat} \) of DP16 was increased with \( T_E \) by 20\%, whereas \( A_{sat}/g_{s-sat} \) of 71BRF was not significantly affected by temperature. \( C_e \) increased \( A_{sat}/E \) at both temperature treatments, with greater increases at \( T_A \) compared with \( T_E \) across both cultivars (Figure 3.5c and d; Table 3.1). With \( C_e \), \( A_{sat}/E \) was increased by 41\% at \( T_A \) and by 31\% at \( T_E \). Increased temperature decreased \( A_{sat}/E \). At \( C_A \), increased temperatures reduced \( A_{sat}/E \) by 22\%, whereas at \( C_e \), increased temperatures reduced \( A_{sat}/E \) by 32\%.

\( C_e \) increased \( Ci/Ca \) by 4\% averaged across all treatments (Figure 3.5e and f; Table 3.1). Elevated temperature decreased \( Ci/Ca \) of DP16 by 6\%; however, \( Ci/Ca \) of 71BRF was not affected by temperature.
Figure 3.5: Effect of [CO₂] and temperature on the ratio of photosynthesis to stomatal conductance ($A_{\text{sat}}/g_{\text{sat}}$, a and b), photosynthesis to transpiration rate ($A_{\text{sat}}/E$, c and d), and intercellular to ambient [CO₂] ratio (Ci/Ca, e and f) of DP16 (white) and 71BRF (shaded). Values represent the mean of 6 plants. Refer to Table 3.1 for a summary of significant main treatment effects and interactions.
$T_E$ decreased $V_{\text{cmax}}$ by 26%; however, there was no effect of $C_E$, or any other interactions on $V_{\text{cmax}}$ (Figure 3.6a and b; Table 3.1). $C_E$ increased $J_{\text{max}}$ by 38% (Figure 3.6c and d; Table 3.1) and $J_{\text{max}}/V_{\text{cmax}}$ by 44% (Figure 3.6e and f; Table 3.1). $J_{\text{max}}/V_{\text{cmax}}$ was increased by 41% with $T_E$. Therefore, carboxylation efficiency was increased with $C_E$ and $T_E$ due to the reductions in $V_{\text{cmax}}$ at $T_E$ and increases in $J_{\text{max}}$ at $C_E$.

![Figure 3.6: Effect of [CO$_2$] and temperature on $V_{\text{cmax}}$ (a and b), $J_{\text{max}}$ (c and d), and $J_{\text{max}}/V_{\text{cmax}}$ (e and f) of DP16 (white) and 71BRF (shaded). Values represent the mean of 6 plants. Refer to Table 3.1 for a summary of significant main treatment effects and interactions.](image-url)
3.4. Discussion

3.4.1. Summary of hypotheses findings

We tested the hypotheses that (1) cotton grown at 640 µL L⁻¹ [CO₂] will have increased plant biomass, increased photosynthesis rates, and decreased stomatal conductance compared with plants grown at 400 µL L⁻¹ [CO₂]; and (2) plants grown at warmer temperatures will have increased rates of growth and development. Our data showed that Cₑ increased biomass and photosynthetic rates compared with plants grown at Cₐ. Plants grown at Tₑ had increased biomass compared with plants grown at Tₐ. We also tested the hypothesis that (3) Sicot 71BRF will have higher photosynthetic rates, lower stomatal conductance and reduced biomass production than DP16, when grown in conditions of warmer temperatures (32°C) and elevated [CO₂]. Our data showed that Sicot 71 BRF had higher photosynthetic rates and produced less biomass than DP16, due to higher leaf area of DP16. Therefore, these data indicate that future, warmer environments may lead to larger cotton plants with potentially greater requirements for water. However, current cultivars such as Sicot 71 BRF may have an advantage over older varieties in future, warmer environments due to a more compact growth habit and higher photosynthetic rates. However, there is no evidence from this study to suggest that DP16 is more positively responsive to projected future climatic conditions than Sicot 71 BRF. Thus, the potential remains to capture further cultivar x [CO₂] and cultivar x temperature interactions to maximise cotton production in future environments.

3.4.2. Impacts of Cₑ and Tₑ on cotton physiology and growth

Our data showed that Cₑ increased photosynthesis of both cultivars, and increased LA at Tₐ, which resulted in greater metabolite availability for growth (Reddy et al., 1998a; Reddy et al., 1995a), so indicate that increased total photosynthate supply is reflected in total dry matter after accounting for changes in respiration. Our data showed that Cₑ affected electron transport in that Jₑmax and therefore Jₑmax/Vₑcmax was increased by CO₂ enrichment, and thus faster assimilation of CO₂ by the plant leading to the observed increased photosynthetic rates. Similarly, Jₑmax and Jₑmax/Vₑcmax were increased with 650 µL L⁻¹ [CO₂] in a Eucalyptus species (Ghannoum et al., 2010b), indicating that elevated [CO₂] increases Jₑmax and Jₑmax/Vₑcmax across a number of species. Cₑ also increased plant biomass and growth of both cultivars, due to faster fixation of CO₂ by the Rubisco enzyme. This is consistent with findings from other studies (Ainsworth and Long, 2005; Mauney et al., 1994; Reddy et al., 1997), although the magnitude of increased biomass is not as large as reported by Mauney et al. (1994) and Reddy et al. (1997). This may be a result of differences in cultivars, temperatures, CO₂ treatments or length of the experimental period. For instance, plants in our experiment were harvested after 38 days, whereas plants in the
experiments conducted by Mauney et al. (1994) were harvested after approximately 152 days. Mauney et al. (1994) attributed increases in biomass and yield to increased early LA; however, the differences between CA and CE treatments may become less apparent later in the season. Therefore, with projected higher CO₂ environments, cotton early vegetative cotton growth is likely to be greater than in current environments.

TE increased early vegetative plant biomass and growth of both cotton cultivars. This is consistent with other studies that temperature within the optimal range for production increases growth and developmental rates (Reddy et al., 1995a; Reddy et al., 1995d). Therefore, as suggested by previous studies (Luo et al., 2014; Reddy et al., 1995a) warmer environments in the future are likely to increase early vegetative cotton production, although this does not account for the increase in incidence of extreme high temperatures which may reduce early vegetative cotton production. However, as CE only increased total LA and plant height at TA, the growth response of cotton grown in future environments may be limited by the interactive effects of temperature and [CO₂], yet total dry matter is not affected by these interactions. This indicates increased internode length and leaf area, which does not necessarily translate to increased biomass accumulation. Therefore, it is possible that the benefits of CE, such as improved plant water use efficiency, may be negated by warmer temperatures as well. Although plant water use was not examined in this study, it is possible that larger plants resulting from these warmer, higher CO₂ environments will have increased water requirements thereby increasing demand for water resources by the cotton industry, given that warmer temperatures increased leaf-level stomatal conductance and transpiration across treatments. Therefore, it will be important for plant water use to be studied in future experiments on the response of cotton to climate change.

CE increased A_sat/g_sat by 41% and Ci/Ca by 4% across all treatments, and CE also increased A_sat/E. Our study demonstrates improvements in A_sat/g_sat and A_sat/E with CE through higher photosynthetic rates, in part because stomatal conductance was not significantly reduced by CE. Similarly, Duursma et al. (2013) reported an increase in instantaneous water use efficiency (ITE; equivalent to A_sat/E) of cotton at elevated [CO₂]. Warmer temperatures decreased A_sat/E efficiency due to greater water use through increased transpiration. Reddy et al. (1995d) showed that canopy water use efficiency was reduced with increasing temperature. Consequently, higher canopy temperatures may occur due to reduced transpiration with CE, which has been observed in FACE experiments on maize (Long et al., 2006). Our data showed that, compared with CA grown plants, A_sat/E was improved in CE_TA treatments, A_sat/E was reduced by CE_TA treatments and there was no significant difference in A_sat/E in CE_CE treatments. This demonstrates that the specific combination of increases in temperature and [CO₂] will determine the level of efficiency of cotton plants in future environments.
3.4.3. Differences in growth and physiology between the two cultivars

Our data showed growth differences between the two cultivars, where DP16 had consistently greater biomass compared with 71BRF. Higher biomass in DP16 can be attributed to greater leaf dry matter from greater leaf number and area, and thus greater light interception by the DP16 plants, despite higher leaf-level photosynthesis of 71BRF plants. Similarly, responsiveness of wheat cultivars to increasing [CO$_2$] was associated with morphological attributes, where greater yield sensitivity to $C_4$ in older cultivars could be attributed to increased tiller and ear number and panicle formation (Manderscheid and Weigel, 1997; Ziska et al., 2004). The compact growth habit and higher leaf-level photosynthetic rates of 71BRF are advantageous in current Australian production systems due to reduced surface area for transpiration.

$C_4$ increased $A_{sat}$ of both cultivars; however, photosynthetic rates were consistently higher for 71BRF. Greater photosynthetic capacity indicates that the modern variety had a photosynthetic advantage, and could potentially lead to higher yields. Studies in Pima cotton have demonstrated that breeding for improved yield has increased both single-leaf photosynthesis and stomatal conductance (Cornish et al., 1991; Percy et al., 1996); however, these did not compare cotton growing in a CO$_2$ enriched environment. In addition to having greater photosynthetic rates, the magnitude of the increase with $C_4$ was higher for 71BRF. This indicates that 71BRF is photosynthetically more responsive to changes in atmospheric [CO$_2$] than the older cultivar, DP16. Stomatal conductance of the different cultivars also responded differently to warmer temperature. $T_E$ increased $g_{s-sat}$ of 71BRF, but DP16 did not respond to $T_E$. There is no evidence that there are any differences in leaf temperature between the two cultivars tested; however, 71BRF had higher rates of transpiration than DP16. Therefore, increased stomatal conductance and transpiration of 71BRF contributed to greater leaf-level water use compared with DP16, although the reduced size and total leaf area of 71BRF may reduce water use at a plant level.

Studies in Pima cotton report genetic variability for stomatal conductance, where the selection for improved heat tolerance has been accompanied by increased stomatal conductance and decreased leaf temperature (Radin et al., 1994). Radin et al. (1994) also found that leaf temperatures of cotton varied among genotypes, yet genotypic differences between 71BRF and DP16 were not evident in our study. This may be due to similar heliotropic movements of the leaves in the two cultivars tested (Radin et al., 1994). However, our data indicated that between the two cultivars tested, there were no cultivar x temperature interactions for $A_{sat}$, LA or total biomass. With the projection for warmer temperatures in cotton regions, consideration of tolerance towards warmer temperatures will be important for maximising yields. Ahmed et al. (1993) reported that a heat-tolerant line of cowpea was
the most responsive to elevated [CO$_2$] with respect to pod production under intermediate or high night temperatures. Therefore, genotypic variations in cotton cultivars may provide opportunities for maximising yields in warmer, future environments, although our data did not indicate that we are currently capturing cultivar x temperature and [CO$_2$] interactions in early stage growth of cotton. However, Cottee et al. (2012; 2010) reported genotypic differences in heat tolerance between cotton cultivars. Our data also showed some differences between cultivars in some physiological parameters ($g_{s\text{-sat}}$, $A_{\text{sat}}/g_{s\text{-sat}}$ and Ci/Ca) for temperature x cultivar interactions, suggesting some elements of differences in heat tolerance between the two cultivars in this study. Therefore, there may be opportunities to take advantage of future changes in climate by capturing genotype x environment interactions.

3.4.4. Conclusions

This study demonstrated that $C_e$ increased biomass and photosynthetic rates compared with plants grown at $C_a$. Plants grown at $T_e$ had increased biomass compared with plants grown at $T_a$. This data also showed that the cultivar Sicot 71BRF had higher photosynthetic rates and produced less biomass than DP16, due to higher LA of DP16. Therefore, future environments may lead to larger cotton plants with potentially greater requirements for water, and thus plants with smaller, more compact growth habits and higher photosynthetic rates ($A_{\text{sat}}$) may have an advantage over older cultivars. Although this study compared the performance of one older variety, it does not exclude the possibility that advantages exist in other older varieties or wild cotton types. There is no current evidence to suggest that older varieties are more positively responsive to future climates than modern varieties. However, the potential remains to capture further cultivar x [CO$_2$] and cultivar x temperature interactions to maximise cotton production in future environments.

These experiments provide the platform for conducting genetic screening trials to elucidate the mechanisms that underlie genotypic differences in productivity under elevated [CO$_2$] and temperature (Ainsworth et al., 2008b). Climate change will challenge plant biologists, agronomists and breeders to provide germplasm that maximises future crop production in the projected climate (Ainsworth et al., 2008a; Ainsworth et al., 2008b). Given that this study has demonstrated that there are genotypic differences in physiological and growth responses to warmer temperatures and elevated [CO$_2$], projected climatic conditions should be considered in the selection of breeding lines for future environments; however, to implement this in breeding programmes is a challenge. In addition, genotypic differences should also be considered in the comparison of growth and physiological responses of cotton to projected climate change.
Chapter 4: Warming negates the positive impact of elevated $[\text{CO}_2]$ on cotton growth and physiology during soil water deficit

4.1. Introduction

Rising $\text{CO}_2$ concentration ($[\text{CO}_2]$), warming and altered precipitation may have significant impacts on the physiology and yield of cotton ($\textit{Gossypium hirsutum}$ L.). Current climate projections indicate that Australia will experience more frequent heatwaves, greater variability in rainfall, an increase in the intensity of droughts, and small decreases in relative humidity (Whetton and Power, 2007). Australian average daily temperatures have increased 0.9 °C from 1910 to 2011, and mean temperatures are projected to rise by 0.6 to 1.5 °C by 2030 compared with the 1980 to 1999 climate (CSIRO and Bureau of Meteorology, 2012). The majority of Australian cotton is irrigated and a significant proportion (15%) of the cultivated land area can be rain-fed (Bange et al., 2005), and highly dependent on the availability of water during the growing season. Maximising water use efficiency (WUE) at all scales is crucial in Australian farming systems, especially during drought. While deficit irrigation and dryland cotton production is a key feature of many production systems worldwide, it remains generally unclear how soil water deficit impacts cotton response to the interactive effects of elevated $[\text{CO}_2]$ and temperature. Given the on-going and projected changes in climate, it is important to understand how rising $[\text{CO}_2]$, temperature and drought will affect Australian cotton production through impacts on plant growth, physiology, water use and water use efficiency (WUE).

The global atmospheric $[\text{CO}_2]$ has increased from a pre-industrial value of about 280 to 400 $\mu$L L$^{-1}$ in 2013 (IPCC, 2013). Increasing atmospheric $[\text{CO}_2]$ is likely to impact plant growth and physiology. Biomass of well-watered cotton was increased up to 40% when grown at 550 $\mu$L L$^{-1}$ $[\text{CO}_2]$ compared with plants grown at 370 $\mu$L L$^{-1}$ $[\text{CO}_2]$ (Mauney et al., 1994; Reddy et al., 1997), and the number of squares was increased 31% when grown at 600 $\mu$L L$^{-1}$ compared with plants grown at 400 $\mu$L L$^{-1}$ $[\text{CO}_2]$ (Yoon et al., 2009). Elevated $[\text{CO}_2]$ generally increases photosynthesis, reduces transpiration and improves WUE of well-watered C$_3$ plants (Ainsworth and Rogers, 2007; Idso et al., 1994; Pallas, 1965; Radin et al., 1987), but this effect may be altered by rising temperature and reduced water availability (Duursma et al., 2013; Lewis et al., 2013). On average, elevated $[\text{CO}_2]$ stimulates light saturated photosynthesis ($A_{sat}$) in C$_3$ plants by 31% (Ainsworth and Rogers, 2007). In cotton, canopy photosynthesis increased 34% for plants grown at 900 $\mu$L L$^{-1}$ $[\text{CO}_2]$ compared with those grown at 350
µL L$^{-1}$ (Reddy et al., 1995d). Elevated [CO$_2$] increased average WUE of cotton by 41 - 52% depending on growth temperatures (Reddy et al., 1995d). Although WUE of cotton may be improved at higher [CO$_2$], it is also necessary to consider total volume of water required by the plants. In a SPAR experiment, Ephrath et al. (2011) found that the rate of water uptake (calculated using time domain reflectometry) for cotton grown at 700 µL L$^{-1}$[CO$_2$] was lower than for plants grown at 350 µL L$^{-1}$[CO$_2$]; however, warmer temperatures are likely to increase the quantity of water lost through evapotranspiration.

Temperature is an important factor in determining the rate of morphological development (Hearn and Constable, 1984). The thermal kinetic window of cotton is between 23.5 °C and 32 °C (Burke et al., 1988) and the optimum temperature of the Australian upland cotton cultivar Sicot 70 BRF is 28 ± 2 °C (Conaty et al., 2012). The sustained upper limit for cotton boll survival is 32 °C (Reddy et al., 1999). Rising temperatures are anticipated to accelerate crop development, increase transpiration, and potentially affect photosynthesis. Plants growing at extremely high temperatures (> 35 °C) during the day assimilate significantly less CO$_2$ than plants grown at 30 °C due to a decrease in the activation state of Rubisco (Salvucci and Crafts-Brandner, 2004); subsequently, these high temperatures reduced biomass by 50% (Reddy et al., 1991a; Reddy et al., 1991b) as well as lowered fruit retention (and therefore yield) to nearly zero (Reddy et al., 1992a). However, sensitivity to temperature is dependent upon the stage of development, with the reproductive stage more sensitive than the vegetative stage (Reddy et al., 1999; Reddy et al., 1992a).

Previous studies (including Chapter 3) have explored the interactive effects of [CO$_2$] and temperature on cotton growth and physiology. Growth of cotton plants in elevated [CO$_2$] is enhanced at most temperatures suitable for growth, but a greater response occurs at near optimum growth temperatures (Reddy et al., 1995a). Although there was a smaller temperature range, the experiment in Chapter 3 demonstrated that elevated [CO$_2$] increased plant biomass across both temperatures (28°C and 32°C). The positive response of plants to high CO$_2$ declined sharply at high temperatures (Reddy 1995b). Likewise, warmer temperatures generally increase plant biomass accumulation, but very high temperatures reduce plant biomass (Reddy et al., 1995a). Cotton grown at elevated [CO$_2$] and warmer temperatures grew taller and had a greater number of mainstem nodes (Reddy et al., 1995c). The number of fruiting organs and the retention of bolls increased with elevated [CO$_2$] and moderately warm temperatures, but declined at very high temperatures (Reddy et al., 1999; Reddy et al., 1995c; Reddy et al., 1995d). Despite these negative effects of warmer temperatures on plant growth characteristics, Lloyd and Farquhar (2008) stated that increased photosynthetic rates at elevated [CO$_2$] should compensate for declines in photosynthesis at higher leaf temperatures or leaf-
to-air vapour pressure deficit in tropical forest trees, thus ameliorating some of the negative effects of high temperature on plant physiology.

Canopy photosynthesis of cotton was higher and transpiration rates were lower in elevated [CO$_2$] compared with ambient [CO$_2$] over a range of temperatures (Reddy et al., 1998a; Reddy et al., 1995d), generating higher canopy water use efficiency (mmol CO$_2$ mol$^{-1}$ H$_2$O) (Reddy et al., 1995d). In general, elevated temperature increased transpiration, but the relative increase in transpiration at higher temperatures was reduced in elevated [CO$_2$] compared with ambient [CO$_2$] in well-watered conditions (Reddy et al., 1995d).

Cotton production is negatively affected by soil water deficit. Stomatal closure minimises water loss through transpiration, but also lowers intercellular CO$_2$ concentration (C$_i$), thereby limiting photosynthesis (Carmo-Silva et al., 2012). Baker et al. (2007) demonstrated that stomatal conductance was very sensitive to initial soil water deficit, whereas photosynthesis decreased under more severe deficits when stomatal conductance values were below 0.4 mol m$^{-2}$ s$^{-1}$. Down-regulation or inhibition of metabolic processes may occur at more severe drought conditions (Flexas and Medrano, 2002). Prolonged water stress also reduces growth and productivity through reduced biomass, loss of fruit and decreased lint yield and quality (Hearn, 1980). Water deficits reduced vegetative growth of cotton by 32%, and water-stressed plants were 16% shorter than irrigated cotton (Pettigrew, 2004b). Water deficits reduced leaf expansion and stem elongation with even greater impacts on reproductive growth (Baker, 1965).

Higher temperatures increase the quantity of water consumed in evapotranspiration (Salvucci and Crafts-Brandner, 2004), which is important in cooling leaves, but lowers leaf level WUE and could potentially also lower whole plant WUE (WUE$_p$: biomass production per unit of crop evapotranspiration). Warmer temperature may increase photosynthesis, but it also increases transpiration rate at a given VPD (Duursma et al., 2013). However, canopy transpiration rates may be lower for plants grown in high [CO$_2$] environments due to reduced stomatal conductance (Ephrath et al., 2011; Reddy et al., 1995d). Reddy et al. (1995d) demonstrated an increase in WUE$_p$ at elevated [CO$_2$], mainly due to increased canopy photosynthesis, and to a lesser degree to reduced canopy transpiration. However, whole plant water use of cotton was 45 - 50% higher at elevated [CO$_2$], despite higher WUE$_p$ (Samarakoon and Gifford, 1996). Therefore, it is important to assess water use of cotton when both temperature and [CO$_2$] are increased and thus understand the interactive effects of elevated temperature, [CO$_2$] and water stress on cotton production.
The objective of this research was to investigate the interactive effects of elevated [CO₂], warming and soil water deficit on biomass production, leaf level physiological responses and whole plant water use and efficiency in cotton. In this study, we tested the hypotheses that (1) cotton grown at 640 µL L⁻¹ [CO₂] will have increased plant biomass, increased leaf photosynthesis rates, and reduced stomatal conductance compared with plants grown at 400 µL L⁻¹ [CO₂]; (2) plants grown at warmer temperatures will have increased rates of development and growth, and higher leaf-level and whole-plant water use than plants at ambient temperature; and (3) that elevated [CO₂] and warmer temperatures will have interactive effects on carbon gain and water use at both a leaf- and whole-plant level, so that higher growth and WUEP in elevated [CO₂] would reduce water requirements of plants grown at warmer temperatures, especially under conditions of soil water deficit. Gaining an understanding of how quickly plants become water stressed and the quantity of water required under different environmental conditions will lead to a better understanding of water use and cotton growth in projected future environments.

4.2. Materials and Methods

4.2.1. Plant material and growing conditions

Cotton (Gossypium hirsutum L. cv, 71BRF [Bollgard II’ Roundup Ready Flex’], CSIRO Australia) (Stiller, 2008) was grown in a naturally-lit, [CO₂] and temperature-controlled glasshouse at the University of Western Sydney, Richmond, Australia. Seeds were sown into 9 L pots containing a mixture of 90% Vertosol clay (Narrabri) and 10% sandy loam soil (field site at the University of Western Sydney, Richmond; see Barton et al. (2010)). Upon emergence, plants were thinned to one plant per pot. Plants were well-fertilised with Multigro® fertiliser (10.1% N, 3.5% P, 5.5% K, 16.3% S, 7.8% Ca) (Incitec Pivot Ltd, Melbourne) and Aquasol® (1.6 g/L) (23.0% N, 4% P, 18.0% K, 0.05% Zn, 0.06% Cu, 0.0013% Mo, 0.15% Mn, 0.06% Fe, 0.011% B) (Hortico, Vic) prior to sowing, and Gran-Am® fertiliser (20.2% N, 24% S) 44 days after planting (DAP). Two glasshouse compartments were set to simulate average temperature (Tₐ: 28/17 °C mid-day/night; “ambient temperature” treatment) and two compartments were set at a daily temperature cycle that was 4 °C higher than the ambient temperature regime (Tₑ: 32/21 °C mid-day/night; “high temperature” treatment). Air temperature was continually adjusted by the temperature-control system and monitored using thermocouples (Ghannoum et al., 2010a). Air temperature was changed 5 times over 24 h to simulate natural field conditions (Appendix 1). For each of the temperature treatments, there were two CO₂ treatments (Target [CO₂] (Tₐ: 400 µL L⁻¹ and Tₑ: 640 µL L⁻¹)). [CO₂] treatments were achieved by the controlled input of CO₂ gas from pressurised cylinders. CO₂ gas (Food grade, AirLiquide, Australia) was injected into the glasshouse bays from
pressurised cylinders through solenoid valves connected to a CO$_2$ monitor/controller (Lambda T, ADC BioScientific Ltd., Hoddesdon, Hertford, UK). CO$_2$ was initially passed through a Purafil® column to eliminate possible ethylene contamination. [CO$_2$] was continuously monitored by logging the voltage output of the CO$_2$ monitors/controllers using a data logger (DL2e, Delta-T Devices Ltd, Cambridge, UK) (Ghannoum et al. 2010).

Relative humidity (mean ± SE) for each treatment were as follows: C$_{E}T_{E}$ (52.8% ± 0.33), C$_{A}T_{E}$ (54.7% ± 0.29), C$_{E}T_{A}$ (67.7% ± 0.33) and C$_{A}T_{A}$ (65.8% ± 0.30). Vapour pressure deficit (VPD) (mean ± SE) was ca. 60% higher in elevated temperature treatments: C$_{E}T_{E}$ (1.6 kPa ± 0.02), C$_{A}T_{E}$ (1.5 kPa ± 0.02), C$_{E}T_{A}$ (0.9 kPa ± 0.01) and C$_{A}T_{A}$ (1.0 kPa ± 0.02). Plants were moved each day within glasshouse bays during the experimental period to prevent differences due to plant position within the glasshouse.

### 4.2.2. Drought treatments

Forty-five days after planting (DAP), 10 plants were randomly selected within each of the four treatments and divided into two groups of five replicate plants. Five control plants were watered daily to field capacity, while the other set of five plants were subjected to a progressive drought. Pots were weighed every morning and water lost from the control plants was replaced. In the drought treatment, water was withheld until visible wilting of plants and net photosynthesis at saturating light ($A_{sat}$) was approximately 40 - 50% of the control plants and soil water content was reduced by approximately 30% (Figure 4.1), at which point plants were considered to be water-stressed. Volumetric soil water use patterns were monitored using time domain reflectometers (TDR, CS616, Campbell Scientific, USA) measured to the bottom of the pots. Plants were re-watered to field capacity after the first drought phase, and remained well watered for a recovery period of 5 days before repeating the process for a second drought cycle. The drought cycle refers to the beginning of the water deficit phase until the end of the 5-day recovery period. Water use was calculated as water lost gravimetrically during the drought phase and water use during the recovery phase was interpolated based on closed water use measurements for each pot and plant growth, as pots were not weighed during the recovery phase. Pot saucers were used to prevent loss of water through drainage. A representative pot without a cotton plant was used to adjust for soil evaporation. WUE$_p$ was calculated as total biomass/total water use (both at 70 DAP).
Figure 4.1: Soil water content (SWC) expressed as a percentage field capacity of the soil for different temperature and \([\text{CO}_2]\) treatments, during two drought cycles and two recovery phases. Circles used for 400 µL L\(^{-1}\) [CO\(_2\)] (CA: a and b), triangles used for 640 µL L\(^{-1}\) [CO\(_2\)] (CE: c and d). Ambient temperature (a and c) is shown in blue, elevated temperature (b and d) is shown in red. Values represent means ± SE of 5 plants (in the water-stressed (open symbols) treatment, in the well-watered (solid symbols) treatment TDR measurement was of one pot). All plants were well-watered during recovery phase (shaded).

4.2.3. Leaf gas exchange measurements

Net photosynthesis at saturating light (\(A_{\text{sat}}\)) and saturating light and elevated CO\(_2\) (\(A_{\text{max}}\)), and stomatal conductance at saturating light (\(g_{s\text{-sat}}\)) and saturating light and elevated CO\(_2\) (\(g_{s\text{-max}}\)) were measured on recently fully expanded leaves using a portable open gas exchange system (LI-6400XT, LI-COR, Lincoln, USA). Gas exchange measurements were taken each day from 45 DAP (31\(^{st}\) March 2011) until the end of the second recovery phase, with exception of the following days: 47 DAP, 48 DAP, 50 DAP and 61 DAP. Both \(A_{\text{sat}}\) and \(A_{\text{max}}\) measurements were conducted at saturating light (photosynthetic photon flux density of 1800 µmol m\(^{-2}\) s\(^{-1}\)), mid-day growth temperature (28 or 32 °C), and VPD was maintained within the range 1.5 - 2.0 kPa for consistency among measurements. \(A_{\text{sat}}\) and \(g_{s\text{-sat}}\) measurements were made between 10 am and 3 pm (Australian Eastern Daylight Time; AEDT) at 400 or 640 µL L\(^{-1}\) [CO\(_2\)], while \(A_{\text{max}}\) and \(g_{s\text{-max}}\) was measured at 1500 µL L\(^{-1}\) [CO\(_2\)]. Each leaf was allowed at least 10 - 15 min to
equilibrate before 5 replicate measurements were recorded. $A_{\text{sat}}$ and $A_{\text{max}}$ were measured in succession on the same leaf.

4.2.4. Plant growth measurements

Plant growth characteristics (height, number of nodes, number of squares/bolls) were assessed twice a week. At the end of the experiment, plants were harvested, separated into vegetative (root, stem, leaf) and reproductive (squares, flowers, bolls) organs. Leaf area was measured using a portable leaf area meter (LI-3100A, LI-COR, Lincoln, NE, USA). Harvested samples were oven-dried at 80 °C for a minimum of 48 h, and weighed. Based on plant growth characteristics (height, node and final biomass), biomass data were interpolated for 70 DAP and compared at this time due to different end dates of the second recovery phase between treatments. Physiological data were compared at the end of the second drought phase on the following dates: $C_{\text{ET}}$ (64 DAP), $C_{\text{ET}}T_A$ (71 DAP), $C_A$ $T_E$ (64 DAP), and $C_A$ $T_A$ (74 DAP).

4.2.5. Statistical analyses

For the leaf gas exchange measurements, each leaf was considered a replicate. Similarly, for the plant growth measurements, each plant was considered a replicate. For both leaf gas exchange and plant growth measurements, five plants in each treatment were measured. Data were analysed by analysis of variance (ANOVA) using Genstat version 13. The assumptions of normality and homogeneity of variances were met for all variables and no transformations were necessary. Means of treatments were compared using least significant difference (lsd) at a 5% level of probability. To compare soil water content and $A_{\text{sat}}$ of water deficit plants, a relative soil water content and relative $A_{\text{sat}}$ was calculated for each day of measurement and plotted (Appendix 2). Relative differences between treatments were calculated by dividing values from water-deficit treatment by well-watered treatment. Relative soil water content was used to minimise variation in the TDR sensors and relative $A_{\text{sat}}$ was calculated to minimise day-to-day variation throughout the drought phase. Photosynthetic responses to each cycle of soil water deficit were analysed using simple linear regression analyses to test for acclimation to water deficits. To test for acclimation to drought conditions in each environment, corresponding $A_{\text{sat}}$ and soil water content during the drought phase for plants in each treatment were compared. To assess the ability of plants to withstand water deficits in each environment, the number of days until drought stress (where $A_{\text{sat}}$ and $g_{s\text{-sat}} \leq 40\%$ of the control plants) for all treatments during each drought phase were analysed using ANOVA (Appendix 3).
4.3. Results

4.3.1. Soil water deficit

There were differences in the rate of decline in soil water content, between the first drought cycle and the second drought cycle, with noticeable differences between T_A and T_E treatments (Figure 4.1). During the first drought phase, plants grown at T_E became water-stressed in 6 - 7 days, compared with 9 days for plants grown at T_A (Appendix 4). Similarly, for the second drought cycle, plants grown at T_E became water-stressed after 7 days, compared with 13 - 15 days for plants grown at T_A. C_E did not affect how quickly plants became water stressed during the first drought phase. During the second drought phase, plants grown at C_E became water-stressed more quickly compared with plants grown at C_A T_A; however at T_E, CO2 treatment did not affect how quickly plants became water stressed.

4.3.2. Vegetative and reproductive biomass production

There were vegetative and reproductive growth benefits from both CO2 and temperature; however, these were greatly reduced under conditions of water stress (Table 4.1; Appendix 5). Interactive effects of [CO2], temperature and water availability impacted vegetative and reproductive biomass production of cotton (Figure 4.2; Table 4.1). Soil water deficits always reduced biomass production compared with well-watered plants. Across both water treatments, C_E increased stem and petiole biomass by 52% at T_A and by 14% at T_E. Across both temperatures, C_E also increased root biomass of well-watered plants. Across water treatments, T_E increased total fruit biomass. Across CO2 treatments, T_E increased root biomass by 42%, only in well-watered plants.

For total vegetative biomass, there was a three-way interaction (P= 0.05; Table 4.2). Relative to C_A T_A plants, vegetative biomass was increased by 55% in C_E T_A plants, 64% in C_A T_E plants, 70% in C_E T_E plants in well-watered conditions. Water deficits always reduced total vegetative biomass (Figure 4.2c and d). C_E increased total vegetative biomass at T_A by 26% in water-stressed plants and by 54% in well-watered plants. At T_E, C_E did not increase total vegetative biomass in either well-watered or water-stressed plants.

Leaf area (Figure 4.3a and b) was reduced by 33% across all treatments with water deficits. Averaged across all treatments, T_E increased leaf area by 17%. C_E increased leaf area by approximately 30% across both water treatments, only at T_A. At T_E, C_E did not increase leaf area in either well-watered or water-stressed plants.
Figure 4.2: Effect of growth temperature, atmospheric [CO₂] and water availability on leaf (a and b), stem & petiole (c and d), root (e and f), total vegetative (g and h) and total fruit (i and j) biomass production (g plant⁻¹) of cotton until 70 DAP. Ambient temperature (Tₐ) is shown in blue, elevated temperature (Tₑ) is shown in red, well watered (wet; shaded) and water-stressed (dry; white). Values represent the mean of 5 plants. Refer to Table 4.2 for a summary of significant main treatment effects and interactions.
Figure 4.3: Effect of growth temperature, atmospheric [CO₂] and water availability on leaf area (cm² plant⁻¹; a and b) of cotton until 70 DAP. Ambient temperature is shown in blue, elevated temperature is shown in red. Values represent the mean of 5 plants. Refer to Table 4.2 for a summary of significant main treatment effects and interactions.
Table 4.1: Three-way ANOVA table for [CO₂], temperature (Temp) and water effects on various parameters of cotton for vegetative biomass until 70 DAP, leaf gas exchange measured at the end of the second drought phase, and plant water use and water use efficiency until 70 DAP. ↑ shows main effect increase; ↓ shows main effect decrease; *, **, *** shows significant interactions at $P \leq 0.05$, 0.01 and 0.001, respectively; and - shows no significant difference at $P > 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>[CO₂]</th>
<th>Temp</th>
<th>Water</th>
<th>[CO₂] x Temp</th>
<th>[CO₂] x Water</th>
<th>Temp x Water</th>
<th>[CO₂] x Temp x Water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vegetative mass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total vegetative mass</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>*</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td><strong>Leaf gas exchange</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{sat}$</td>
<td>↑</td>
<td>-</td>
<td>↓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$g_{s\text{ sat}}$</td>
<td>↓</td>
<td>-</td>
<td>↓</td>
<td>**</td>
<td>***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water use</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>***</td>
</tr>
<tr>
<td>Water Use Efficiency</td>
<td>↑</td>
<td>↓</td>
<td>-</td>
<td>**</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(WUE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2: Three-way ANOVA table for [CO$_2$], temperature (Temp) and water effects on various parameters of cotton for vegetative and reproductive biomass until 70 DAP, leaf gas exchange measured at the end of the second drought phase, and plant water use and water use efficiency until 70 DAP. Values in bold represent significance at $P<0.05$. Least significant difference (lsd) at $P<0.05$ are shown for significant $^a$ main effects, $^b$ two-way interactions, and $^c$ three-way interactions.

<table>
<thead>
<tr>
<th>Vegetative mass and leaf area</th>
<th>[CO$_2$]</th>
<th>Temp</th>
<th>Water</th>
<th>lsd$^a$</th>
<th>[CO$_2$] x Temp</th>
<th>[CO$_2$] x Water</th>
<th>Temp x Water</th>
<th>lsd$^b$</th>
<th>[CO$_2$] x Temp x Water</th>
<th>lsd$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>0.003</td>
<td>0.001</td>
<td>0.001</td>
<td>0.044</td>
<td>0.913</td>
<td>0.224</td>
<td></td>
<td></td>
<td>0.029</td>
<td>1.542</td>
</tr>
<tr>
<td>Stem/petiole</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.021</td>
<td>0.427</td>
<td>0.664</td>
<td>1.582</td>
<td>0.094</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>0.005</td>
<td>0.001</td>
<td>0.001</td>
<td>0.328</td>
<td>0.002</td>
<td>0.001</td>
<td>0.939</td>
<td>0.564</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total vegetative mass</td>
<td>0.018</td>
<td>0.001</td>
<td>0.001</td>
<td>0.018</td>
<td>0.122</td>
<td>0.069</td>
<td></td>
<td>0.050</td>
<td>3.997</td>
<td></td>
</tr>
<tr>
<td>Leaf area (cm$^2$)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.038</td>
<td>0.175</td>
<td>0.146</td>
<td>189.800</td>
<td>0.069</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Reproductive mass            |          |      |       |        |                 |                  |             |        |                        |        |
| Total fruit                  | 0.643    | 0.001| 0.043 | 0.022  | 0.889           | 0.181            | 0.790       | 0.532  |

| Leaf gas exchange            |          |      |       |        |                 |                  |             |        |                        |        |
| $A_{sat}$                    | 0.019    | 0.086| 0.001 | 2.893  | 0.959           | 0.732            | 0.298       | 0.064  |
| $A_{max}$                    | 0.632    | 0.266| 0.001 | 0.005  | 0.222           | 0.001            | 0.091       | 0.115  |
| $g_{s-sat}$                  | 0.001    | 0.328| 0.001 | 0.006  | 0.001           | 0.294            | 0.091       | 0.115  |
| $g_{s-max}$                  | 0.073    | 0.731| 0.001 | 0.021  | 0.056           | 0.620            | 0.109       | 0.054  |
| $A_{sat}/g_{s-sat}$          | 0.001    | 0.003| 0.001 | 0.001  | 0.091           | 0.089            | 28.920      | 0.320  |
| $A_{sat}/E$                  | 0.001    | 0.127| 0.001 | 1.155  | 0.607           | 0.061            | 0.191       | 0.529  |
| Ci/Ca                        | 0.150    | 0.136| 0.001 | 0.061  | 0.091           | 0.870            | 0.155       | 0.870  |

| Water                        |          |      |       |        |                 |                  |             |        |                        |        |
| Water use                    | 0.047    | 0.001| 0.001 | 0.293  | 0.133           | 0.001            | 0.587       | 0.380  |
| Water Use Efficiency (WUE)   | 0.002    | 0.001| 0.937 | 0.029  | 0.811           | 0.640            | 0.558       | 0.522  |
4.3.3. Photosynthesis and stomatal conductance

The interactive effects of [CO₂], temperature and water availability affected leaf level photosynthesis and stomatal conductance of cotton. Soil water deficits generally reduced physiological functioning. Cₑ increased Aₘₐₓ by 19% (Figure 4.4a, b; Table 4.2). Each drying cycle caused a decline in photosynthetic rate (Aₛₐₜ, Figure 4.6) of cotton, while well-watered cotton maintained relatively constant Aₛₐₜ. Cotton grown at Tₑ became water stressed more quickly when water was withheld than plants grown at Tₐ, shown by the faster decline in Aₛₐₜ compared with the fully watered treatment. Aₘₐₓ was increased by 95% with Cₑ in water-stressed plants grown at Tₐ, but otherwise Cₑ did not affect Aₘₐₓ (Figure 4.4c, d). The impact Tₑ had on Aₘₐₓ of cotton was variable, where Aₘₐₓ was increased by 14% in well-watered plants grown at Cₐ, but reduced by 53% in water-stressed plants grown at Cₑ.

Cₑ also decreased gₛₛₛₑ but only at Tₑ. For plants grown at CₑTₑ, by the end of the second drought treatment, gₛₛₑ (Figure 4.4 e, f; Table 4.1; Table 4.2) was reduced by 51% and gₛₛₑₑ (Figure 4.4g, h; Table 4.1; Table 4.2) was reduced by 45%, compared to CₐTₑ. Cₑ reduced gₛₛₑ of well-watered plants by 39%; however, there was no significant effect of Cₑ on water-stressed plants. Water deficits reduced gₛₛₑ by 89% in plants grown at Cₐ and by 83% in plants grown at Cₑ. Tₑ increased gₛₛₑ of plants grown at Cₐ, but not of plants grown at Cₑ.
Figure 4.4: Effect of growth temperature, atmospheric [CO₂] and water availability on photosynthetic rates at 1800 μmol m⁻² s⁻¹ light and growth [CO₂] (A_{sat}, a and b), photosynthetic rate at 1800 μmol m⁻² s⁻¹ light and saturating [CO₂] of 1500 μL L⁻¹ [CO₂] (A_{max}, c and d), stomatal conductance rates at 1800 μmol m⁻² s⁻¹ light (g_{s-sat}, e and f), and stomatal conductance rate at 1800 μmol m⁻² s⁻¹ light and 1500 μL L⁻¹ [CO₂] (g_{s-max}, g and h) of cotton at the end of the second drought phase. Ambient temperature (T_A) is shown in blue, elevated temperature (T_E) is shown in red, well watered (wet; shaded) and water-stressed (dry; white). Values represent the mean of 5 leaves. Refer to Table 4.2 for a summary of significant main treatment effects and interactions.
$A_{sat}/g_{sat}$ was increased by 139% with water deficits across all [CO$_2$] and temperature treatments (Figure 4.5a, b) due to a combination of increased $A_{sat}$ and decreased $g_{sat}$. $T_E$ increased $A_{sat}/g_{sat}$ by 98% in well-watered plants and 164% in water-stressed plants grown at $C_E$. $C_E$ increased $A_{sat}/E$ by 55% and water deficits increased $A_{sat}/E$ by 75% (Figure 4.5c, d). Water deficit reduced Ci/Ca by 39% (Figure 4.5e, f).

Figure 4.5: Effect of growth temperature, atmospheric [CO$_2$] and water availability on photosynthesis to stomatal conductance ratios ($A_{sat}/g_{sat}$, a and b), photosynthesis to transpiration rate ($A_{sat}/E$, c and d), and intercellular to ambient [CO$_2$] ratio (Ci/Ca, e and f) of cotton at the end of the second drought phase. Ambient temperature ($T_A$) is shown in blue, elevated temperature ($T_E$) is shown in red, well watered (wet; shaded) and water-stressed (dry; white). Values represent the mean of 5 leaves. Refer to Table 4.2 for a summary of significant main treatment effects and interactions.
Our data indicated that there were no temperature effects on the rate of recovery of $A_{\text{sat}}$ (Figure 4.6), $g_{\text{sat}}$ (Figure 4.7), $A_{\text{max}}$ (Appendix 6), or $g_{\text{max}}$ (Appendix 7). There is also no evidence that $C_{\text{E}}$ improved photosynthetic or stomatal recovery from drought stress. When data of photosynthesis and measured soil water deficit were compared across drought cycles using regression analyses, we could not detect any evidence of acclimation to drought (Appendix 3).

Figure 4.6: Photosynthesis at saturating light ($A_{\text{sat}}$, 1800 $\mu$mol m$^{-2}$ s$^{-1}$) for well-watered (closed symbol) and water-stressed (open symbol) plants grown at ambient ($C_{\text{A}}$: circles) and elevated ($C_{\text{E}}$: triangles) [CO$_2$], and ambient ($T_{\text{A}}$: blue) and elevated ($T_{\text{E}}$: red) temperatures. Values represent means ± SE of 5 leaves. All plants were well-watered during the recovery phase (shaded).
Figure 4.7: Stomatal conductance at saturating (1800 µmol) light ($g_{s\text{-sat}}$, mol m$^{-2}$ s$^{-1}$) for well-watered and water-stressed plants grown at ambient and elevated [CO$_2$], and ambient and elevated temperatures. Circles used for C$_A$: 400 µL L$^{-1}$ [CO$_2$], triangles used for C$_E$: 640 µL L$^{-1}$ [CO$_2$]. Ambient temperature (T$_A$) is shown in blue, elevated temperature (T$_E$) is shown in red. Values represent means ± SE of 5 leaves. All plants were well-watered during the recovery phase (shaded).

4.3.4. Whole plant water use

C$_E$ increased whole plant water use of cotton by 7% (Figure 4.8, Table 4.1) across both temperature treatments. Plants that were water-stressed used 26 - 37% less water than well-watered cotton across all treatments (Figure 4.8a and b, Table 4.1). Warmer temperatures increased whole plant water use of cotton in all treatments. T$_E$ increased water use of well-watered plants on average 72%, and increased water use of water-stressed plants by 64%. WUE$_P$ (Figure 4.8c and d) was increased with C$_E$ at T$_A$, but not at T$_E$. Compared with plants grown at C$_A$T$_A$, on average WUE$_P$ of cotton grown at C$_E$T$_A$ was increased by 26%. Elevated temperature decreased WUE$_P$ at C$_A$, but not at C$_E$. At C$_A$, a rise in temperature decreased WUE$_P$ by 17%.
Figure 4.8: (a and b) Whole plant water use (kg plant\(^{-1}\)) and (c and d) whole plant water use efficiency (g kg\(^{-1}\)) of well-watered (wet; shaded) and water-stressed (dry; white) cotton grown at ambient (C\(_{A}\): 400 \(\mu\)L L\(^{-1}\) [CO\(_2\)]) and elevated (C\(_{E}\): 640 \(\mu\)L L\(^{-1}\) [CO\(_2\)]) and ambient (T\(_{A}\): blue) and elevated (T\(_{E}\): red) temperatures until 70 DAP. Values represent the mean of 5 plants. Refer to Table 4.2 for a summary of significant main treatment effects and interactions.

4.4. Discussion

C\(_{E}\) increased photosynthetic rates (\(A_{sat}\)) across all treatments, but stomatal conductance (\(g_{s,sat}\)) was reduced by C\(_{E}\) only at T\(_{E}\). C\(_{E}\) increased total vegetative biomass at T\(_{A}\), but not at T\(_{E}\), with greater increases in well-watered plants compared with water-stressed plants, thereby indicating that leaf-level responses were not the same as plant-level responses. These results led to partial acceptance of
the first hypothesis that cotton grown at 640 µL L⁻¹ [CO₂] will have increased plant biomass, increased photosynthesis rates, and reduced stomatal conductance compared with plants grown at 400 µL L⁻¹ [CO₂]. The data also partially supported the third hypothesis that Cₑ and Tₑ combined would have interactive effects on carbon gain and water use. Tₑ increased total vegetative biomass and increased whole plant water use of cotton in all treatments, in agreement with the second hypothesis that cotton grown at warmer temperatures will have increased rates of development and growth, and higher plant water use than plants grown at ambient temperature. At Tₑ, an increase in [CO₂] increased water use by the plant; however, WUEₚ was also improved. At Tₑ, Cₑ did not improve WUEₚ of cotton, thus indicating that Cₑ did not alleviate the negative effects of Tₑ and leading to rejection of the third hypothesis. Therefore, our study indicated that Cₑ does not mitigate the negative effect of Tₑ on water use and WUEₚ, in either well-watered or water-stressed conditions.

4.4.1. Elevated [CO₂] may ameliorate moderate soil water deficit

Cₑ impacted cotton growth, physiology and water use, although the magnitude of the benefit of Cₑ was largely dependent upon air temperature and water availability. Our data showed that Cₑ increased plant biomass, particularly at Tₐ; however, the benefit of Cₑ was reduced at warmer temperatures. This has also been shown to occur in other crops where Cₑ increased wheat biomass and grain yield at 2 °C above ambient temperature, but did not enhance biomass and yield at 4 °C or 6 °C above ambient temperature (Dias de Oliveira et al., 2013). Similarly, Cₑ did not increase soybean growth and physiology at 3.5 °C warmer temperatures (Ruiz-Vera et al., 2013). In addition, our data did not suggest that Cₑ improved plant response to water deficits or improved photosynthetic recovery from drought stress. Other studies demonstrated that the benefit of Cₑ was reduced at warmer temperatures, as high temperatures (> 35 °C) are detrimental to mid- and late-season growth and boll retention (Reddy et al., 1995a; Reddy et al., 1997), indicating that the same interactions between Cₑ and a range of warmer temperatures are seen throughout all stages of growth for cotton.

As Cₑ increased Aᵣₛ, it is likely that Cₑ increased biomass through greater photosynthesis and metabolites available for growth, and enhanced early season light interception (Reddy et al., 1995a). In addition, the response in total biomass production was similar to the pattern of LA, suggesting that increased surface area for photosynthesis may have led to increased total biomass. Bunce (1998) found that gₛ of soybean, barley and wheat responded to Cₑ more at high VPD and was less responsive at low VPD, thereby suggesting that the response of plants to Cₑ can vary depending on other environmental conditions. Similarly, our data showed that Cₑ decreased gₛ, but only at Tₑ. Reduced rates of photosynthesis were most likely one reason for lower biomass for water-stressed plants grown at Cₑ compared with well-watered plants. Total vegetative biomass was higher in water-
stressed \( C_T T_D \) plants compared with \( C_A T_A \) plants, although the magnitude was lower than the increase in well-watered plants. Therefore, there is the potential for \( C_e \) to mitigate the negative effects (in terms of reduced vegetative biomass) of water-stress at temperatures experienced similar to \( T_A \). It is important to note that, as suggested by a number of other studies (Dugas et al., 1994; Hileman et al., 1994; Hunsaker et al., 1994; Kimball et al., 1994; Mauney et al., 1994; Reddy et al., 1995d; Samarakoon and Gifford, 1996), the benefits of \( C_e \) arise from increased WUE through greater biomass production and increased plant size, rather than a reduction in overall water use, and therefore cotton production is likely to require more water in higher-CO\(_2\) environments.

4.4.2. Elevated temperature increased water use and exacerbated moderate drought stress

Our data showed that warmer temperatures increased both plant water use (Figure 4.8a and b) and the rate (Figure 4.1) at which water was used, indicating that warmer temperatures (increasing VPD by 60%) exacerbated moderate drought stress. As plants became increasingly water-stressed during the drought cycles, photosynthetic rates and stomatal conductance declined (Figure 4.6 and Figure 4.7). Warmer temperatures have been shown to increase transpiration rates of cotton (Duursma et al., 2013; Reddy et al., 1995d), and similarly our data showed that warmer temperatures increased whole plant water use of cotton in all treatments. Cotton grown at \( T_A \) were able to withstand water deficits for much longer than those grown at \( T_E \), due to reduced leaf biomass and lower evaporative demand of plants grown in a warmer environment (Figure 4.3a and b, Figure 4.8). Plants grown at \( T_E \) used water much more quickly during both drought cycles, regardless of CO\(_2\) treatment. In addition, cotton grown at \( T_E \) used more water in both CO\(_2\) treatments, probably due to increased leaf area and biomass and thus greater surface area over which transpiration occurred. Warmer temperatures have been shown to increase plant growth and therefore leaf area and leaf area index (LAI) of cotton (Reddy et al., 1995a; Yoon et al., 2009). Increased leaf surface area may offset decreased stomatal conductance per unit leaf area on whole canopy evapotranspiration (Allen, 1999; Morison and Gifford, 1984). Our data showed increased leaf area and plant water use with warmer temperatures. Although \( C_e \) decreased \( gs_{\text{sat}} \) by 51% in the \( T_E \) treatment, averaged across all treatments, \( T_E \) increased plant water use by 68%. This impacts both leaf- and plant-level WUE, where there were significant reductions in WUE of cotton with warmer temperatures (Hileman et al., 1994), indicating that cotton production will require more water in warmer environments.
4.4.3. Interactive effects of elevated \([\text{CO}_2]\) and elevated temperature during soil water deficit

This study demonstrated that \([\text{CO}_2]\), temperature and water deficits have interactive effects on physiology, growth and consequently water use and efficiency of cotton. Our data showed that \(C_E\) decreased stomatal conductance of both well-watered and water-stressed plants only at \(T_E\). Reddy et al. (1998a) suggested elevated \([\text{CO}_2]\) could ameliorate the negative effect of high temperature on Rubisco, causing up-regulation of photosynthesis in cotton leaves, although high temperatures in that study were 36/28 °C compared with 32/21 °C in our study. Despite decreased stomatal conductance at warmer temperatures, our data do not indicate that the effects of \(C_E\) on photosynthesis were temperature dependent within the optimal temperature range for cotton, as \(C_E\) increased \(A_{\text{sat}}\) by 19% across both temperature treatments.

\(C_E\) increased total vegetative biomass of cotton grown at \(T_A\) by 26 - 54%, depending on water treatment, yet there was no increase at \(T_E\) (Figure 4.2). Leaf area followed the same pattern, indicating that the plants responded to elevated \([\text{CO}_2]\) at some temperatures, despite different patterns in photosynthetic rates (Figure 4.3). Similarly, a study investigating the interactive effects of \(C_E\), warmer temperatures and drought on wheat have shown severe reductions in biomass and grain yield caused by terminal drought were partially ameliorated by \(C_E\) and temperature, provided that the temperature was not > 2 °C above ambient (Dias de Oliveira et al., 2013). Therefore, \(C_E\) may potentially ameliorate the negative effect of drought, through increased photosynthetic rates and biomass production, but that may be dependent on growth temperature. However, our data indicated that greater productivity in cotton may require increased water use. In addition, \(C_E\) did not increase biomass at \(T_E\) suggesting that elevated \([\text{CO}_2]\) may not offset detrimental effects of higher temperature and VPD, which is an assumption in many models (Lloyd and Farquhar, 2008). Therefore, this highlights the importance of developing and validating models in conjunction with glasshouse and field experiments.

\(C_E\) increased total vegetative biomass and leaf area and improved WUE\(_P\) of cotton grown at \(T_A\). WUE\(_L\) (\(A_{\text{sat}}/E\)) was increased with \(C_E\), however this was averaged across all temperature and water treatments. Therefore, this indicates that the improvements in WUE\(_P\) of cotton are more associated with relative efficiency of leaf area and biomass production than improvements in WUE\(_L\). Overall, both \(T_E\) and \(C_E\) increased plant water use of cotton, indicating that cotton grown in warmer future environments will have higher water requirements. Therefore, \(C_E\) at current temperatures will increase WUE\(_P\), but there will be limited benefit of \(C_E\) at warmer temperatures, which are predicted for future climates.
4.4.4. Conclusions

This study investigated the interactive effects of elevated [CO₂], warming and soil water deficit on biomass production, leaf level physiological responses and whole plant water use and efficiency in cotton. Cₑ increased vegetative biomass and decreased stomatal conductance; however, these responses were dependent on growth temperature. Cₑ increased vegetative biomass, leaf area and improved WUEₚ only at Tₐ whereas, warmer temperatures negated the positive response of improved WUEₚ to Cₑ. Cotton grown at Tₐ tolerated water deficit to a better extent than plants grown at Tₑ, indicating that cotton may be more susceptible to long dry periods in projected warmer environments. Tₑ was the driving factor for increased water use in cotton; however, Cₑ also increased water use to a lesser extent, despite improvements in WUEₚ with Cₑ at Tₐ. Therefore, growth benefits of Cₑ may occur at Tₐ, but Cₑ will not mitigate negative effects, such as increased water use, of rising temperature on cotton growth and physiology in future environments. These findings indicate that cotton plants may use more water at higher temperatures and thus crops may need to be irrigated more frequently, thereby irrigation management in cotton production systems may need to be reviewed for future environmental conditions, although further research should test these concepts in field based studies.
Chapter 5: Environmental effects on the relationship of leaf-level conductance and photosynthesis to VPD

5.1. Introduction

Atmospheric vapour pressure deficit (VPD) is the difference between the amount of moisture the air can hold when it is saturated and ambient moisture in the air (Bureau of Meteorology, 2011), measured in Pascals. VPD varies throughout the season. In cotton growing regions, low VPDs are more likely to occur early in the season with higher relative humidity and cooler temperatures. High VPDs are likely to occur later in the growing season, with lower relative humidity and warmer temperatures resulting in a greater difference in vapour pressure between the leaf and the atmosphere. In addition, VPD changes throughout the day as increased ambient air temperature usually leads to a simultaneous increase in ambient air VPD (Pettigrew et al., 1990).

The environmental stimuli that affect stomatal opening and closing are light, intercellular [CO$_2$], air humidity, and plant and soil water deficits (Grantz, 1990; Knox et al., 2005; Xue et al., 2004). Soil water status and VPD are important environmental parameters that influence plant gas exchange (Xue et al., 2004). If air flowing over a leaf changes from high to low humidity, the transpiration rate of a plant will increase, guard cells will lose turgor and stomatal aperture will decrease (Knox et al., 2005; Lange et al., 1971). By reducing stomatal aperture, the leaf can restrict water loss. Changes in humidity alter transpiration, energy balance and tissue temperature.

Plants respond to changes in VPD between the leaf and the atmosphere, through changes in stomatal response (Grantz, 1990). Increasing VPD linearly increases the transpiration rate at leaf level (Rawson et al., 1977; Yong et al., 1997) despite a decrease in stomatal conductance, and similar trends have been observed in leaves of glasshouse grown cotton plants (Duursma et al., 2013; Slatyer and Bierhuizen, 1964). By avoiding high $E$ that would otherwise be caused by increasing VPD, stomatal closure avoids the corresponding decline in plant water potential (Oren et al., 1999). Studies have also shown that stomatal conductance ($g_s$) decreases with increasing VPD, although the precise mechanism for this correlation is not clear (Conaty et al., 2014; Yong et al., 1997). In most cases, $g_s$ decreases exponentially with increasing VPD. Leaf-to-air vapour pressure difference during midday measurements of stomatal response to carbon dioxide affected the magnitude of the response (Bunce, 1998). On days when VPD was low, no significant change in $g_s$ occurred in increased [CO$_2$]; however, when VPD was higher, $g_s$ decreased by 24 - 52% within a few minutes (Bunce, 1998). Xue et
al. (2004) demonstrated that responses of gas exchange parameters to VPD were related to soil water potential in field-grown wheat. Stomatal conductance was very sensitive to VPD, and decreased with increased VPD even at relatively high soil water potential (-0.09 MPa). Leaf transpiration rate increased as VPD increased at high soil water potential (-0.09 MPa), but decreased as VPD increased under water stress. Therefore, the response of \( g_s \) to VPD cannot be explained as a feedback mechanism, i.e. the decrease in \( g_s \) was not due to increased transpiration at high VPD (Xue et al., 2004). Greater transpiration rates at high VPD may lead to greater plant water use during the season, and thus it is necessary to understand the impacts of VPD on the physiology of field-grown cotton.

There is also evidence that increasing VPD can cause inhibition of photosynthesis unrelated to stomatal closure (Morison and Gifford, 1983; Pettigrew et al., 1990). However Duursma et al. (2013) found that in cotton, photosynthesis was relatively insensitive to VPD as it decreased on average only 13% from the maximum photosynthetic rate over the range of VPD (1 to 4 kPa). Rawson et al. (1977) compared the VPD response of a number of C\(_3\) species, including wheat, soybean, sunflower and sorghum, to step changes in VPD over the range 0.8 - 2.7 kPa. They found little or no response to VPD in these species, which were grown under high light, were well-watered, and maintained at a mean temperature of 26 °C. Franks and Farquhar (1999) found that wheat and broad bean were the least sensitive to changes in VPD (although sample sizes were small). Therefore, crop plants may have inadvertently been selected for high \( g_s \) in the interest of maximising productivity, and high \( g_s \) may contribute to very high rates of transpiration under natural conditions (Franks and Farquhar, 1999).

Responses of \( g_s \) to increasing VPD generally follow an exponential decrease described by several empirical functions (Oren et al., 1999). Stomatal models are based on the theory of “optimal” behaviour. These models assume that stomata function in such a way that the total loss of water during a day is a minimum for the total amount of carbon taken up (Cowan and Farquhar, 1977). A unified model adapted by Medlyn et al. (2011) used the theory of optimal \( g_s \) based on the theoretical argument that stomata should act to minimise the amount of water used per unit carbon gained. The Medlyn et al. (2011) model uses a combination of two models: the Cowan and Farquhar (1977) theory of optimal stomatal behaviour and the Farquhar et al. (1980) model of photosynthesis. Glasshouse experiments showed that cotton is very responsive to VPD, where the model adapted by Duursma et al. (2013) describes an equation for \( g_s \), responding to atmospheric \([\text{CO}_2]\) and leaf-to-air vapour pressure deficits. However, these models are yet to be tested on field-grown cotton.

As the majority of Australian cotton is irrigated, the area planted to cotton depends on the availability of irrigation water for the season. Irrigation is essential to achieve potential yield in cotton grown in Eastern Australia, as in-season precipitation is sometimes insufficient to meet crop water demand.
(Tennakoon and Hulugalle, 2006). The estimated mean seasonal evapotranspiration is 735 mm (Tennakoon and Milroy, 2003), but potential seasonal evapotranspiration may exceed 1000 mm due to low humidity and high temperature in most of the cotton growing areas (Tennakoon and Hulugalle, 2006). Plant water availability during critical growth periods may affect physiological processes and productivity. Higher temperatures alone increase the quantity of water consumed in evapotranspiration (Salvucci and Crafts-Brandner, 2004), which is important in cooling leaves. With global warming, there is likely to be increases in both day-time and night-time temperatures (IPCC, 2014). If the diurnal temperature range remains constant, global warming will lead to an increase in VPD because the saturated vapour pressure curve is steeper at higher temperatures than at lower temperatures (Kirschbaum, 2004). However, there is likely to be a shift in climatic zones, with differences between regions and continents such as projections for wetter regions throughout India and northern tropical Africa and drier throughout nearly all other land regions (Sherwood and Fu, 2014). With potentially warmer temperatures, changes in rainfall distribution and altered VPD in future climates, it is important to understand how VPD impacts leaf-level physiology of field-grown cotton as these will lead to a broader understanding of crop responses to projected future climates.

Although there have been some studies testing the response of cotton to varied VPD in the glasshouse, VPD experiments on field-grown Australian Bt cotton are limited. In addition, some of the studies on the VPD response of cotton only examine the response over a narrow range of VPD (Yong et al., 1997); however, other studies (Bunce, 2006) test the response to a greater range of VPD. Therefore, these concepts should be tested in field environments in Australia.

Improved understanding of cotton physiological response to VPD may assist in validation and improvement of models and irrigation scheduling in cotton (Conaty et al., 2014). Therefore, it could be useful to quantify the understanding of leaf physiological responses and integrate to the canopy scale (Hammer and Wright, 1994; Milroy and Bange, 2003) to gain a broader understanding of altered environments on crop production. For instance, cotton crop simulation models such as OZCOT use canopy-level gas exchange measurements to predict cotton growth and yield. Therefore, improving field-based knowledge and understanding of leaf gas exchange to changes in VPD may lead to improved model predictions for cotton grown in future environments, particularly with higher VPD associated with warmer temperatures.

The objective of this research was to assess the impact of altered VPD on leaf-level physiology of cotton grown in Australian field conditions and examine the environmental variables that influence changes in stomatal and photosynthetic response. In this study, we tested the hypotheses that (1) increased $V_{PD_{L}}$ will reduce stomatal conductance in the field; (2) that variation in stomatal...
conductance and photosynthetic rates can be explained by changes in growth conditions and consequently variables that describe the environmental factors; and (3) that the Instantaneous Transpiration Efficiency (ITE; equivalent to $A_{sat}/E$) model developed using cotton grown in glasshouse conditions (Duursma et al., 2013) can be used to estimate $A_{sat}/E$ of field-grown cotton.

5.2. Materials and Methods

5.2.1. Experimental design and plot management

Cotton (*Gossypium hirsutum*, cv. 71BRF [Bollgard II® Roundup Ready Flex®], CSIRO Australia) was grown at the Australian Cotton Research Institute (ACRI), Narrabri during the 2011/12 season. Cotton was planted on three dates within the sowing window (early (S1) = 5th October 2011; mid (S2) = 9th November 2011; late (S3) = 30th November 2011) to generate different VPD environments for measurements (Figure 5.1).

The three irrigation treatments were (a) fully watered – non-stressed (NS); (b) limited water- early stress (ES); and (c) limited water- late stress (LS). The first irrigation was skipped for the early stress treatment. One irrigation event was skipped during the early boll-fill stage for the late stress treatment. Plastic was used to cover the ground during the water stress treatments to reduce the risk of rainfall prematurely alleviating the stress. However, substantial high rainfall and flooding during the season led to the exclusion of measurements in some of the treatments (S1LS).

Each plot consisted of two rows of furrow-irrigated cotton with an additional two-row buffer around each plot (a total of four rows per plot). Plants were irrigated down the centre three rows to minimise the lateral movement of water from the fully irrigated to the water-stressed plots. Each row was 66 m long, except for the three control irrigation treatments, which were each 22 m long. A row spacing of 1 m was used with a sowing density of 14 plants m$^{-2}$. The field layout is shown in Figure 5.1. Replication was achieved through measuring gas exchange on a number of different plants within a plot, rather than replication of the plots themselves.

Experiments were managed according to current Australian practices, except for imposed irrigation treatments as outlined.
Figure 5.1: Field layout for experiment depicting combination of sowing times and water treatments for each plot.

Daily weather conditions, including minimum and maximum air temperatures and rainfall events were obtained from the Myall Vale weather station (Figure 5.2).
Figure 5.2: Daily minimum (°C, blue) and maximum (°C, red) air temperature and rainfall (mm, grey) at ACRI, Narrabri from 5 October 2011 to 23 May 2012.

5.2.2. Leaf gas exchange

Net photosynthesis at saturating light \((A_{\text{sat}})\) and stomatal conductance \((g_{s_{\text{sat}}})\) were measured on recently fully expanded leaves using a portable open gas exchange system (LI-6400XT, LI-COR, Lincoln, USA). Leaf gas exchange measurements were taken at saturating light (photosynthetic photon flux density of 2000 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)), 400 \(\mu\)L L\(^{-1}\) \([CO_2]\), and block temperature was set to the anticipated mid-day temperature. A comparison between the block and air temperatures is shown in Appendix 8. VPD response curves were achieved by controlling VPD (temperature x relative humidity) at the leaf surface within the IRGA chamber. Measurements began on full-bypass of air, representing natural ambient humidity conditions. These initial measurements were referred to as the ‘ambient’ dataset. Water from the air was slowly scrubbed using the desiccant drierite (W.A. Hammond Drierite Co., USA), increasing VPD, by approximately 0.5 kPa for each measurement. All gas exchange measurements, including these VPD response curves, were referred to as the ‘complete’ dataset. The number of measurements captured for each response curve varied because of differences in the range of VPD generated by the IRGA at each time and day.
Measurements were made between the hours of 10 am to 3:30 pm (Australian Eastern Daylight Time; AEDT), over 3 - 4 consecutive days at different times during the water stress treatment. Measurements during the same period of consecutive days were made on the same recently mature leaf (approx 3rd leaf from the top of the plant on the first day of measurement), which had been tagged. Each leaf was allowed approximately 2 min to equilibrate before the reading was recorded. Leaf gas exchange measurements of the equivalent control (i.e. non water-stressed treatment) plants were taken on the same day as the water-stressed plants. Two to three leaves from each plot were measured per day. Mean ambient VPD_L and air temperature on each day of measurement is presented in Appendix 9. Plant g_s-sat and A_sat responses to ambient VPD_L are shown in Appendix 10.

5.2.3. Leaf water potential

Leaf water potential (ψ_l) was measured using the pressure chamber (Corvallis, OR, USA) to coincide with gas exchange measurements. These were taken weekly during water stress treatments. Leaf water potential was measured at solar noon.

5.2.4. Soil water

A neutron probe was used to monitor soil water content every 0.2 m to a depth of 1.2 m. These measurements were taken every 10 days, and weekly during water stress treatments (Appendix 11). Volumetric soil water content (VSWC %) was calculated using a formula, which has been calibrated for soils in an adjacent field, with the same soil classification at ACRI (Ward et al., 1999). VSWC % = 0.0006x + 24.225 where x is the count measurement at each depth (Warren Conaty, pers comm.). VSWC % was averaged across all depths (i.e. 0 - 120 cm).

5.2.5. Canopy temperature

Wireless, battery-operated SmartCrop infrared thermometers (Smartfield Inc., Lubbock, TX, USA) were used to monitor canopy temperature in each plot. Sensors were periodically repositioned to maintain them at 20 - 30 cm above the canopy pointing south (to reduce the effects of specular reflectance) at an angle of 70° to the vertical for the duration of the measurement period. Where possible, two sensors were placed towards the centre of each plot; however, some plots only had one sensor due to a limited number of sensors available. Stress hours were recorded by the SmartCrop sensors as the calculated time that the canopy temperature was above 28 °C (Conaty, 2011). This was used to calculate accumulated temperature stress hours (ASH) between irrigation events.
5.2.6. Plant growth measurements

Plant growth characteristics (height, number of nodes) of 20 representative plants from each plot were assessed approximately every 10 days to monitor plant growth. These are summarised in Appendix 12.

5.2.7. Statistical analysis

Testing treatment effects

For the complete dataset, a linear mixed-effects model (R version 3.1.0.) was used to analyse the treatment effects on stomatal conductance. This was used to show the relationship between stomatal conductance and VPD. The model fitted in R was:

$$g_{s\text{-sat}} = \log VPD_L + TBlk + TBlk^2 + \text{Sowing} + \text{WaterTrt} + \text{Sowing} \times \text{WaterTrt}$$ \hspace{1cm} (1)

where; $VPD_L$ is leaf-level vapour pressure deficit (kPa), $TBlk$ is block temperature of the cuvette ($^\circ$C), Sowing is the sowing treatment, and waterTrt is the water treatment.

For the complete dataset, the same linear mixed-effects model (1) was used to analyse the treatment effects on photosynthesis. Using Genstat version 16 (VSN International, Hemel Hempstead, UK), REML was then used to test treatment effects on $g_{s\text{-sat}}$ and photosynthesis of cotton exposed to ambient VPD in the field (i.e. non-scrubbed measurements).

Testing environmental effects

Generalised linear models were used to link the responses of field-grown cotton to the treatment effects with the overall responses of the plants to the biological and environmental responses. To test model effects, data were analysed using Genstat version 16 (VSN International, Hemel Hempstead, UK) by stepwise regression using Generalised Linear Models. This method was used both for the ambient VPD dataset and the complete dataset for both stomatal conductance and photosynthesis. A number of variables were tested, but the best maximal model for both stomatal conductance and photosynthesis was found to be: $VPD_L \times \text{Plant} \times T_l-T_a \times \text{ASH}$, where; $VPD_L$ is leaf-level vapour pressure deficit, Plant is the individual plant, $T_l-T_a$ is the difference between leaf and air temperature and ASH is accumulated temperature stress hours. A table of these analyses is found in Appendix 13. Stomatal conductance data were transformed using a logarithmic transformation, which improved the $R^2$ over a linear regression (from $R^2= 0.387$ to $R^2= 0.428$).
Testing the $A_{sat}/E$ (ITE) model

Duursma et al. (2013) showed that for cotton grown in a controlled environment glasshouse (in two CO$_2$ and temperature treatments) that the following equation gave satisfactory fits to measured ITE when VPD was varied independently of temperature and other environmental drivers:

$$ITE = \frac{A}{E} = \frac{C_aP_a}{g_1D_s^{k+D_s}}$$

where ITE is the ratio of photosynthesis to transpiration ($\mu$mol mmol$^{-1}$), $C_a$ is atmospheric [CO$_2$], $P_a$ is the atmospheric pressure (kPa), $g_1$ is a parameter, $D_s$ is the leaf-to-air vapour pressure deficit (kPa), and $k$ is an empirical parameter. Based on the assumption that stomata respond optimally to changes in VPD, $k$ would equal 0.5.

To test whether this model, and the parameters estimated by Duursma et al. (2013) are adequate to estimate $A_{sat}/E$ in field conditions, Eq. (2) was fitted to a “well-watered” subset of the VPD measurements of field grown cotton using R. The “well-watered” subset was based on VPD data within the first five days of gas exchange measurements for each treatment, and used because it is known that the model is not appropriate for water-stressed conditions (Remko Duursma, pers. comm). Predicted (modelled) $A_{sat}/E$ was compared with measured $A_{sat}/E$ using parameters estimated from (a) the fit to field data and (b) glasshouse prediction data. The root mean square error (RMSE) was used to indicate the goodness of fit for how well the model predicted the measured $A_{sat}/E$ values. The mean absolute difference (MAD) was used as a measure of the difference between modelled estimates and measured values.

5.3. Results

5.3.1. Treatment effects on cotton physiology

The response of $g_{sat}$ and $A_{sat}$ to VPD, for each sowing time for all data generated is shown in Figure 5.3, depicting a decline in both $g_{sat}$ and $A_{sat}$ with increasing VPD. The data showed that there was a significant sowing x water treatment interaction on $g_{sat}$ of the complete dataset predictions ($P=0.035$; Figure 5.3, Table 5.1, Figure 5.4); however, there were no interactive effects of sowing x water treatment on $g_{sat}$ for initial ambient field VPD measurements ($P=0.084$; Table 5.1). Sowing time was not significant as a predictor for the relationship between $g_{sat}$ and VPD, ($P=0.07$; Figure 5.3, Table 5.1, Figure 5.5); however, temperature of the Licor cuvette did have a significant effect. Water treatment also had a significant effect on $g_{sat}$ for both the complete dataset ($P=0.035$, Figure 5.3, Table 5.1) and ambient VPD measurements ($P=0.019$; Table 5.1).
Sowing time had a significant effect on photosynthesis for both the complete and ambient datasets (P= 0.001; Table 5.1). The data also showed that temperature of the Licor cuvette had a significant effect on \(A_{sat}\) of cotton using the complete dataset (\(P= 0.001; \) Table 5.1). Water treatment had a significant effect on \(A_{sat}\) in the complete measurements (\(P= 0.016; \) Table 5.1), but not in the ambient dataset (\(P= 0.189; \) Table 5.1). There were no interactive sowing x water treatment on photosynthesis of cotton in the complete dataset or exposed to ambient VPD\(_t\) (\(P> 0.05; \) Table 5.1).
Figure 5.3: (a) Stomatal conductance ($g_{s\text{-sat}}$) and (b) photosynthesis ($A_{sat}$) for VPD response curves for each sowing treatment of field-grown cotton. Sowing treatments are coloured red (S1), blue (S2) and green (S3). Lines represent each VPD curve and hence includes the complete dataset.
Table 5.1: P-values for the treatment effects on stomatal conductance ($g_{s\text{-sat}}$) and photosynthesis ($A_{\text{sat}}$) of the complete (ANOVA) and ambient (REML) datasets; where VPD<sub>L</sub> is vapour pressure deficit of the leaf, TBlk is block temperature of the cuvette (°C), Sowing is the sowing treatment and WaterTrt is the water treatment. *, **, *** show significant differences at $P \leq 0.05$, $0.01$ and $0.001$, respectively. Figures in bold represent significance at $P<0.05$.

<table>
<thead>
<tr>
<th>Treatment Effect</th>
<th>$g_{s\text{-sat}}$ Complete</th>
<th>ambient</th>
<th>$A_{\text{sat}}$ Complete</th>
<th>ambient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log (VPD&lt;sub&gt;L&lt;/sub&gt;)</td>
<td>0.001***</td>
<td>-</td>
<td>0.001***</td>
<td>-</td>
</tr>
<tr>
<td>TBlk</td>
<td>0.001***</td>
<td>-</td>
<td>0.001***</td>
<td>-</td>
</tr>
<tr>
<td>TBlk&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.001***</td>
<td>-</td>
<td>0.001***</td>
<td>-</td>
</tr>
<tr>
<td>Sowing</td>
<td>0.074</td>
<td>0.334</td>
<td>0.001***</td>
<td>0.001***</td>
</tr>
<tr>
<td>WaterTrt</td>
<td>0.035*</td>
<td>0.019*</td>
<td>0.016*</td>
<td>0.189</td>
</tr>
<tr>
<td>Sowing x WaterTrt</td>
<td>0.036*</td>
<td>0.084</td>
<td>0.809</td>
<td>0.214</td>
</tr>
</tbody>
</table>
Figure 5.4: The predictions for water treatment (early stress (ES), late stress (LS) and non-stressed (NS)) and sowing time (S1, S2 and S3) on (a) stomatal conductance \( g_{\text{sat}} \) and (b) photosynthesis \( A_{\text{sat}} \) of field-grown cotton using Eq. (1). The points are predictions that account for other variables in the model that are not shown in the plot.
Figure 5.5: The predictions of how sowing time (S1: red, S2: green, and S3: blue) and VPD$_L$ affect (a) stomatal conductance ($g_{s-sat}$) and (b) photosynthesis ($A_{sat}$) of field-grown cotton using Eq. (1). The points are predictions that account for other variables in the model that are not shown in the plot.
5.3.2. Comparison of environmental effects on stomatal conductance and photosynthesis of field-grown cotton using Generalised Linear Models

Generalised linear models used to assess the environmental effects on $g_{s\text{-sat}}$ of the ambient VPD measurements indicated that VPD$_{a}$ accounted for 39.5% of the variation. The addition of the variables Plant (+ 4.8%), $T_r-T_a$ (+ 16.1%) and ASH (+ 7.1%) accounted for a total of 67.5% of the variation, indicating these factors were all significant predictors of $g_{s\text{-sat}}$. Therefore, the best fitting regression analysis for $g_{s\text{-sat}}$ in the ambient VPD dataset was VPD$_{a}$ + Plant + $T_r-T_a$ + ASH.

In comparison, the same generalised linear models used to assess the environmental effects on $g_{s\text{-sat}}$ of the complete dataset indicated that the VPD$_{a}$ alone accounted for 32.3% of the variation. The cumulative addition of Plant (+ 4.3%), $T_r-T_a$ (+ 32.8%) and Plant x $T_r-T_a$ (+ 0.8%) increased accountable variation to a total of 70.2%. Therefore, the best fitting regression analysis for $g_{s\text{-sat}}$ in the complete dataset was VPD$_{a}$ + Plant + $T_r-T_a$ + Plant x $T_r-T_a$.

Generalised linear models used to assess the environmental effects on photosynthesis of ambient VPD measurements indicated that VPD$_{a}$ accounted for 28.9% of the variation. The cumulative addition of ASH (+ 17.1%) and VPD$_{a}$ x $T_r-T_a$ (+ 9.5%) improved the model by accounting for a total of 55.5% of the variation. Therefore, the best fitting regression analysis for photosynthesis at ambient VPD was VPD$_{a}$ + ASH + VPD$_{a}$ x $T_r-T_a$.

In comparison, the same generalised linear models were used to assess the environmental effects on photosynthesis of the complete dataset. VPD$_{a}$ accounted for 16.8% of the variation, and the cumulative addition of $T_r-T_a$ (+ 15.4%), ASH (+ 6.3%), Plant x $T_r-T_a$ (+ 1.9%) and VPD$_{a}$ x ASH (+ 1.2%) increased accountable variation to a total of 41.6%. Thereby, the best fitting regression for the photosynthesis with the complete dataset was VPD$_{a}$ + $T_r-T_a$ + ASH + Plant x $T_r-T_a$ + VPD$_{a}$ x ASH.

5.3.3. Estimating $A_{sat}/E$ for field conditions using the Duursma et al. (2013) model

The $A_{sat}/E$ response to VPD is shown in Figure 5.6. $A_{sat}/E$ showed a strong response to VPD. The comparison of modelled and measured $A_{sat}/E$ using estimated $g_1$ and $k$ parameters is shown in Figure 5.7. Estimated parameter values of field grown cotton were $g_1 = 4.35$ (95% confidence interval (CI) = 4.24 - 4.47) and $k = 0.59$ (95% CI = 0.53 – 0.64). In addition, for the comparison of modelled and measured $A_{sat}/E$ for field data MAD= 0.546, whereas using the glasshouse data prediction MAD= 0.551.
Figure 5.6: $A_{sat}/E$ response to VPD of “well-watered” field-grown cotton. Black solid line represents model fit using $g_1$ and $k$ estimates from field data. Blue dashed line represents $g_1$ and $k$ model prediction based on cotton grown in the glasshouse.

Figure 5.7: Comparison of modelled and measured $A_{sat}/E$ using Eq. (2) where $g_1$ and $k$ parameters are from (a) field data and (b) glasshouse data prediction from Duursma et al. (2013). Also shown are the 1:1 lines (black). (a) RMSE= 0.714; MAD= 0.546 and (b) RMSE does not apply; MAD= 0.551.
5.4. Discussion

Environmental conditions in a field can greatly influence crop physiology and yield (Pettigrew et al., 1990), and therefore it is important to assess the impact of the environment on physiology of field-grown cotton as warmer temperatures, changes in rainfall distribution and altered VPD are projected in the future for Australian cotton regions. In this study, we found that increased VPD may reduce stomatal conductance in field-grown cotton; that variation in stomatal conductance and photosynthetic rates can be explained by changes in growth conditions and consequently variables that describe environmental factors, such as VPD; and that the $A_{sat}/E$ (ITE) model developed using cotton grown in glasshouse conditions can be used to estimate $A_{sat}/E$ of field-grown cotton.

In this study, a large proportion of variation in $g_{s-sat}$ was accounted for by the VPD environment. We found that VPD$_L$ alone accounted for 32.3 and 39.5% of the variation in $g_{s-sat}$ for the complete and ambient gas exchange measurements, respectively. Similar to numerous other studies (Duursma et al., 2013; Oren et al., 1999), our data showed a general decline in $g_{s-sat}$ with increased VPD. Our study highlights that although VPD$_L$ accounts for a large proportion of the cumulative variation in $g_{s-sat}$, there were still a number of other variables that influenced variation in stomatal response, including the plant (4.3%), $T_r - T_a$ (32.8%) and Plant x $T_r - T_a$ (0.8%) interactions. Nonetheless, we could only account for c. 70% of variation in $g_{s-sat}$. Therefore, 30% of the variation in $g_{s-sat}$ is due to something that we either did not measure or analyse. For example, Duursma et al. (2013) developed models to describe the stomatal response to environmental factors of cotton grown in the glasshouse, where conditions such as growth temperatures were highly controlled, unlike in the field. Variables that were accounted for included VPD, assimilation rate and atmospheric [CO$_2$] (Duursma et al., 2013). Therefore, when these models are used for field-based studies, there may be unexplained variation depending on the antecedent growth conditions of the crop, which may include factors such as nutrient status of an individual leaf, and leaf angle affecting light-interception.

For photosynthetic responses, VPD$_L$ accounted for only 16.8% of the variation in the complete dataset, and accounted for 28.9% of the variation in photosynthetic rates for the ambient gas exchange measurements. $T_r - T_a$ and ASH were also important factors for plant photosynthetic response. Adding $T_r - T_a$ increased the variation accounted for by 15.4% in the complete dataset but was not a significant variable in the ambient dataset. In addition, adding the variable ASH increased the variation accounted for by 6.3% in the complete dataset and by 17.1% in the ambient dataset. The addition of Plant x $T_r - T_a$ and VPD$_L$ x ASH were significant interactions for photosynthetic response in the complete dataset, whereas the VPD$_L$ x $T_r - T_a$ interaction was significant in the ambient dataset. Other studies reported a lack of response of photosynthesis to altered VPD (Rawson et al., 1977; Yong et al., 1997), although in
these experiments temperatures were generally held constant during the study. Similarly, Duursma et al. (2013) found that photosynthesis was relatively insensitive to VPD, with a 13% decrease in maximum photosynthesis over 1 - 4 kPa, but reported higher photosynthetic rates of cotton grown at warmer air temperatures resulting in a higher transpiration rate at a given VPD, again highlighting the impact of temperature effects on photosynthesis. Therefore, in comparison, these studies allowed a better identification of the direct effects of VPD, but not allowed the independent effect of temperature to be observed. Given that both T\textsubscript{l}-T\textsubscript{a} and ASH have accounted for variation in photosynthetic rates, this highlights the importance of how warmer temperatures may affect photosynthesis of cotton grown in future, warmer climates, regardless of the small direct impact of VPD on photosynthesis.

The A\textsubscript{sat}/E model fit to the field data suggests that the g\textsubscript{s} and k parameters used in the glasshouse can also be used to estimate A\textsubscript{sat}/E in the field. Therefore, this indicates that the A\textsubscript{sat}/E model developed using cotton grown in the glasshouse is also applicable to cotton grown in the field and highlights that controlled environment glasshouse studies can be successfully utilised to further our understanding of leaf-level physiological responses to environmental conditions. In addition, these studies are useful when attempting to scale from leaf to canopy level responses. Thus, this improves our ability to predict the effect of climate change on crop water use efficiency (Duursma et al., 2013). However, limitations were that although plants were grown in the field, A\textsubscript{sat}/E was measured using the cuvette of the Licor, where wind speeds, and thus boundary layer conductance, were high (Grantz and Vaughn, 1999). Boundary layer conductance can affect leaf temperature, and transpiration rates at a given stomatal conductance, and therefore may not represent actual gas exchange in the field. Therefore, the combination of canopy and leaf-level measurements may be the most useful in describing cotton response to the environment. However, the success in using the A\textsubscript{sat}/E model in both glasshouse and field-grown cotton is promising for the validation of other simulation models. For example, the OZCOT cotton crop simulation model currently does not account for physiological changes in canopy photosynthesis or transpiration in response to VPD\textsubscript{l}. Therefore, a better understanding of the physiological responses may improve our predictions of growth and water use, especially with the simulation of future environments.

5.4.1. Conclusions

VPD\textsubscript{l} accounted for a large proportion of the variation in g\textsubscript{s-sat} and photosynthesis, with smaller percentages attributed to other factors such as the individual plant, T\textsubscript{l}-T\textsubscript{a}, ASH and VPD\textsubscript{l} x T\textsubscript{l}-T\textsubscript{a}, Plant x T\textsubscript{l}-T\textsubscript{a} and VPD\textsubscript{l} x ASH interactions. Using generalised linear models, c. 70% of variation in g\textsubscript{s-sat} was accounted for by VPD\textsubscript{l}, Plant, T\textsubscript{l}-T\textsubscript{a}, ASH and Plant x T\textsubscript{l}-T\textsubscript{a} interactions. Similarly, a total 42 - 56% of
variation in photosynthetic rate was accounted for by VPD\textsubscript{L}, T\textsubscript{p}-T\textsubscript{a}, VPD\textsubscript{L} x T\textsubscript{p}-T\textsubscript{a}, Plant x T\textsubscript{p}-T\textsubscript{a} and VPD\textsubscript{L} x ASH interactions. However, a proportion of the variation in \(g_{s\text{-sat}}\) and photosynthesis were not explained by these measurements.

Data from this study can be used for \(A_{\text{sat}}/E\) models and can potentially be used to inform crop simulation models to account for possible impacts of climate change on crop production. In conjunction with information of cotton canopy temperature response (Conaty et al., 2014), a better understanding of VPD may aid our understanding of physiological responses of field-grown cotton and lead to better management of cotton production in future environments.
Chapter 6: Effects of elevated CO\textsubscript{2} and temperature on field-grown cotton

6.1. Introduction

The global atmospheric [CO\textsubscript{2}] has increased from pre-industrial value of about 280 μL L\textsuperscript{-1} to 400 μL L\textsuperscript{-1} in 2013 (CSIRO and Bureau of Meteorology, 2012; IPCC, 2013), and will continue to rise in the future, affecting plant physiology and growth. Elevated atmospheric [CO\textsubscript{2}] generally stimulates photosynthesis, leading to increased crop growth and yield, especially in C\textsubscript{3} species (Ainsworth and Long, 2005; Reddy et al., 1995d). Elevated [CO\textsubscript{2}] also often decreases stomatal aperture, which impacts conductance of CO\textsubscript{2} and water vapour through stomata, which can improve leaf-level water-use efficiency (Ainsworth and McGrath, 2010; Kimball and Idso, 1983), but may also lead to a reduction in transpirational cooling of plant leaves and an increase in leaf temperature (Kimball et al., 2002; Morison and Gifford, 1984). Although elevated [CO\textsubscript{2}] contributes to enhanced plant growth and improved leaf-level water use efficiency (WUE) (Reddy et al., 1995d; Samarakoon and Gifford, 1996), there may be a downscaling of these positive impacts due to higher growth temperature and increased whole plant water use as a consequence of greater leaf area (Samarakoon and Gifford, 1996). Greater variability in precipitation and increasing air temperature may substantially offset the positive effects of rising CO\textsubscript{2} on plant growth (Hatfield et al., 2011).

A range of experimental systems, including environmental chambers, glasshouses, Soil-Plant-Atmosphere Research (SPAR) units, open-top chambers (OTC), and Free Air CO\textsubscript{2} Enrichment (FACE) facilities, have been developed to expose plants to elevated atmospheric [CO\textsubscript{2}] (Ainsworth et al., 2008b; Kimball et al., 2002; Kimball et al., 1997; Long et al., 2004; Mauney et al., 1994; Reddy et al., 2001; Reddy and Reddy, 1998). In addition, experimental systems capable of measuring whole canopy gas exchange, while simultaneously controlling [CO\textsubscript{2}] include OTC, horizontal-flow-through field chambers, whole tree chambers and naturally-lit SPAR facilities (Baker et al., 2014b). In controlled environmental chambers and glasshouses, individual plants are typically grown in pots, and light, water, humidity and nutrients are controlled. Therefore, there are often higher levels of environmental control than in field conditions, but such facilities may restrict root growth, which can negatively influence photosynthetic capacity, shoot growth and harvestable yield potential, and thus reduce the response to CO\textsubscript{2} stimulation (Ainsworth and McGrath, 2010; Arp, 1991). In addition, there are also more rapid fluctuations in soil water status, root temperature, and typically an artificial canopy arrangement affecting light interception, air circulation and temperatures that are not
representative of conditions in the field. Limitations in glasshouse, SPAR and OTC facilities led to the development of FACE systems.

Large scale FACE experiments allow the exposure of plants to elevated [CO\(_2\)] under natural and fully open-air conditions. FACE technology uses an array of pipes to release CO\(_2\)-enriched air or pure CO\(_2\) gas upwind of the plots to maintain an elevated concentration on the target area, rather than confinement structures. Therefore, FACE relies on natural wind and diffusion to disperse the CO\(_2\) across the experimental area (Ainsworth and Long, 2005; Hendrey and Kimball, 1994) and thus these systems encounter problems with CO\(_2\) enrichment when wind speeds are low. There is also evidence to suggest that cyclically varying or surging [CO\(_2\)], as occur in FACE studies, may misrepresent the response of plants to long-term constant [CO\(_2\)] with the same mean [CO\(_2\)] (Bunce, 2012). Responses to pulses of CO\(_2\) were related to both the extent of the change and the duration of CO\(_2\) enrichment (Evans and Hendrey, 1992), but Holtum and Winter (2003) found lower mean rates of net photosynthesis when [CO\(_2\)] varied compared with constantly elevated [CO\(_2\)]. In addition, environmental variables such as temperature, light and humidity cannot easily be controlled at a field scale (Kimball et al., 1997). For further details on these different systems, refer to literature review (Chapter 2).

FACE and SPAR experiments in cotton have shown that elevated [CO\(_2\)] increased biomass production, lint yield and plant water use efficiency (Ephrath et al., 2011; Mauney et al., 1994; Radin et al., 1987), increased photosynthetic rates and decreased transpiration rates (Reddy et al., 1998a; Reddy et al., 1995b). Samarakoon and Gifford (1996) found that cotton grown in the glasshouse at 700 µL L\(^{-1}\) [CO\(_2\)] used more water than plants grown at ambient [CO\(_2\)], due to a very large increase in leaf area. In contrast, Ephrath et al. (2011) found in a SPAR study that soil water use of plants grown under elevated [CO\(_2\)] was significantly lower than those grown under ambient [CO\(_2\)] for both water-stressed and well-watered plants. However, FACE experiments showed that 550 µL L\(^{-1}\) [CO\(_2\)] did not significantly affect evapotranspiration (ET) of cotton compared with plants grown at 370 µL L\(^{-1}\) [CO\(_2\)] (Dugas et al., 1994; Hunsaker et al., 1994; Kimball et al., 1994). These experiments suggest that the direct impact of elevated [CO\(_2\)] is unlikely to result in a need for increased irrigation to maintain cotton yields. However, ET may change if concomitant changes in climate occur, such as warming, higher VPD, increased frequency of heat shocks and altered rainfall distribution. It is important to understand the magnitude of the expected changes and mechanisms involved in crop response to elevated [CO\(_2\)] in order to adapt our agricultural systems and accurately model future food supply (Ainsworth and McGrath, 2010). In addition, assessments have not been undertaken on high input/high yielding
cotton systems in Australia, which may have different patterns of crop growth patterns compared to previous climate change studies into cotton.

Canopy EvapoTranspiration and Assimilation (CETA) chambers are open systems that have been used to measure canopy gas exchange of pot-grown and field-grown cotton plants in the US (Baker et al., 2014a; Baker et al., 2009). Measurements of CO\textsubscript{2} and H\textsubscript{2}O fluxes are important for understanding the impacts of the environment on crop productivity. Both single-leaf and whole-canopy gas exchange provide a highly sensitive measure of the degree of stress to which a crop is exposed. However, whole canopy net assimilation is more highly correlated with crop growth and final yield than leaf-level measurements of net-assimilation (Baker et al., 2009). CETA chambers can accurately estimate transpiration (E) across different dates and a wide range of canopy LAI (Baker et al., 2009), and may be used to generate elevated [CO\textsubscript{2}] in the canopy (Baker et al., 2014b). Internal air temperatures in some open system chambers can increase by as much as 2 to 5 °C compared with outside ambient air. CETA chambers can use a programmable data logger to control a variable speed fan, which in previous studies has limited chamber temperature to 0.5 °C above ambient air temperature, provided there was sufficient water to cool the system via latent energy exhausted out of the exit tubing (Baker et al., 2014a; Baker et al., 2014b). Reported maximum air changes for the CETA systems were 7.2 chamber volumes min\textsuperscript{-1} (Baker et al., 2014a). In addition, placing a chamber over a crop canopy often alters environmental variables, including reducing photosynthetically active radiation (PAR) by approximately 13%, which may affect canopy gas exchange (Baker et al., 2014a).

Cotton production systems in Australia are considerably different from those in many other parts of the world, including Maricopa AZ where FACE studies on field-grown cotton were conducted. Many other studies exploring the effects of climate change on cotton have not been conducted in the field (Bunce and Nasyrov, 2012; Reddy et al., 1998a; Thomas et al., 1993). The majority of Australian cotton is produced in intensive broadacre systems under furrow irrigation, and with high fertiliser inputs. In Australia during the late 1980s, the optimum N fertiliser rates averaged 145 kg ha\textsuperscript{-1} for rotation, and 189 and 210 kg ha\textsuperscript{-1} for min- and max-till, respectively (Constable et al., 1992). During this time, average cotton yields in Australia ranged from 1500 to 2600 kg lint ha\textsuperscript{-1} (Constable et al., 1992). In modern Australian irrigated cotton systems, nitrogen is applied at rates between 150 - 230 kg N ha\textsuperscript{-1} (Braunack, 2013). In comparison, approximately 130 kg N ha\textsuperscript{-1} was applied to the FACE experiments in Maricopa AZ during the early 1990s at which time average yields in Arizona were 1100 - 1200 kg lint ha\textsuperscript{-1} (Mauney et al., 1994). These differences between the systems may have implications for growth and water use of cotton.
The objective of this research was to (1) construct CETA chambers that will elevate [CO\textsubscript{2}] in the field and to evaluate the utility of the chambers for the purpose of field-based climate change studies; and (2) identify the impacts of increased atmospheric [CO\textsubscript{2}] and elevated temperature on whole canopy physiology of field-grown cotton in high-input production systems. In this study, we tested the hypotheses that (1) CETA chambers can be used to elevate [CO\textsubscript{2}] for assessing climate change effects on early growth of field-grown cotton in Australian conditions; (2) elevated [CO\textsubscript{2}] and temperature will increase whole canopy photosynthetic rate and plant biomass compared with ambient [CO\textsubscript{2}]; and (3) elevated [CO\textsubscript{2}] and temperature will increase water use, and increase plant water use efficiency (biomass/water used) of high input field-grown cotton.

6.2. Materials and methods

6.2.1. Plant materials and growing conditions

This experiment was conducted at the Australian Cotton Research Institute (ACRI), Narrabri during the 2012-13 cotton growing season. However, timing of the experiment was off-set from the typical Australian cotton season due to concerns of very high temperatures inside the CETA chambers during the hottest summer months. A comparison of radiation and temperatures over a few sample dates is shown in Appendix 14. The transgenic cotton variety Sicot 71 BRF (Stiller, 2008) was sown at 14.2 seeds m\textsuperscript{-1} over 24 m x 8 rows on 19\textsuperscript{th} February 2013, following pre-irrigation on 14\textsuperscript{th} February 2013. The plots were prepared according to current production methods and plants were well-fertilised. Plants were irrigated on 12\textsuperscript{th} April 2013 (52 DAP [days after planting]).

CETA chambers used in CO\textsubscript{2} enrichment studies were similar to chambers described by Baker et al. (2009), and modified to allow for greater control of [CO\textsubscript{2}] inside the chamber according to Baker et al. (2014b). Chambers were constructed from aluminium framework covered in transparent lexan (GE Polymershapes, Coppell, TX). The chambers were 0.75 x 1 m and 1 m in height. A variable speed squirrel cage type blower (Dayton Electric Manufacturing, Niles, IL) was connected to an aluminium duct with flexible tubing. Air was pushed through these ducts into a cone shaped entrance duct, covered with lexan. A perforated lexan sheet with 2.5 cm diameter holes separated the entrance from the main plant chamber. Air from the chamber passed through another sheet of perforated lexan before exiting the system via the top of an exit-chamber (measuring 0.2 x 0.75 x 1 m). Inlet air was measured at the entrance of the cone and exit air was sampled from the exit-chamber. Air flow was measured in the aluminium duct with a pitot tube and static ports connected to a pressure transducer (Serta Systems, Inc., Boxborough, MA).
A vacuum pump (Cole-Parmer, Vernon Hills, IL) pulled gas samples at a flow rate of 3 L min\(^{-1}\) from the entrance and exit points through gas sample lines (Nylotube-12, New Age Industries, Southhampton, PA). Entrance and exit air sample streams were measured at 10 s intervals, using a solenoid valve controlled by the datalogger to switch between the two lines. An infrared gas analyser (IRGA) (LI-COR 7000, LI-COR, Inc., Lincoln, NE) was used to measure entrance and exit CO\(_2\) and H\(_2\)O in the air sample stream. The datalogger recorded IRGA readings, air (T\(_{in}\) and T\(_{out}\)) and soil temperatures, photosynthetic flux density measured using quantum sensors (LI-COR, Inc., Lincoln, NE) for plants inside the chambers but not for control plants. Therefore, [CO\(_2\)] and soil temperatures were not recorded for the control plants, and other conditions such as air temperature, humidity and canopy temperature were monitored as detailed in Section 6.2.2.

Six aluminium bases (110.5 cm x 85.5 cm x 25.0 cm) were inserted approximately 5 cm into the ground. CETA chambers were set on top of four of the six bases and the remaining two were reference plots without either chambers or elevated [CO\(_2\)] (C\(_{ct}\) treatment). One cm thick weather-strip foam was glued to the bottom of the chamber frame, and held to the base with small C-clamps to seal the chambers. Chambers were set up over the plants on 3\(^{rd}\) April 2013 (43 DAP). On that date, plants were 17 ± 0.3 cm in height with 6 ± 0.096 nodes (mean ± SE). CO\(_2\) was injected into chambers from 4\(^{th}\) April 2013 (44 DAP) until the 1\(^{st}\) May 2013 (71 DAP).

Each chamber was connected to a datalogger CR-3000 (Campbell Scientific Inc., Logan, UT) and infrared gas analyser (LI-COR 7000, LI-COR, Inc., Lincoln, NE). Two chambers were designated as ambient [CO\(_2\)], with no additional changes to atmospheric [CO\(_2\)] (C\(_{a}\) treatment). CO\(_2\) cylinders were connected to two of the chambers which were designated as elevated [CO\(_2\)] and maintained at 650 µL L\(^{-1}\) [CO\(_2\)] (C\(_{e}\) treatment). CO\(_2\) injected into the chamber was regulated by an Omega flow controller (OMEGA Engineering, Stamford, CT), based on incoming gas sample measurements. A 5-stage feedback control algorithm was used by the datalogger to make adjustments to the CO\(_2\) injection flow rate at 20 s intervals based on measured chamber inlet [CO\(_2\)] (Baker et al., 2014b). The CO\(_2\) line was connected to the fan to mix CO\(_2\) with the air and distribute it throughout the chamber.

Equipment for operation of the chambers was housed in a small shed located in the centre of the experiment. Due to limitations in the length of cables, chambers were positioned on both sides of the shed, with one C\(_{c}\), C\(_{a}\), C\(_{e}\) plot on the northern side and one C\(_{c}\), C\(_{a}\) and C\(_{e}\) on the southern side (Figure 6.1). Therefore, overall treatment arrangement was: two ambient [CO\(_2\)] chambers (C\(_{a}\)), two elevated [CO\(_2\)] chambers and 2 control areas (C\(_{c}\)). There were 2 - 3 buffer rows between chambers.
Figure 6.1: CETA chambers used to generate CO₂-treatments in the field during 2013 at ACRI Narrabri.

6.2.2. Environmental monitoring

Soil moisture was monitored in each of the chambers and in one control plot. Green-light-red-light (GLRL) (Odyssey Dataflow Systems, Christchurch, NZ) capacitance sensors were installed in the middle of the cotton row within each chamber (only 1 GLRL sensor for C₃ treatment) to measure soil water content (SWC) at the following depths: 10, 30, 50, 70 and 90 cm. Soil water measurements were logged every 3 hours. Volumetric soil water content (VSWC %) was calculated using the formula, which has been calibrated for soils at ACRI (Tony Nadelko, pers comm.): -76.525x³ + 223.89x² – 218.51x + 71.399, where x is the calibrated sensor reading (raw value divided by sensor calibration constant) at each depth.

Soil water content was averaged at each depth for each treatment. Plants were irrigated at 52 DAP (12th April 2013) by filling each of the bases with the same amount of water. △SW1 was calculated as the change in VSWC between 43 and 51 DAP and △SW2 was calculated as the change in VSWC between 53 and 73 DAP.
Air temperature (°C) and relative humidity (RH %) were not controlled, but were measured inside the CETA chamber using Tiny Tag Ultra (Gemini Data Loggers, West Sussex, UK) sensors, which were positioned at the top of the canopy (only 1 for Cc treatment). These sensors were not housed in a miniature Stephenson screen and were repositioned as the canopy grew. Air temperature was also monitored using thermocouples at the air entry and exit ports of the chamber (CA and CE), and logged by the CR-3000 datalogger (CR-3000, Campbell Scientific, Inc., Logan, UT). Soil temperature was measured in the CETA chambers using a thermocouple buried 5 cm beneath the soil surface; soil temperature was not measured for Cc plots. Daily temperature, RH and VPDa were defined as those between 8 am and 6 pm (Australian Eastern Daylight Time; AEDT). VPDa was calculated from the Tiny Tag data using the following equations:

\[ VPD_a = es - ea \]

Where:

\[ es = 0.6108 \times e^{\left(\frac{17.27 \times Ta}{Ta + 237.3}\right)} \]

\[ ea = \left(\frac{RH}{100}\right) \times es \]

where Ta is air temperature in °C and RH is relative humidity in % (Conaty et al., 2014; Ham, 2005).

Apogee infrared thermometers (IRT) (Apogee IRT, model SI-121, Apogee Instruments Inc., Logan, UT) were used to monitor canopy temperature inside the chambers (CA and CE treatments). Wireless, battery-operated “SmartCrop™” IRT (Smartfield Inc., Lubbock, TX, USA) sensors were used to monitor canopy temperature of control plots outside the chambers (Cc treatments). Sensors were positioned 20 to 30 cm above the crop canopy, and repositioned as the crop grew to maintain this distance. As described by Conaty et al. (2012), sensors were positioned to point south thereby reducing specular reflectance, and at an angle to the vertical of 70°, which resulted in an approximate field of view of 0.5 m² thus ensuring there was no interference from exposed soil. Daily canopy temperatures were defined as those between 8 am and 6 pm (AEDT).

6.2.3. Leaf gas exchange measurements

The photosynthetic (A) response to internal [CO₂] (Ci) curves (ACi) were measured using automated programmes for the Licor 6400 open photosynthesis system. ACi curves were obtained by changing the CO₂ concentration entering the leaf cuvette in steps under a constant, saturating PAR of 2000 µmol m⁻² s⁻¹ and a cuvette temperature of 26 °C. The steps for CA and CE treatments were 400, 380,
200, 150, 75, 40, 0, 50, 100, 200, 380, 400, 650, 900, 1300, 1700, 2000 µmol mol\(^{-1}\), and the C\(_t\) treatment followed the same steps starting at 650 µmol mol\(^{-1}\). The leaves were given at least two minutes to equilibrate to each new condition before the auto-programme recorded the measurement after stability was reached. The Farquhar-type C\(_3\) photosynthetic model as described by Sharkey et al. (2007) was used to derive maximal carboxylation rate \(V_{\text{cmax}}\) and maximal photosynthetic electron transport rate \(J_{\text{max}}\) using non-linear curve fitting which minimises the differences between observed and predicted photosynthesis. Final ACi curves were measured on the 30\(^{th}\) April and 1\(^{st}\) May 2013 (70 - 71 DAP). Leaf gas exchange measurements were taken on two to three recently mature leaves (3\(^{rd}\) leaf from the top of the plants) in each replicate between 9 am – 3 pm (AEDT), with measurements for each treatment randomised throughout the day to take into account the time of day. The chambers were removed between 9 am – 3 pm on each of these days in order to take these measurements. Temperature and relative humidity were recorded by the Tiny Tag sensors during this time; however, removal of the chambers resulted in all plants being exposed to natural atmospheric [CO\(_2\)] which was not recorded by the dataloggers.

### 6.2.4. Canopy gas exchange calculations

The CETA chambers were designed to simulate the cuvette of the LICOR 6400. Canopy-level photosynthesis and transpiration calculations were derived from equations in the LICOR 6400 manual (LI-COR, 2011) and Baker et al. (2009). These calculations did not account for soil respiration.

### 6.2.5. Plant growth measurements

Following removal of the chambers on the 2\(^{nd}\) May 2013 (72 DAP), plants from each of the three treatments were harvested and processed for biomass. Plants from each treatment had reached squaring (mean number of squares ± SE: C\(_C\) = 5.0 ± 0.5; C\(_A\) = 5.5 ± 0.7; C\(_E\) = 9.8 ± 1.0) and plants inside the chambers had just reached flowering (mean number of flowers and green bolls ± SE: C\(_C\) = 0.0 ± 0.0; C\(_A\) = 0.04 ± 0.04; C\(_E\) = 0.233 ± 0.126). Each plant was processed individually for height, nodes, stem, leaf, square and boll biomass, and for leaf area. Samples were dried at 80 °C using a forced-air oven for 7 days and weighed.

### 6.2.6. Statistical analysis

Data was analysed by residual maximum likelihood (REML) using Genstat version 16. REML analysis was used to test for significance of adding CO\(_2\) treatment (comparing C\(_A\) and C\(_E\)) to the model for biomass and harvest data at 72 DAP. These analyses were performed due to an uneven number of replicate plants inside each chamber. Changes in volumetric soil water content (VSWC) prior to
irrigation, post irrigation and total change in VSWC were also analysed by REML using Genstat version 16. In addition, the total change in VSWC for each treatment was analysed by regression analysis using SigmaPlot version 12. A two-tailed t-test was used to test the difference in slope from 1.0. Acceptance of the null hypothesis where $\beta = 1$, indicates that there is no bias towards one treatment using more water than another across the whole data set. SAS version 9.3 was used to calculate $A$ and ET and to obtain data summaries of each chamber. R version 3.1.0 was used to fit curves to ACi data and generate $V_{cmax}$ and $J_{max}$ coefficients. Genstat version 16 was then used to analyse the coefficients by REML. Where necessary, data was transformed to meet analysis assumptions. Data were assessed at a $P=0.05$ level of significance.

6.3. Results

6.3.1. Chamber environment

The environment inside the chambers ($C_A$ and $C_E$) varied with external field conditions. Daily air temperature was on average 4 °C warmer inside the chamber than outside and at times was higher in $C_E$ than $C_A$ (Figure 6.2a). Mean daily relative humidity was on average $7.5 \pm 0.77$ % drier inside the chambers than outside (Figure 6.2b). On average, daily VPDa was 1.6 kPa higher inside the chambers compared with the $C_c$ treatment (Figure 6.3). Average hourly fluctuations for one sample day are shown in Appendix 15. Mean daily [CO$_2$] inside the $C_A$ chambers was consistent over the experimental period, averaging $387 \pm 0.8$ µL [CO$_2$] L$^{-1}$ (Figure 6.2c). Mean daily [CO$_2$] inside the $C_E$ chambers was more variable averaging $626 \pm 6.8$ µL [CO$_2$] L$^{-1}$, but consistently at least 200 µL L$^{-1}$ higher than $C_A$ chambers. Changes in [CO$_2$] each minute averaged between -0.009 and -0.005 µL L$^{-1}$ for $C_A$ treatments and between -0.001 and -0.002 µL L$^{-1}$ for $C_E$ treatment for two sample dates (Appendix 16), indicating that overall [CO$_2$] inside the chambers was relatively stable (Appendix 17 and Appendix 18). Mean daily canopy temperatures (Figure 6.4a) were similar in the $C_A$ and $C_E$ treatments and rarely exceeded 30 °C. Mean daily canopy temperatures were $27 \pm 0.3$ °C inside the chambers and $28 \pm 0.5$ °C in the $C_c$ treatment. Mean daily soil temperature was $25 \pm 0.3$ °C and $24 \pm 0.5$ °C for $C_A$ and $C_E$ chambers, respectively (Figure 6.4b).
Figure 6.2: Average daily (a) air temperature, (b) relative humidity and (c) \([\text{CO}_2]\) from 8 am – 6 pm (AEDT) for ambient \(\text{CO}_2\) (\(C_A\), circle), elevated \(\text{CO}_2\) (\(C_E\), triangle) and control (\(C_C\), square) for 43 - 72 DAP. Values represent mean ± SE of two chambers (sample size of one in the control treatment). Target \([\text{CO}_2]\) was 650 µL L\(^{-1}\), data range between 300 - 800 µL L\(^{-1}\) with a gap in data at 54 DAP due to malfunction of dataloggers (panel c). Average daily \([\text{CO}_2]\) for \(C_C\) was not monitored.
Figure 6.3: Average daily VPDs from 8am - 6pm (AEDT) for ambient CO₂ (CA, circle), elevated CO₂ (CE, triangle) and control (CC, square) for 43 - 72 DAP. Values represent mean ± SE (sample size of one in the control treatment).
Figure 6.4: Average daily (a) canopy temperature (°C) and (b) soil temperature (°C) between 8 am and 6 pm (AEDT) for ambient CO₂ (Cₐ: white circle), elevated CO₂ (Cₑ: black triangle) and no chamber (Cₖ: white square) for 43 - 72 DAP. Values represent mean ± SE. Gap in data at 54 DAP due to malfunction of dataloggers. Cₖ data not available for soil temperature.

6.3.2. Soil water content and water use

The change in volumetric soil water content (VSWC %) throughout the profile was calculated to compare plant water use between each treatment (Cₖ, Cₐ and Cₑ) before (43 - 51 DAP; Figure 6.5a) and after (53 - 73 DAP; Figure 6.5b) irrigation, which occurred at 52 DAP. Using REML, there was no significant difference in any of the comparisons between treatments or depths. Using a t-test, our data showed that the slope of each treatment comparison is equal to 1, and therefore there is no bias of
any treatment using more water than another. This suggests that overall there was no difference in plant-level water use between C_c and C_A (Figure 6.6a), C_c and C_e (Figure 6.6b), and C_A and C_e treatments (Figure 6.6c).

Figure 6.5: Change in volumetric soil water content (VSWC %) to a depth of 90 cm for (a) 43 - 51 DAP and (b) 53 - 73 DAP for C_c, C_A and C_e treatments measured using green-light-red-light (GLRL) sensors. Values represent mean. Horizontal bars for C_A and C_e treatments represent SE of two GLRL sensors (sample size of one in the control treatment). Plants were irrigated at 52 DAP. There were no significant differences between treatments across depths for either of the time periods.
Figure 6.6: Change in the sum of volumetric soil water content (VSWC) between (a) Cc and CA (Adj R² = 0.712); (b) Cc and CE (Adj R² = 0.704; and (c) CA and CE (Adj R² = 0.922). Data are for individual chambers. T-tests showed that the slopes of each were not significantly different from 1.0. Also shown are the 1:1 lines (dashed).
6.3.3. Plant growth and biomass

C_E increased leaf (51%), stem (86%) and total vegetative (67%) biomass compared with the C_A treatment (Figure 6.7a-c; Table 6.1; P< 0.05). C_E increased total fruit biomass by 59% compared with C_A (Figure 6.7d; P= 0.025). Cotton grown at C_E were 17% taller (Figure 6.8a; P= 0.001), had 51% greater leaf area (Figure 6.8c; P= 0.001) than plants grown at C_A, and had a 9% greater number of nodes (Figure 6.8b; P= 0.025). Despite warmer air temperatures inside the chambers, there was no significant difference in biomass or the number of nodes between the C_E and C_A treatments; however, C_A increased height by 30% and leaf area by 15% compared with C_E.

Table 6.1: Statistical analyses for plant biomass, harvest and water count data. REML analysis was used to test for differences between C_A and C_E chamber treatments for biomass and harvest at 72 DAP. * represents significance at P< 0.05, ** represents significance at P< 0.01 and *** represents significance at P< 0.001. Values in bold represent significance at P< 0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plant biomass</strong></td>
<td></td>
</tr>
<tr>
<td>Leaf (g plant(^{-1}))</td>
<td>0.001***</td>
</tr>
<tr>
<td>Stems (g plant(^{-1}))</td>
<td>0.001***</td>
</tr>
<tr>
<td>Total vegetative biomass (g plant(^{-1}))</td>
<td>0.001***</td>
</tr>
<tr>
<td>Total fruit biomass (g plant(^{-1}))</td>
<td>0.025*</td>
</tr>
<tr>
<td><strong>Physiology</strong></td>
<td></td>
</tr>
<tr>
<td>(A_{sat})</td>
<td>0.007***</td>
</tr>
<tr>
<td>(g_{s-sat})</td>
<td>0.038*</td>
</tr>
<tr>
<td>(E)</td>
<td>0.027*</td>
</tr>
<tr>
<td>(A_{sat}/g_{s-sat})</td>
<td>0.001*</td>
</tr>
<tr>
<td>(A_{max})</td>
<td>0.774</td>
</tr>
<tr>
<td>(g_{s-max})</td>
<td>0.540</td>
</tr>
<tr>
<td>(V_{cmax})</td>
<td>0.115</td>
</tr>
<tr>
<td>(J_{max})</td>
<td>0.263</td>
</tr>
<tr>
<td>(J_{max}/V_{cmax})</td>
<td>0.795</td>
</tr>
<tr>
<td><strong>Harvest</strong></td>
<td></td>
</tr>
<tr>
<td>Height (cm plant(^{-1}))</td>
<td>0.001***</td>
</tr>
<tr>
<td>Leaf Area (cm(^2) plant(^{-1}))</td>
<td>0.001***</td>
</tr>
<tr>
<td>Nodes (plant(^{-1}))</td>
<td>0.025*</td>
</tr>
</tbody>
</table>
Figure 6.7: Effect of ambient CO₂ (CA), and elevated CO₂ (CE) on (a) leaf, (b) stem, (c) total vegetative and (d) total fruit dry biomass production (g plant⁻¹) of cotton at 72 DAP. Values represent the mean of plants in two chambers. Refer to Table 6.1 for significant differences.
Figure 6.8: Final (a) plant height (cm plant⁻¹) (b) number of nodes (plant⁻¹) and (c) leaf area (cm² plant⁻¹) of cotton grown at ambient CO₂ (Cₐ) and elevated CO₂ (Cₑ). Values represent the mean of plants in two chambers at 72 DAP. Refer to Table 6.1 for significant differences.
6.3.4. Leaf and canopy physiology

$C_\varepsilon$ increased leaf-level $A_{\text{sat}}$ by 20% and $A_{\text{sat}}/g_{\text{s-sat}}$ by 63% (Figure 6.9; Table 6.1; $P<0.05$). Leaf-level $g_{\text{s-sat}}$ was reduced by 25% and $E$ was reduced by 18% with $C_\varepsilon$ (Figure 6.9; Table 6.1; $P<0.05$). $C_\varepsilon$ did not affect leaf-level $A_{\max}$ or $g_{\text{s-max}}$ compared with $C_A$ (Figure 6.10). $C_\varepsilon$ did not affect $V_{\text{cmax}}, J_{\max}$ or $J_{\max}/V_{\text{cmax}}$ (Figure 6.11; Table 6.1). There was no significant difference in leaf-level physiology measurements between $C_\varepsilon$ and $C_A$, with the exception of a 14% reduction in $E$ and an 18% increase in $A_{\text{sat}}/g_{\text{s-sat}}$ with $C_A$.

Whole canopy photosynthesis and transpiration of plants grown at $C_\varepsilon$ at two representative dates, at the beginning and end of the experiment, are shown in Appendix 19. Whole canopy gas exchange data for plants grown at $C_A$ has been excluded due to equipment malfunction.
Figure 6.9: Effect of ambient CO$_2$ ($C_A$) and elevated CO$_2$ ($C_E$) on cotton (a) photosynthesis, $A_{sat}$; (b) stomatal conductance, $g_{s-sat}$; (c) transpiration, $E$; and (d) photosynthetic efficiency, $A_{sat}/g_{s-sat}$. Measurements were made at 70 and 71 DAP between 9 am and 3 pm (AEDT). Values represent the mean of 11 plants. Refer to Table 6.1 for significant differences.
Figure 6.10: (a) $A_{\text{max}}$ and (b) $g_{\text{s-max}}$ of cotton grown with ambient CO$_2$ ($C_A$) and elevated CO$_2$ ($C_E$). Measurements were made at 70 and 71 DAP between 9 am and 3 pm (AEDT). Values represent the mean of 11 plants. Refer to Table 6.1 for significant differences.
Figure 6.11: Final (a) $V_{cmax}$, (b) $J_{max}$ and (c) $J_{max}/V_{cmax}$ of cotton grown with ambient CO$_2$ (C$_A$) and elevated CO$_2$ (C$_E$). Measurements were made at 70 and 71 DAP between 9 am and 3 pm (AEDT). Values represent the mean of 11 plants. Refer to Table 6.1 for significant differences.
6.4. Discussion

CETA chambers elevated atmospheric [CO$_2$] and generated warmer temperatures in the field thereby supporting the first hypothesis that CETA chambers are useful for the purpose of climate change research on crop species. Our data showed that biomass of cotton was increased inside chambers with C$_E$ compared with C$_A$ thereby partially supporting the second hypothesis; however whole canopy photosynthesis data would have strengthened the understanding of these changes, had it been available. Our data showed that C$_E$ increased plant biomass without noticeable changes in volumetric soil water content; however, again understanding of plant water use and water use efficiency would have been improved with whole canopy measurements.

6.4.1. CETA chambers as a method for elevating [CO$_2$] in the field

CETA chambers were successfully used to elevate atmospheric [CO$_2$] of field-grown cotton. Advantages of these systems are that they do not require as much CO$_2$ as larger-scale FACE experiments and that plants can be grown in the field, thereby eliminating effects associated with plants grown in pots and better capture crop and canopy effects. In addition, data from the CETA chambers indicate that fluctuations in [CO$_2$] were minimal. The greatest difference in [CO$_2$] was 22.0 µL L$^{-1}$ min$^{-1}$, compared with FACE systems where [CO$_2$] can fluctuate by more than 100 µL L$^{-1}$ within one minute (Bunce, 2011; Bunce, 2012). CETA chambers are a suitable method for studying the response of field grown cotton to some aspects of projected climatic changes, as climate change projections in Australia are for elevated atmospheric [CO$_2$], warmer temperatures and lower air relative humidity (CSIRO and Bureau of Meteorology, 2014; Whetton and Power, 2007).

However, some of the limitations of these chambers include warmer air temperatures and substantially lower relative humidity, and thus higher VPD$_a$ relative to ambient conditions. For these reasons, comparisons can only be made between the C$_A$ and C$_E$ chamber treatments, and hence are the only treatments discussed. As temperature and humidity conditions are similar throughout all four chambers, the effect of elevated [CO$_2$] can be assessed by comparing C$_A$ and C$_E$ grown cotton plants. Without the capacity for cooling, controlled temperature treatments are difficult to study using CETA chambers, and hence care must be taken in comparisons between C$_E$ and chamber treatments. Average daily temperature inside the chamber was around 4 °C warmer than average daily temperature recorded outside the chamber. The greatest differences between C$_E$ and chamber air temperatures occurred in the first couple of days after applying the chamber treatments and in the couple of days prior to irrigation. This suggests that the initial plant response, such as transpiration, to the altered environment may be different from how cotton would respond over longer periods of
exposure. Similarly, warmer air temperatures in the chambers prior to irrigation at 52 DAP may be due to decreased transpiration in response to water deficits, but would need to be tested using whole canopy gas exchange measurements. Warmer temperatures generally increased thermal time (day degrees) for cotton inside the chamber which consequently increased rate of development, and can potentially lead to higher photosynthetic rates (Reddy et al., 1995b; Reddy et al., 1991b) due to greater leaf area; however, these changes in development and physiology were not observed between Cc and Ca treatments (Appendix 20). While air temperature differences were measured, these generally did not translate to measured differences in growth and physiology between Cc and Ca treatments at the end of the experiment, with the exception of increased plant height (30%), increased A_sat/g_sat (18%) and reduced leaf-level transpiration (16%) (Appendix 20). The effects of warmer temperatures in the chambers may have been exacerbated if the experiment had been conducted over the hottest summer months.

The chambers allow for the study of multiple effects of climate change such as warmer temperatures and altered humidity and VPDₐ, whereas some FACE facilities are limited in this respect. However, as temperature has many effects on plant biochemistry and physiology in addition to altering Cₐ effects, it is currently not possible to differentiate temperature and VPDₐ effects by using CETA chambers. Modifications to the chamber systems could potentially be made to humidify the incoming air and thus reduce the VPDₐ differences between the chamber and non-chamber environments, although it is likely that temperature and VPDₐ effects may still be a limitation of CETA systems. Reddy et al. (1998a) showed that leaf photosynthesis in cotton benefited more from elevated [CO₂] at warm temperatures than at low growth temperatures. Although our study did not compare the effect of elevated [CO₂] at different temperatures, our data indicate that the environment inside the chambers did not reach temperatures that caused reductions in growth and development of cotton (Reddy et al., 1992a; Reddy et al., 1991b). However, again very high temperatures may have been a limitation if the experiment had been conducted during the hottest summer months.

Another possible constraint of the CETA chambers is the physical limitation in handling large plants. For our study of early growth and physiology, this was not a problem as plants did not outgrow the chambers; however, in longer-term studies taking plants to maturity, or in studies of taller varieties of cotton, CETA chambers must be modified to allow for taller plants. However, despite limitations of the CETA chambers, they provide valuable comparisons of some important responses of field-grown cotton exposed to elevated atmospheric [CO₂] in projected environmental conditions.
6.4.2. Effect of C\textsubscript{E} on field-grown cotton

Our data showed that C\textsubscript{E} increased biomass of well-watered, field-grown cotton. Pot and field experiments have shown that C\textsubscript{E} increased biomass and photosynthesis, as well as reducing transpiration of cotton (Mauney et al., 1994; Reddy et al., 1995d). Similarly, our data also showed that C\textsubscript{E} increased leaf-level $A_{\text{sat}}$, and reduced both leaf-level $g_{s\text{-sat}}$ and $E$. These changes resulted in an increase in $A_{\text{sat}}/g_{s\text{-sat}}$, indicating that C\textsubscript{E} may have physiological benefits for field-grown cotton. However, our data showed that there were no change in leaf-level $V_{\text{cmax}}$, $J_{\text{max}}$ or $J_{\text{max}}/V_{\text{cmax}}$ as would normally be expected after long-term exposure to C\textsubscript{E} environments (Ainsworth and Rogers, 2007; Bernacchi et al., 2005; Singh et al., 2013a). Other experiments using a variety of plants have shown that C\textsubscript{E} generally reduces $V_{\text{cmax}}$ and increases $J_{\text{max}}$, indicating a shift away from Rubisco and towards electron transport (Ainsworth and Rogers, 2007; Harley et al., 1992). For cotton grown in the glasshouse (Chapter 3), C\textsubscript{E} increased $J_{\text{max}}$ and $J_{\text{max}}/V_{\text{cmax}}$, although there were no significant differences in $V_{\text{cmax}}$. These differences may partially be due to the combination of warmer temperatures and altered VPD\textsubscript{i} inside the chambers maintaining biochemistry and enzyme activity compared with these other studies. Warmer temperatures have also been shown to reduce $V_{\text{cmax}}$ and increase $J_{\text{max}}/V_{\text{cmax}}$ of cotton grown at constant temperature cycles in the glasshouse (Chapter 3). Alternatively, the lack of differences in biochemistry may be due to plants being in equivalent environments during the period of gas exchange measurements, given that all the chambers had to be removed for measurements. However, this would assume that the plants equilibrate to the altered environmental conditions very quickly.

Our data showed that C\textsubscript{E} increased total vegetative biomass by 67%, whereas the increase in biomass due to elevated [CO\textsubscript{2}] was 37% in FACE experiments (Mauney et al., 1994). Although this may be partly explained by the difference between absolute and relative chamber effects (Kimball et al., 1997) or reduced plant response to fluctuations of [CO\textsubscript{2}] in FACE experiments (Bunce, 2012), the higher levels of stimulation may also be due to increased temperature, which is also projected for future climates but provides some confounding effects in terms of analyses. In addition, Mauney et al. (1994) increased [CO\textsubscript{2}] to 550 $\mu$L L\textsuperscript{-1} for 144 days, whereas our study elevated [CO\textsubscript{2}] to 626 $\mu$L L\textsuperscript{-1} for 28 days. Therefore, early growth of cotton may be stimulated by C\textsubscript{E}, but differences between plants exposed to C\textsubscript{A} and C\textsubscript{E} may become reduced with time as overall growth slows. Other possible reasons for differences in the magnitude of the biomass response to C\textsubscript{E} could be differences in nutritional inputs (Singh et al., 2013b) or soil type, although, these concepts should be explored further.

The analysis of changes in measurements of soil capacitance showed that there were no differences in soil water use among treatments during the experimental period. Similarly, a number of FACE
studies have shown that elevated [CO\textsubscript{2}] to 550 µL L\textsuperscript{-1} did not have any effect on ET of well-watered, well-fertilised cotton (Dugas et al., 1994; Hunsaker et al., 1994; Kimball et al., 1994). This varies from glasshouse studies, where plants grown at 700 µL L\textsuperscript{-1} [CO\textsubscript{2}] used more water than cotton grown at ambient conditions due to a very large leaf area response (Samarakoon and Gifford, 1995). Our data also showed a 51% increase in leaf area. However, Mauney et al. (1994) reported an increase in leaf area index (LAI) of cotton with elevated [CO\textsubscript{2}] during one period of early season growth, but thereafter found no consistent differences in LAI attributable to the FACE environment. Therefore, as this experiment was conducted on early season cotton plants, it is possible that later canopy development may negate early season differences. Despite an increase in leaf area, our data suggested there was no significant difference in water extraction. Increased biomass without significant changes in water consumption indicates that plant water use efficiency was increased, as has been suggested by other studies (Hileman et al., 1994; Hunsaker et al., 1994). However, this study was conducted only on early season cotton growth and therefore did not account for later season canopy development or the distribution of resources during the reproductive phase. In addition, glasshouse studies (Chapter 4) have indicated that C\textsubscript{E} increased plant water use in early-stage growth of cotton. Therefore, we did not obtain a definitive answer to the integrated effects of C\textsubscript{E} on plant water use, but this study indicates that further studies should be conducted to explore the integrated environmental effects of climate change on field-grown cotton in Australian production systems. In addition, data from this study and other studies can be used to inform crop simulation models to account for the possible impacts of climate change on crop production and therefore shape management decisions for cotton production in future environments.

6.4.3. Conclusions

This study investigated the impacts of increased atmospheric [CO\textsubscript{2}] on whole canopy physiology of field-grown cotton in high-input production systems. CETA chambers were a successful method of increasing atmospheric [CO\textsubscript{2}] of field-grown cotton, despite limitations with increased temperature and altered humidity and VPD\textsubscript{a} that limit comparisons between ambient field conditions and chamber experiments. C\textsubscript{E} increased early stage biomass of well-watered, field-grown cotton. Although there were some changes in leaf-level physiology measurements, our data indicate that there were no large changes in leaf-level biochemistry. In this study, we did not obtain a definitive answer to the integrated effects of C\textsubscript{E} on plant water use. Therefore, due to these conflicting findings particularly around water use, this study indicates that further studies should be conducted to explore the integrated environmental effects of climate change in field-grown cotton in Australian production systems.
Chapter 7: General discussion

Current climate models predict that Australia will have more heatwaves, changes in rainfall distribution, an increase in the intensity of droughts, and small decreases in relative humidity (CSIRO and Bureau of Meteorology, 2014; Whetton and Power, 2007). Changes in CO₂, temperature, precipitation and consequently atmospheric VPD under these scenarios of climate change present a challenge to crop production, and may have significant impacts on the physiology and yield of cotton, and therefore on the Australian cotton industry.

Understanding the implications of varied environmental conditions for agricultural crops is critical for developing cropping systems resilient to stresses induced by climate change (Ainsworth and McGrath, 2010; Stokes and Howden, 2010). The Australian cotton industry is characterised by high input management and highly mechanised farming operations (Hearn and Fitt, 1992). The majority of Australian cotton is grown using furrow-irrigation and Australian bred cotton cultivars adapted to Australian climate and soil conditions (Liu et al., 2013; Roth et al., 2013). In order to maintain high fibre quality and high yielding cotton production in the projected future Australian environment, it is necessary to develop a greater understanding of the physiological and growth response of cotton to these projected changes in climate. Although projected rainfall trends are difficult to ascertain due to high variability, water may become limiting across south-eastern Australia (CSIRO and Bureau of Meteorology, 2014; IPCC, 2014) and consequently there may be reduced water available to cotton growers. Using a combination of glasshouse and field-based studies, this thesis investigated the integrated effects of projected climatic change (elevated [CO₂], warmer temperature, altered VPD and soil water deficit) on the physiology, growth and water use of cotton in high-yielding, high-input cotton systems in Australia.

7.1.1. Genotypic differences in response to climate change

It is possible that current cotton varieties may have an advantage over older varieties in future elevated [CO₂] and temperature environments (Chapter 3). Although my study showed there were no genotypic differences in growth responses to warmer temperature and elevated [CO₂], the modern variety Sicot 71BRF had consistently lower vegetative biomass compared with the older variety, DP16. This study also showed that there were genotypic differences in physiological responses to warmer temperature and elevated [CO₂], where photosynthesis of both genotypes responded positively to Cₚ, but Aₚₚ was consistently higher for the current variety (Sicot 71BRF) than the older variety (DP16). Greater photosynthetic capacity of Sicot 71BRF may increase photosynthetic efficiency and may indicate that the newer variety is physiologically more responsive to changes in atmospheric [CO₂].
than the older cultivar DP16. However, in this pot experiment these interactive effects were not translated into increased growth responses.

Stomatal conductance of the two genotypes responded differently to warmer temperature. $T_e$ increased $g_{\text{sat}}$ of 71BRF by 23%, but $g_{\text{sat}}$ of DP16 did not respond to $T_e$. This suggests that Sicot 71BRF may transpire and cool the leaf surface more efficiently than the older variety, contributing to improved heat dissipation. However, increased stomatal conductance of Sicot 71BRF may also contribute to greater leaf-level water use, although the smaller total leaf area of Sicot 71BRF relative to DP16 may reduce water use at a plant level.

Given that there were some physiological differences between the two cultivars tested in this study, genotypic variations may also partially explain differences in the magnitude of cotton response in FACE and SPAR experiments (Hendrix et al., 1994; Hunsaker et al., 1994; Inoue et al., 1990; Mauney et al., 1994; Reddy et al., 1995d) that have investigated the effects of elevated $[\text{CO}_2]$ on cotton growth and development, as different cotton varieties were used in these studies. Breeding of Australian cotton varieties may have inadvertently selected plants better suited for rising atmospheric $[\text{CO}_2]$ and warmer temperatures compared with older varieties, despite studies in wheat (Ziska, 2008; Ziska et al., 2004), and oat (Ziska and Blumenthal, 2007) that have shown that newer lines were less responsive than older lines to rising $[\text{CO}_2]$. However, wheat and oats are determinate monocots and thus different growth habit and distribution of resources (such as to roots and secondary growth) may contribute to different growth responses between the species. In addition, given that this is based on the single comparison of one old and new cultivar, grown in pots in a controlled environment, further exploration of these interactions are necessary to assess the impact breeding has had on genotype suitability for future environments. However, genotypic differences to projected climatic conditions should be considered in comparisons of growth and development responses between studies.

7.1.2. Physiological responses of cotton to the environment

The studies within this thesis suggest that the integrated effects of climate change are likely to impact cotton physiology, growth and water use. Plants respond to their physical environment, including VPD. For both $g_{\text{sat}}$ and photosynthesis, VPD$_l$ accounts for a large proportion of the variation, with generally smaller percentages attributed to other factors such as individual plant, $T_r-T_a$, ASH, and VPD$_l \times T_r-T_a$, Plant $\times T_r-T_a$ and VPD$_l \times$ ASH interactions. In cotton, stomatal conductance is very responsive to altered VPD (Duursma et al., 2013), and Chapter 5 demonstrated that VPD$_l$ accounted for 67 - 70% of the variation in stomatal conductance for field-grown cotton. By including plant, $T_r-T_a$, ASH and Plant $\times T_r-T_a$ interactions, total variability in $g_{\text{sat}}$ that could be accounted for was approximately 69%. In
comparison, VPD\textsubscript{L} accounted for 42 - 56% of the variation in photosynthetic rates. Therefore, for both \(g_{s\text{-sat}}\) and photosynthesis there was still a large proportion of variation not accounted for, indicating that part of the variation was due to something that we did not measure or analyse. However, given the physiological responses of cotton to VPD\textsubscript{L}, VPD should be considered in all future climate change studies, and should also be a consideration for developing methods of elevating [CO\textsubscript{2}] in field-based studies. For instance, FACE experiments do not alter ambient VPD conditions, but projected climatic scenarios indicate that VPD may be altered with warmer temperatures and changes in rainfall patterns. Therefore, systems such as the CETA chambers (Chapter 6) may be beneficial in studying these altered VPD environments, but consequently add layer of complexity and restrict comparisons between external and chamber environments.

### 7.1.3. Effects of elevated [CO\textsubscript{2}] on cotton physiology and growth

Elevated [CO\textsubscript{2}] increases photosynthetic rates, decreases stomatal conductance and increases biomass production (Idso et al., 1994; Mauney et al., 1994; Reddy et al., 1995d). Similarly, my data indicated that \(C_E\) impacts cotton growth, physiology and water use, although the magnitude of the response is largely dependent on air temperature and water availability, as well as cultivar to a lesser extent. The magnitude of the effect of elevated [CO\textsubscript{2}] on \(A_{\text{sat}}\) of cotton was variable. In the glasshouse studies, \(C_E\) increased \(A_{\text{sat}}\) of 71BRF by 43% in the variety experiment (Chapter 3), and increased \(A_{\text{sat}}\) by 19% in the drought experiment (Chapter 4). In well-watered field-grown cotton plants, leaf-level \(A_{\text{sat}}\) was increased by 20% (Chapter 6). Therefore, the increase in leaf-level \(A_{\text{sat}}\) with \(C_E\) is also likely to depend on the combination of other environmental conditions.

\(C_E\) also increased the biomass and leaf area of field-grown cotton. Given that dry matter production and leaf area are the product of leaf photosynthesis, it is possible that canopy level photosynthesis was enhanced by higher leaf area as well as by higher leaf-level photosynthetic rates. Other studies have also shown \(C_E\) to increase photosynthesis (Reddy et al., 1995d; Zhao et al., 2004). Both glasshouse and field studies showed \(C_E\) did not affect \(V_{\text{cmax}}\), and \(C_E\) also did not affect \(J_{\text{max}}\) or \(V_{\text{cmax}}/J_{\text{max}}\) ratio of field-grown cotton. Similarly Zhao et al. (2004) showed there were no differences in \(V_{\text{cmax}}\) or \(J_{\text{max}}\) for cotton grown at 360 and 720 \(\mu\text{L} \text{ L}^{-1} [\text{CO}_2]\), using SPAR facilities. Therefore, using a range of different methods to elevate [CO\textsubscript{2}], there have been no changes to \(V_{\text{cmax}}\) or \(J_{\text{max}}\) with \(C_E\) suggesting that there has not been any acclimation to \(C_E\) in these experiments.

\(C_E\) increased leaf- and plant-level WUE at \(T_a\), but at \(T_E\) the benefits of \(C_E\) may be negated. \(C_E\) reduced \(g_{s\text{-sat}}\) of field-grown cotton by 25% (Chapter 6), whereas in the glasshouse, \(C_E\) only reduced \(g_{s\text{-sat}}\) at warmer (32/21 °C) temperatures (Chapter 4). Therefore, warmer glasshouse temperatures (32/21 °C)
may be representative of field conditions inside the CETA chambers, where average daily air temperatures inside the CETA chambers were often warmer than 32 °C. Although Reddy et al. (1998a) showed that leaf photosynthesis in cotton benefited more from elevated [CO₂] at warm (36/28 °C) temperatures than at low (26/18 °C) growing temperatures, our data suggest that warmer temperatures (32/21 °C) are likely to increase both plant biomass and water use. Cₑ increased plant water use at Tₑ, although WUEₚ was improved, whereas increased water consumption at Tₑ resulted in lower WUEₚ regardless of atmospheric [CO₂]. Therefore, whole plant growth and WUEₚ may be increased in Cₑ at Tₐ (but increase total water use), but Cₑ will not mitigate the negative effects of Tₑ on WUE of cotton grown in future environments.

In addition, cotton grown at Tₐ tolerated water deficit to a greater extent than plants grown at Tₑ, indicating that cotton may be more susceptible to long dry periods in projected warmer environments. Consequently, warmer temperatures in future production systems may lead to increased demand for the limited water resources in Australian cotton systems and therefore, suggests that irrigation and agronomic management may need to be altered for future environmental conditions. The CETA chamber experiment indicated that there was no detectable difference in whole plant water use, although it was difficult to separate the effects of warmer temperature and elevated [CO₂] in this study and there was only one set of measurements for the control, and thus requires further research to explore plant water use under variable environmental scenarios.

### 7.2. Suggested future work

This study evaluated the responses of Australian cotton growth and physiology to integrated factors of projected climatic change. However, there are several opportunities for further research as a result of this study, as summarised below:

- **Assessment and validation of physiology and production models of plant response to VPD using data collected from field-grown plants, so that the knowledge gained can be extrapolated to other locations and projected climate scenarios.**

- **Further investigation of the physiological and growth responses of a wider range of cotton varieties to projected climatic change (warmer temperatures, elevated [CO₂], altered VPD and water deficits) in Australian production systems.**

- **Field studies that encompass a range of temperature, CO₂ and VPD environments, soil types and water and nutrient regimes.** These studies should be extended through the full growth cycle because later canopy development may negate early season differences, and thus would
require larger chambers than the CETA chambers with more sophisticated temperature control. Field studies should inform management options for adaptation to future climate scenarios.

7.3. Conclusions

The integrated responses of Australian cotton varieties to warmer temperatures, elevated atmospheric [CO$_2$], and altered VPD were assessed in this thesis. Cotton responds strongly to changes in VPD, and hence the VPD environment should be characterised in future climate change studies. Elevated [CO$_2$] impacts cotton growth, physiology and water use, although the magnitude is largely dependent on air temperature and water availability. With elevated [CO$_2$], there are benefits of increased leaf and plant level WUE; however, glasshouse experiments indicate that warmer temperatures may negate the positive impact of increased WUE with elevated [CO$_2$]. Glasshouse experiments indicate that warmer growing temperatures may increase plant water use and reduce tolerance of water deficits, potentially leading to increased demand for water in Australian cotton production systems; however, this is yet to be determined for plants grown in the field. Therefore, modern cultivars with smaller, more compact growth habits and higher photosynthetic capacity may have an advantage over older cultivars in terms of water use, but there is currently no evidence to suggest that older cultivars are more responsive to elevated [CO$_2$] and warmer temperatures than modern cultivars. These studies also have explored the utility of CETA chambers to assess the integrated effect of projected climate change for cotton grown in the field. Despite limitations of these chambers in terms of meaningful comparisons between chamber and non-chamber treatments, CETA chambers proved a successful method of elevating atmospheric [CO$_2$] and applying conditions of a projected climate to field-grown cotton.
References


Amthor J.S. (2000) Direct effect of elevated CO$_2$ on nocturnal in situ leaf respiration in nine temperate deciduous tree species is small. Tree Physiology 20: 139-144.


129


air CO₂ enrichment (FACE) and differential irrigation. Agricultural and Forest Meteorology 70: 189-207.


LI-COR (2011) Using the LI-6400/LI-6400 XT portable photosynthesis system., Lincoln, NE.


Appendix

Appendix 1: Average daily air temperature (°C) inside the glasshouse for ambient (blue) and elevated (red) temperature treatments. Values represent the mean ± SE of two rooms.
Appendix 2: (a) photosynthetic response ($A_{sat}$, $\mu$mol m$^{-2}$ s$^{-1}$) to soil water availability (% of field capacity) (b) stomatal conductance ($g_{sat}$, mol m$^{-2}$ s$^{-1}$) in response to changing soil water availability (% of field capacity) (c) transpiration ($E$, mol m$^{-2}$ s$^{-1}$) to soil water availability, and (d) transpiration efficiency ($A/E$, $\mu$mol mol$^{-1}$) to soil water availability (% of field capacity). Circles used for $C_A$: 400 $\mu$L L$^{-1}$ [CO$_2$], triangles used for $C_E$: 640 $\mu$L L$^{-1}$ [CO$_2$]. Ambient temperature ($T_A$) is shown in blue, elevated temperature ($T_E$) is shown in red.
Appendix 3: Photosynthetic acclimation of each of the water-stressed plants grown in each of the four treatments (CA: 400 µL L⁻¹ [CO₂]; CE: 640 µL L⁻¹ [CO₂]; TA: 28/17 °C (day/night); TE: 32/21 °C (day/night).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA TA</td>
<td>0.081</td>
</tr>
<tr>
<td>CA TE</td>
<td>0.670</td>
</tr>
<tr>
<td>CE TA</td>
<td>0.940</td>
</tr>
<tr>
<td>CE TE</td>
<td>0.840</td>
</tr>
</tbody>
</table>
Appendix 4: Number of days until drought stress for each drought phase (D1 and D2) for each CO$_2$ (CA: 400 µL L$^{-1}$; CE: 640 µL L$^{-1}$) and temperature (TA: 28/17 °C; TE: 32/21 °C) treatment. Values represent means ± SE of 5 plants. ANOVA for [CO$_2$] and temperature effects on the number of days until drought stress. F-values in bold represent significance at P< 0.05.

<table>
<thead>
<tr>
<th></th>
<th>CA$_A$</th>
<th>CA$_E$</th>
<th>CE$_A$</th>
<th>CE$_E$</th>
<th>[CO$_2$]</th>
<th>Temperature</th>
<th>[CO$_2$] x Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SE</td>
<td>mean</td>
<td>SE</td>
<td>mean</td>
<td>SE</td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>8.6 ± 0.2</td>
<td>7 ± 0</td>
<td>9 ± 0</td>
<td>6.8 ± 0.6</td>
<td>0.756</td>
<td>0.001</td>
<td>0.357</td>
</tr>
<tr>
<td>D2</td>
<td>14 ± 0</td>
<td>5.8 ± 0.2</td>
<td>12 ± 0</td>
<td>6 ± 0</td>
<td><strong>0.001</strong></td>
<td><strong>0.001</strong></td>
<td><strong>0.001</strong></td>
</tr>
</tbody>
</table>
Appendix 5: Effect of temperature, atmospheric [CO₂] and water availability on vegetative and reproductive biomass of cotton until 70 DAP. Values represent mean ± SE of 5 plants.

<table>
<thead>
<tr>
<th>Water trt</th>
<th>Ambient Temperature</th>
<th>Ambient + 4°C Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>400 µL L⁻¹ [CO₂]</td>
<td>640 µL L⁻¹ [CO₂]</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vegetative growth</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf dry mass (g plant⁻¹)</td>
<td>well watered</td>
<td>7.7 ± 0.286</td>
</tr>
<tr>
<td></td>
<td>water stressed</td>
<td>5.9 ± 0.479</td>
</tr>
<tr>
<td>Stem &amp; petiole dry mass (g plant⁻¹)</td>
<td>well watered</td>
<td>9.8 ± 0.500</td>
</tr>
<tr>
<td></td>
<td>water stressed</td>
<td>6.9 ± 0.479</td>
</tr>
<tr>
<td>Root dry mass (g plant⁻¹)</td>
<td>well watered</td>
<td>3.4 ± 0.334</td>
</tr>
<tr>
<td></td>
<td>water stressed</td>
<td>3.0 ± 0.275</td>
</tr>
<tr>
<td>Total vegetative dry mass (g plant⁻¹)</td>
<td>well watered</td>
<td>20.9 ± 0.925</td>
</tr>
<tr>
<td></td>
<td>water stressed</td>
<td>15.7 ± 1.214</td>
</tr>
<tr>
<td><em>Reproductive growth</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fruit dry mass (g plant⁻¹)</td>
<td>well watered</td>
<td>0.9 ± 0.112</td>
</tr>
<tr>
<td></td>
<td>water stressed</td>
<td>0.9 ± 0.160</td>
</tr>
</tbody>
</table>
Appendix 6: Photosynthetic capacity ($A_{\text{max}}, \mu\text{mol m}^{-2}\text{s}^{-1}$) of well-watered and water-stressed cotton grown at ambient and elevated $[\text{CO}_2]$ and ambient and elevated temperatures, measured at 1800 $\mu\text{mol m}^{-2}\text{s}^{-1}$ light and 1500 $\mu\text{L L}^{-1}[\text{CO}_2]$. Circles used for 400 $\mu\text{L L}^{-1}[\text{CO}_2]$, triangles used for 640 $\mu\text{L L}^{-1}[\text{CO}_2]$. Ambient temperature is shown in blue, elevated temperature is shown in red. Values represent means ± SE of 5 leaves. All plants were well watered during the recovery phase (shaded).
Appendix 7: Capacity of stomatal conductance ($g_{s\text{-max}}$, mol m$^{-2}$ s$^{-1}$) of well-watered and water-stressed cotton grown at ambient and elevated [CO$_2$] and ambient and elevated temperatures, measured at 1800 µmol m$^{-2}$ s$^{-1}$ light and 1500 µL L$^{-1}$ [CO$_2$]. Circles used for CA: 400 µL L$^{-1}$ [CO$_2$], triangles used for CE: 640 µL L$^{-1}$ [CO$_2$]. Ambient temperature (T$_A$) is shown in blue, elevated temperature (T$_E$) is shown in red. Values represent means ± SE of 5 leaves. All plants were well watered during the recovery phase (shaded).
Appendix 8: Comparison between ambient air temperature and block temperature (°C) for gas exchange measurements on cotton during the 2011-12 season. Also shown is the 1:1 line (dashed).
Appendix 9: Mean ambient VPD\textsubscript{l} (kPa) and air temperature (°C) and respective standard errors of the mean (SE) for each treatment on each day of measurement.

<table>
<thead>
<tr>
<th>DAP</th>
<th>Date</th>
<th>Sowing treatment</th>
<th>Water treatment</th>
<th>VPD\textsubscript{l} (kPa)</th>
<th>Air temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mean</td>
<td>SE</td>
</tr>
<tr>
<td>86</td>
<td>30/12/2011</td>
<td>S1</td>
<td>NS</td>
<td>1.03</td>
<td>±0.117</td>
</tr>
<tr>
<td>86</td>
<td>30/12/2011</td>
<td>S1</td>
<td>ES</td>
<td>1.08</td>
<td>±0.093</td>
</tr>
<tr>
<td>64</td>
<td>31/12/2011</td>
<td>S2</td>
<td>NS</td>
<td>1.50</td>
<td>±0.180</td>
</tr>
<tr>
<td>104</td>
<td>9/02/2012</td>
<td>S2</td>
<td>NS</td>
<td>1.06</td>
<td>±0.159</td>
</tr>
<tr>
<td>71</td>
<td>9/02/2012</td>
<td>S3</td>
<td>NS</td>
<td>1.05</td>
<td>±0.051</td>
</tr>
<tr>
<td>71</td>
<td>9/02/2012</td>
<td>S3</td>
<td>ES</td>
<td>1.04</td>
<td>±0.011</td>
</tr>
<tr>
<td>104</td>
<td>9/02/2012</td>
<td>S2</td>
<td>ES</td>
<td>0.98</td>
<td>±0.180</td>
</tr>
<tr>
<td>75</td>
<td>13/02/2012</td>
<td>S3</td>
<td>NS</td>
<td>1.97</td>
<td>±0.072</td>
</tr>
<tr>
<td>75</td>
<td>13/02/2012</td>
<td>S3</td>
<td>ES</td>
<td>1.57</td>
<td>±0.101</td>
</tr>
<tr>
<td>109</td>
<td>14/02/2012</td>
<td>S2</td>
<td>NS</td>
<td>1.96</td>
<td>±0.131</td>
</tr>
<tr>
<td>109</td>
<td>14/02/2012</td>
<td>S2</td>
<td>LS</td>
<td>1.40</td>
<td>±0.193</td>
</tr>
<tr>
<td>109</td>
<td>14/02/2012</td>
<td>S2</td>
<td>ES</td>
<td>1.51</td>
<td>±0.179</td>
</tr>
<tr>
<td>77</td>
<td>15/02/2012</td>
<td>S3</td>
<td>NS</td>
<td>1.40</td>
<td>±0.111</td>
</tr>
<tr>
<td>110</td>
<td>15/02/2012</td>
<td>S2</td>
<td>LS</td>
<td>1.24</td>
<td>±0.013</td>
</tr>
<tr>
<td>77</td>
<td>15/02/2012</td>
<td>S3</td>
<td>ES</td>
<td>1.36</td>
<td>±0.055</td>
</tr>
</tbody>
</table>
Appendix 10: (a) Stomatal conductance ($g_{s\text{-sat}}$), (b) photosynthesis ($A_{\text{sat}}$) and (c) photosynthetic efficiency ($A_{\text{sat}}/g_{s\text{-sat}}$) at ambient VPD for each of the three sowing times: early sowing (S1, circle), mid sowing (S2, triangle), and late sowing (S3, square). (d) Stomatal conductance ($g_{s\text{-sat}}$) (e) photosynthesis ($A_{\text{sat}}$) and (f) photosynthetic efficiency ($A_{\text{sat}}/g_{s\text{-sat}}$) at ambient VPD for each of the three water treatments: non stressed (NS, black), early stress (ES, green) and late stress (LS, red). Ambient VPD is defined as first measurement of the VPD curve before air has been passed through drierite. Therefore, VPD at each point in time reflects actual field conditions (non-scrubbed measurements).
Appendix 11: Average volumetric soil water content (VSWC %) from 0 - 120 cm for each sowing time (S1: circle, S2: triangle and S3: square) and water treatment (NS: black, ES: green and LS: red) during the experimental period, measured using the neutron probe.
Appendix 12: Average (a) plant height and (b) nodes by days after planting (DAP) for each plot for each sowing time (S1: circle, S2: triangle and S3: square) and water treatment (NS: black, ES: green and LS: red). Values represent mean ± SE.
Appendix 13: Analysis of stepwise regression using Generalised Linear Models, for the maximal model VPD$_L$ x Plant x T$_{l-Ta}$ x ASH. P-values for terms sequentially added to the model are shown in the table, where values in bold represent significance at P< 0.05. % represents a marginal term, where the interaction was not able to be included in the model.

<table>
<thead>
<tr>
<th>Term added to model</th>
<th>$g_{s sat}$ Complete</th>
<th>Ambient</th>
<th>$A_{sat}$ Complete</th>
<th>Ambient</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPD$_L$</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Plant</td>
<td>0.001</td>
<td>0.036</td>
<td>0.311</td>
<td>0.172</td>
</tr>
<tr>
<td>T$_{l-Ta}$</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.074</td>
</tr>
<tr>
<td>ASH</td>
<td>0.377</td>
<td>0.003</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>VPD$_L$ x Plant</td>
<td>0.082</td>
<td>0.813</td>
<td>0.913</td>
<td>0.544</td>
</tr>
<tr>
<td>VPD$<em>L$ x T$</em>{l-Ta}$</td>
<td>0.356</td>
<td>0.983</td>
<td>0.787</td>
<td>0.003</td>
</tr>
<tr>
<td>Plant x T$_{l-Ta}$</td>
<td><strong>0.032</strong></td>
<td>0.092</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VPD$_L$ x ASH</td>
<td>0.775</td>
<td>0.892</td>
<td>0.03</td>
<td>0.403</td>
</tr>
<tr>
<td>Plant x ASH</td>
<td>%</td>
<td>0.116</td>
<td>0.852</td>
<td>0.548</td>
</tr>
<tr>
<td>T$_{l-Ta}$ x ASH</td>
<td>0.574</td>
<td>0.496</td>
<td>0.584</td>
<td>0.308</td>
</tr>
<tr>
<td>VPD$<em>L$ x Plant x T$</em>{l-Ta}$</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>0.284</td>
</tr>
<tr>
<td>VPD$_L$ x Plant x ASH</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>VPD$<em>L$ x T$</em>{l-Ta}$ x ASH</td>
<td>0.942</td>
<td>0.748</td>
<td>%</td>
<td>0.507</td>
</tr>
<tr>
<td>Plant x T$_{l-Ta}$ x ASH</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>VPD$<em>L$ x Plant x T$</em>{l-Ta}$ x ASH</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
</tbody>
</table>

**Total df** 186 44 186 44

**Total SS** 19.6 3.8 2129.0 542.5
Appendix 14: An example of changes in radiation (MJ m$^2$ day$^{-1}$), and maximum and minimum air temperature (°C) in Narrabri over a cotton season. October and December periods are long term averages based on available data since 1957, whereas February dates are actual radiation levels and temperatures during the experimental period. (Source: CottAssist)

<table>
<thead>
<tr>
<th>From</th>
<th>To</th>
<th>Radiation (MJ m$^2$ day$^{-1}$)</th>
<th>Maximum air temperature (°C)</th>
<th>Minimum air temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Oct-12</td>
<td>12-Dec-12</td>
<td>1744</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>20-Dec-12</td>
<td>1-Mar-13</td>
<td>1770</td>
<td>34</td>
<td>19</td>
</tr>
<tr>
<td>19-Feb-13</td>
<td>2-May-13</td>
<td>1385</td>
<td>28</td>
<td>13</td>
</tr>
</tbody>
</table>
Appendix 15: Average hourly (a) air temperature, (b) relative humidity, and (c) VPD inside the chambers and control plot on the 6th April 2013 (46 DAP) from 8 am – 6 pm (AEDT). Values represent mean ± SE of two chambers for ambient [CO$_2$] (C$_A$; circle) and elevated [CO$_2$] (C$_E$; triangle), and one control plot (C$_C$; square).
Appendix 16: Minimum, maximum and mean change in [CO₂] each minute for ambient (Cₐ) and elevated (Cₑ) CETA chambers for 6th April 2013 (46 DAP) and 25th April 2013 (65 DAP).

<table>
<thead>
<tr>
<th></th>
<th>6-Apr-13</th>
<th></th>
<th>25-Apr-13</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA</td>
<td>CE</td>
<td>CA</td>
<td>CE</td>
</tr>
<tr>
<td>Minimum</td>
<td>-13.0</td>
<td>-13.2</td>
<td>-5.8</td>
<td>-11.9</td>
</tr>
<tr>
<td>Maximum</td>
<td>22.5</td>
<td>22.0</td>
<td>4.9</td>
<td>5.7</td>
</tr>
<tr>
<td>Mean</td>
<td>-0.009</td>
<td>-0.001</td>
<td>-0.005</td>
<td>-0.002</td>
</tr>
<tr>
<td>SE Mean</td>
<td>0.051</td>
<td>0.069</td>
<td>0.025</td>
<td>0.040</td>
</tr>
</tbody>
</table>
Appendix 17: [CO$_2$] of ambient (C$_A$; blue circle) and elevated (C$_E$; red triangle) CETA chambers averaged over 1 min for (a) 6$^{th}$ April 2013 [46 DAP] and (b) 25$^{th}$ April 2013 [65 DAP]. Values represent mean ± SE of two chambers (data is for one elevated [CO$_2$] chamber on the 6$^{th}$ April, due to equipment malfunction).
Appendix 18: Change in $[\text{CO}_2]$ each minute (delta $[\text{CO}_2]$ ($\mu$L L$^{-1}$ min$^{-1}$)) for ambient (a and b, C_A; blue circle) and elevated (c and d, C_E; red triangle) CETA chambers for 6th April 2013 (46 DAP; a and c) and 25th April 2013 (65 DAP; b and d). Values represent mean of two chambers (data is for one elevated $[\text{CO}_2]$ chamber on the 6th April, due to equipment malfunction).
Appendix 19: Average hourly photosynthesis ($A_{sat}$; a and b) and transpiration ($E$; c and d) for plants inside elevated [CO$_2$] chambers on the 6th April 2013 (46 DAP; a and c) and 25th April 2013 (65 DAP; b and d) from 8 am - 6pm (AEDT). Data for ambient [CO$_2$] chambers has been excluded due to equipment malfunction.
Appendix 20: Table of means and standard errors (SE) for plant biomass, physiology and harvest parameters of cotton grown in control (C<sub>C</sub>), ambient [CO<sub>2</sub>] (C<sub>A</sub>) or elevated [CO<sub>2</sub>] (C<sub>E</sub>) CETA chambers. Also shown are F-values for REML analyses between C<sub>C</sub> and C<sub>A</sub>, and C<sub>A</sub> and C<sub>E</sub> treatments. Values in bold represent significance at P< 0.05.

<table>
<thead>
<tr>
<th></th>
<th>C&lt;sub&gt;C&lt;/sub&gt; mean</th>
<th>SE</th>
<th>C&lt;sub&gt;A&lt;/sub&gt; mean</th>
<th>SE</th>
<th>C&lt;sub&gt;E&lt;/sub&gt; mean</th>
<th>SE</th>
<th>F-value C&lt;sub&gt;C&lt;/sub&gt; compared with C&lt;sub&gt;A&lt;/sub&gt;</th>
<th>F-value C&lt;sub&gt;A&lt;/sub&gt; compared with C&lt;sub&gt;E&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plant biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total vegetative biomass (g plant&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>6.6</td>
<td>0.44</td>
<td>7.0</td>
<td>0.42</td>
<td>11.8</td>
<td>0.95</td>
<td>0.476</td>
<td>0.001</td>
</tr>
<tr>
<td>Total fruit (g plant&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.2</td>
<td>0.03</td>
<td>0.4</td>
<td>0.06</td>
<td>0.6</td>
<td>0.09</td>
<td>0.005</td>
<td>0.025</td>
</tr>
<tr>
<td><strong>Physiology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A&lt;sub&gt;sat&lt;/sub&gt;</td>
<td>19.0</td>
<td>0.62</td>
<td>18.9</td>
<td>0.89</td>
<td>22.8</td>
<td>1.94</td>
<td>0.940</td>
<td>0.007</td>
</tr>
<tr>
<td>g&lt;sub&gt;S-sat&lt;/sub&gt;</td>
<td>0.3</td>
<td>0.01</td>
<td>0.2</td>
<td>0.02</td>
<td>0.2</td>
<td>0.02</td>
<td>0.144</td>
<td>0.038</td>
</tr>
<tr>
<td>E</td>
<td>5.0</td>
<td>0.21</td>
<td>4.2</td>
<td>0.21</td>
<td>3.5</td>
<td>0.19</td>
<td>0.021</td>
<td>0.027</td>
</tr>
<tr>
<td>A&lt;sub&gt;sat&lt;/sub&gt;/g&lt;sub&gt;S-sat&lt;/sub&gt;</td>
<td>70.3</td>
<td>1.51</td>
<td>83.2</td>
<td>2.49</td>
<td>135.2</td>
<td>11.61</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt;</td>
<td>82.9</td>
<td>4.12</td>
<td>87.5</td>
<td>5.94</td>
<td>73.3</td>
<td>6.25</td>
<td>0.532</td>
<td>0.115</td>
</tr>
<tr>
<td>J&lt;sub&gt;max&lt;/sub&gt;</td>
<td>187.7</td>
<td>9.25</td>
<td>169.3</td>
<td>7.79</td>
<td>147.5</td>
<td>17.32</td>
<td>0.144</td>
<td>0.263</td>
</tr>
<tr>
<td>J&lt;sub&gt;max&lt;/sub&gt;/V&lt;sub&gt;max&lt;/sub&gt;</td>
<td>2.3</td>
<td>0.08</td>
<td>2.0</td>
<td>0.07</td>
<td>2.0</td>
<td>0.17</td>
<td>0.008</td>
<td>0.795</td>
</tr>
<tr>
<td><strong>Harvest</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm plant&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>34.2</td>
<td>0.89</td>
<td>44.4</td>
<td>1.44</td>
<td>51.9</td>
<td>1.44</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Leaf area (cm&lt;sup&gt;2&lt;/sup&gt; plant&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>388.0</td>
<td>22.64</td>
<td>445.6</td>
<td>25.05</td>
<td>673.5</td>
<td>47.10</td>
<td>0.095</td>
<td>0.001</td>
</tr>
<tr>
<td>Nodes (plant&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>10.8</td>
<td>0.25</td>
<td>11.2</td>
<td>0.29</td>
<td>12.1</td>
<td>0.31</td>
<td>0.358</td>
<td>0.025</td>
</tr>
</tbody>
</table>