Investigating the main and interactive effects of vapour pressure deficit, soil water deficit, warmer temperatures and elevated CO$_2$ on cotton growth and physiology

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Abstract

Climate change factors such as rising temperature, atmospheric carbon dioxide concentration \([\text{CO}_2]\) and increased drought frequency and intensity have been shown to affect cotton physiology and productivity. To investigate the effect of climate change on cotton, we divided our study into two parts, of which the first study focused on the effect of vapour pressure deficit (VPD) and soil water deficit (SWD) on stomatal response and the second study focused on the effect of increased temperature (T\text{E}) and elevated [CO\text{_2}] (C\text{E}) on cotton growth and physiology. We conducted both experiments in an environmentally controlled glasshouse to imitate the projected drier, warmer and increased [CO\text{_2}] conditions. Our goal was to use cotton to improve the understanding of the physiological and growth response of C3 crops to changing climate.

The first experiment was developed as climate change has driven increasing air temperature and longer warm seasons throughout Australia. These changes have resulted in a drier atmosphere (higher vapour pressure deficit, VPD) and drier soil (high soil water deficit, SWD) that contributes to cotton stress and therefore, reduces cotton growth and physiological performance. This study investigated the effect of atmospheric and soil drought on cotton physiology by experimentally controlling air temperature (22, 26, 30 and 34\degree\text{C}), humidity, and soil moisture content (100\% and 50\% field capacity) to determine the physiological responses of cotton to these factors individually, and in combination. A novel approach in this study was to assess the two components (temperature and relative humidity) of VPD to examine the relative contribution of each factor to plant response to VPD, and to determine whether SWD affected this relationship. Higher SWD decreased stomatal conductance (\(g_s\)), and higher VPD further reduced \(g_s\). Higher SWD and VPD also reduced transpiration (\(E\)), but to a lesser degree than the reduction in \(g_s\). In contrast, photosynthesis (\(A\)) was not affected by the change in SWD, nor very responsive to VPD or temperature. The stomatal model predicted that well-watered cotton would increase whole-plant water use more than cotton grown in SWD under elevated temperature.

The focused climate change factors for the second experiment included elevated temperature (T\text{E}) and increased [CO\text{_2}] (C\text{E}), as they will generally increase plant source strength, while loss of fruits will decrease sink strength. The relationship between source-sink strength in cotton is likely to determine the impact of future climate factors on cotton production. The association
between physiological functions and the change in sink strength will also enable us to assess acclimatisation in cotton under future climate. To investigate the source-sink relationship in cotton, we grew cotton plants in the glasshouse under the following climate conditions: (1) ambient \([\text{CO}_2]\) (420 ppm) and ambient temperature (32/21°C); (2) elevated \([\text{CO}_2]\) (640 ppm) and ambient temperature (32/21°C); (3) ambient \([\text{CO}_2]\) (420 ppm) and elevated temperature (36/25°C); and (4) elevated \([\text{CO}_2]\) (640 ppm) and elevated temperature (36/25°C), and then removed 50% of the fruits (FR) from half of the plants in each growth condition, to assess source-sink relationships. We found that FR plants exhibited greater vegetative biomass and square (reproductive buds) production but no effect of FR on the total biomass was observed. Our results showed that \(T_E\) decreased reproductive biomass more significantly under ambient \([\text{CO}_2]\) (CA) than under the CE condition. \(T_E\) increased open boll retention by an average of one fruit per plant. \(T_E\) decreased greater reproductive biomass under CA than under CE condition. \(T_E\) increased the partitioning of carbon to vegetative and existing reproductive parts, rather than promoting new fruit growth. \(A\) and \(g_s\) were not affected by the fruit removal. There was an interactive effect between CE and time; CE increased \(A\) during the early stage then decreased at increasing days after planting (DAP). CE decreased \(g_s\) while \(T_E\) increased \(g_s\) at increasing DAP. There was no interactive effect between climate factors and fruit removal on vegetative, reproductive, total biomass or leaf gas exchange traits. There was no correlation between \(A\) and the production of plant biomass. The results of this study suggested that elevated climate factors can alter biomass partitioning and a higher carbon assimilation rate does not indicate a greater biomass production. Also, CE did not fully alleviate the negative effects of extreme temperature on sink strength in cotton.
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Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

....................................................
(Signature)
Author contributions

Chapter 1: The background section of this chapter was part of my thesis proposal.

Chapter 2: This study was funded by CRDC as part of a collaborative project between Western Sydney University and CSIRO in Narrabri, NSW. The project was designed by Professor David Tissue and Dr Katrina Broughton (CSIRO). Renee Smith and I were responsible for plant watering and data collection for water content, plant growth and leaf gas exchange data. Dr Katrina Broughton was responsible for overseeing the experiment, data collection (i.e. leaf gas exchange data) and transportation of the LI-6800 portable photosynthesis system (LI-COR, Lincoln, USA). I was also responsible for the data interpretation and writing for this study.

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Declaration

I used a professional copyediting service for this thesis. The editor was Karin Hosking.
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Chapter 1

General Introduction

Background

Rising temperature, atmospheric carbon dioxide concentration [CO$_2$] and increased drought frequency and intensity have been shown to affect cotton productivity. This research is important as cotton is a major crop that generated over $1 billion to the Australian economy in 2016–2017 (ABS 2018), and there are unidentified impacts from climate change on cotton productivity that need further investigation. According to the cotton production research conducted by the International Cotton Advisory Committee (ICAC) (Bange et al. 2016) and other studies (Broughton et al. 2017; Osanai, Tissue, Bange, Braunack, et al. 2017), warming temperature and drought generate negative impacts on cotton productivity. However, these studies did not investigate the effect of atmospheric drought (vapour pressure deficit, VPD) which has been shown to affect plant physiology. The Bange et al. (2016) report indicates that elevated temperature causes higher soil evaporation and transpiration rates which can intensify crop water stress in drought. It is important to explore the interactive effect of VPD and soil drought as warmer climate contributes to higher VPD, which results in more frequent drought events (Lobell et al. 2015). Additionally, it is unclear about the relative effects of these environmental factors on physiology and growth.

Moreover, the rise of [CO$_2$] and increased temperature have been shown to individually alter the source-sink relationship in crop physiology and biomass accumulation (Barrett & Gifford 1995). Likewise, increased [CO$_2$] has been shown to downregulate photosynthesis capacity to balance the sink capacity with the available resource (Ko & Piccinni 2009; Broughton et al. 2017). However, the interactive effects of elevated [CO$_2$] and temperature on the source-sink
relationship in cotton are still unclear and require further investigation. The results of this research are, therefore, expected to contribute to better understanding of cotton acclimation and to improve management of cotton farms with the projected increase in temperature, \([\text{CO}_2]\), and more frequent drought events.

The main focus of the following sections includes plant responses to VPD, soil water deficit (SWD), warmer temperature and elevated \([\text{CO}_2]\). The section on drought effects explores how VPD (or reduced RH), elevated temperature and SWD affect plant physiological function. Ultimately, the section on climate change factors describes the effect of elevated temperature and increased \([\text{CO}_2]\) on plant source-sink relationships and gas exchange activity.

**Literature review**

**Plant response to vapour pressure deficit and soil water deficit**

*Effect of vapour pressure deficit and its components on leaf gas exchange*

Vapour pressure deficit (VPD) is projected to rise throughout Australia as a result of increased temperature and reduced rainfall (BOM 2018) which can deteriorate plant physiological function. For example, rising VPD decreases \(g_s\) which leads to decreased leaf carbon intake (Ko & Piccini 2009; Duursma et al. 2013). It also decreases leaf water potential (\(\Psi_{\text{leaf}}\)) (Conaty et al. 2014; Pettigrew et al. 1990) and increases the transpiration rate \((E)\) in cotton (Duursma et al. 2013). Moreover, rising VPD may increase intrinsic water use efficiency (WUE\(_i\)) but not growth (Zhang et al. 2019). Thus, our study focused on the response of cotton to rising VPD as it is an important climate factor that can affect plant physiology.

VPD consists of two components: temperature and RH (Anderson 1936). Stephens et al. (2018) indicated that increasing temperature was the main factor that contributed to rising VPD, rather than reduction in humidity, particularly in Australia. In general, plants respond to temperature and RH differently. For example, photosynthesis \((A)\) can be reduced by stomatal closure and lower Rubisco carboxylation rates as a result of lower RH and extreme temperature, respectively (Li et al. 2018). Moreover, \(g_s\) was more responsive to reduced RH
than to changes in temperature (Ball, Woodrow & Berry 1987). \( E \) may be controlled by temperature (Duursma et al. 2013) and by the response of \( g_s \) to decreased RH (Drake & Salisbury 1972). Our study, therefore, aimed to disentangle the physiological and stomatal response of cotton to VPD and the relative contribution of these two components (relative humidity and temperature) when cotton is grown in soil water deficit.

**Response of plant to soil water deficit**

Soil water deficit (SWD) has been observed throughout Australia (BOM 2018), and many studies have shown that it can downgrade plant physiology (Broughton et al. 2017; Hejnák et al. 2015; Osanai, Tissue, Bange, Braunack, et al. 2017). Broughton et al. (2017) found that SWD decreased \( g_s \) and \( E \). On the other hand, a reduction in \( A \) caused by SWD has also been observed in various crop species such as cotton (Chastain et al. 2014), maize, sorghum (Kakani et al. 2011) and groundnut (Chakraborty et al. 2016). Pettigrew (2004) found that SWD restricted growth and development in cotton. Moreover, Li et al. (2019) found that SWD reduced \( g_s \) and \( A \) before the disruption of plant hydraulic function occurred. Therefore, part of our study aimed to investigate the effect of SWD on growth, leaf gas exchange and hydraulic function. Moreover, we investigated the effect of SWD on the relationship between VPD components because changes in this relationship may also affect plant physiology.

**Stomatal conductance under drought and warmer conditions and the unified stomatal model**

Stomatal conductance (\( g_s \)) is the key regulator for leaf gas exchange activity, and it is responsive to rising VPD, elevated temperature and SWD conditions. For example, stomatal closure decreases transpiration (\( E \)) at rising VPD (e.g. Ambrose et al. 2010; Marchin et al. 2016). Broughton et al. (2017) found that SWD reduced \( g_s \) which led to lower \( A \). Additionally, rising VPD induces stomatal closure, which can also reduce \( A \) as a result of decreased intercellular [\( \text{CO}_2 \)] (\( C_i \)) (Duursma et al. 2013). Finally, elevated temperature (32°C) stimulated \( g_s \) (Broughton et al. 2017); however, extreme temperature (36°C) decreased \( g_s \) (Reddy, Reddy & Hodges 1998) in cotton. Drought has been a major problem in cotton growing areas and therefore, research into the combined effect of rising VPD, warmer temperature and SWD on cotton physiology is needed. We aimed to determine whether climate factors or soil water content would generate stronger effects on the physiological performance of cotton under future climate.
Our study used the unified stomatal model (Medlyn et al. 2011) to explain the stomatal response in cotton to rising VPD and elevated temperature at different soil water levels. The unified stomatal model is shown in Eq. (1),

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

where $g_0$ and $g_1$ are fitted stomatal conductance parameters. Vapour pressure deficit at the leaf surface is indicated by $D_s$, leaf-to-air atmospheric CO$_2$ concentration ($C_a$), and net photosynthesis ($A$) (Medlyn et al. 2011). This model allows us to investigate plant water use efficiency via stomatal behaviour under a warmer and drier climate. For example, Medlyn et al. (2011) suggested in their study that the increase in $g_1$ will result in decreasing WUE. On the other hand, this model should allow us to identify which environmental factor (atmospheric drought or soil drought) has more effect on cotton plant physiological performance.

**Source-sink relationship response to elevated temperature and increased [CO$_2$]**

*Source strength and sink strength*

The sugar transported via phloem to different parts of a plant generates a source-sink relationship, which is the supply and demand for resources within plants, and this relationship can be altered by climate factors (Lemoine et al. 2013). In general, plant productivity may be determined by the source strength or sink strength; the concept of ‘strength’ concerns the site of sugar allocation or preferable parts of plants that will receive or deliver sugar. In other words, the sink is the part of a plant receiving photosynthates and the source is the photosynthate provider (Wardlaw 1990), see Figure 1.1. According to a review by Smith, Rao & Merchant (2018), sink strength is commonly referred to as the product of sink activity (e.g. growth and carbon allocation) and sink amount (e.g. reproductive parts or yield). Source strength can be determined by plant organs that perform photosynthesis which generally act as the resource (photosynthate) supply; these parts may include leaves, stem, fruits and any parts that contain chlorophyll (Wardlaw 1990). Marcelis (2004) suggested that plant vegetative growth can determine the source-sink balance. They further suggested that changes in reproductive growth can be affected by a reduction in source strength and competition with existing reproductive parts. Wardlaw (1990) summarised that plant allocation of
photosynthate was preferentially used to support the growth of fruit and seeds rather than vegetative growth under ambient conditions. Nevertheless, alterations in environmental conditions may change the source-sink relationship, as summarised by Lemoine et al. (2013), which may be particularly evident in future climate conditions. Biomass partition may shift between vegetative and reproductive components under rising temperature and increasing [CO₂]. Therefore, this study aimed to understand the relative effects of elevated [CO₂] and air temperature on source-sink relationships in cotton grown by experimentally manipulating sink strength and activity.

**Figure 1.1** Simplified conceptual diagram of this study representing the source-sink relationship in a plant without any changes in atmospheric conditions (Smith, Rao & Merchant 2018; Wardlaw 1990).

**Effect of elevated temperature and reduced sink strength on plant growth and physiology**

Tₑ has been shown to affect growth and development in cotton. Broughton et al. (2017) showed that Tₑ increased total vegetative biomass and reproductive biomass. Similarly, Tₑ increases seed cotton yield, boll number and boll size. These findings may suggest that Tₑ promotes both source and sink strength; however, Tₑ can reduce sink strength by detaching cotton bolls, thereby leaving a reduced number of young bolls on branches (Zhao et al. 2005).

On the other hand, Saleem et al. (2018) observed that plants offset the loss of fruit by promoting new fruit growth and increasing boll retention on plants under ambient temperature. Nevertheless, we are uncertain whether plants will duplicate this behaviour under Tₑ conditions and therefore, part of our study aimed to investigate the effect of Tₑ on the source-sink relationship.
Plant physiology has been shown to be affected by T_E but not by changes in sink strength. For example, T_E increased g_s (Broughton et al. 2017) and E (Reddy, Reddy & Hodges 1998) but there was no change in g_s even when plants loss a significant amount of fruit load (Bustan et al. 2016; Li et al. 2007). On the other hand, A was not significantly affected by T_E (Broughton et al. 2017; Duursma et al. 2013). In contrast, Zhao et al. (2005) found that there was a slight increase in A when cotton was grown under a warmer temperature (36°C). They suggested that fruit shedding (reduced sink strength) may have slowed down leaf senescence and therefore, did not lower the photosynthetic rate. Therefore, our study aimed to explore the effect of change in fruit load on plant physiology under T_E.

Effect of elevated [CO_2] on plant growth and physiology

Another focused climate factor in this study is elevated [CO_2] (C_E), which has been shown to affect plant growth and leaf gas exchange activity. For growth, C_E increased vegetative biomass and reproductive biomass by increasing the number of nodes and the number of bolls per plant, respectively (Osanai, Tissue, Bange, Anderson, et al. 2017). For physiological performance, C_E decreased g_s and E but increased A (Broughton et al. 2017; Ko & Piccinni 2009; Reddy, Reddy & Hodges 1998) which means that C_E may improve whole-plant water use efficiency (Broughton et al. 2017) and intrinsic water use efficiency (Osanai, Tissue, Bange, Anderson, et al. 2017). However, Barrett & Gifford (1995) found that C_E stimulated carbon allocation to leaves and therefore, increased leaf thickness which may decrease A. We were unable to determine whether the source-sink relationship has a role in these leaf gas exchange factors, or vice versa, under C_E conditions. We aimed to identify whether there is a significant relationship between plant biomass allocation and leaf physiology and whether C_E conditions will affect this relationship in cotton.

Interactive effect of elevated temperature and increased [CO_2] on plant growth and physiology

There are interactive effects of T_E and C_E on cotton growth. Broughton et al. (2017) showed that the combined effect of these climate factors increased vegetative biomass and reproductive biomass in cotton. Additionally, the height of cotton plants grown under T_E and C_E conditions also increased significantly (Osanai, Tissue, Bange, Anderson, et al. 2017). However, Osanai, Tissue, Bange, Anderson, et al. (2017) and Tan et al. (2015) indicated that T_E is the dominant factor that can accelerate the development of cotton and wheat,
respectively. The faster growth and development in plants indicated that the utility of available resources is beyond an efficient rate (Poorter & De Jong 1999) and thus, under C\textsubscript{E} and T\textsubscript{E} plants are likely to deplete the available resources faster. The results from Allen et al. (2018), however, are not in line with the other studies mentioned; they found that T\textsubscript{E} mainly increased vegetative biomass and restricted reproductive growth in soybeans. The inconsistencies between studies are likely due to different crop species and temperatures used in each study. Nevertheless, we aimed to address whether plants would allocate their biomass towards particular components, either vegetative or reproductive, under T\textsubscript{E} and C\textsubscript{E} conditions. As mentioned, we removed half of the fruit load of plants and investigated the growth development of both vegetative and reproductive components under T\textsubscript{E} and C\textsubscript{E} conditions.

In general, the interactive effect of C\textsubscript{E} and T\textsubscript{E} decreased \( g_s \) but increased \( A \) (Broughton et al. 2017; Reddy, Reddy & Hodges 1998). However, a review by Becklin et al. (2017) indicated that C\textsubscript{E} is unlikely to alleviate the negative effect of T\textsubscript{E} on plant physiology. This conclusion is also consistent with Broughton et al. (2017) who found that C\textsubscript{E} may promote growth and photosynthesis but also increase water usage. Also, C\textsubscript{E} did not decrease Rubisco activity or cotton carbon assimilation (Harley et al. 1992). Nevertheless, further investigation of the relationship between the source-sink relationship and leaf gas exchange as \( A \) may not be the parameter responsible for increased sink strength under T\textsubscript{E} and C\textsubscript{E} conditions.

In summary, it is unclear whether plants will allocate their resources towards vegetative or reproductive growth and whether there is a link between source-sink capacity and leaf gas exchange under future climates. In this study, we removed fruits from plants to alternate the source-sink capacity and investigated the main and interactive effects of T\textsubscript{E} and C\textsubscript{E} on this relationship. We also examined the association between source-sink strength and \( A \) to determine whether higher photosynthetic rates would enhance plant growth.

**Thesis overview**

**Thesis objectives**

The main objective of this thesis was to investigate the response of cotton to climate change factors including drought, warmer air temperatures and elevated atmospheric [CO\textsubscript{2}]. We
conducted two experiments to investigate: 1) the effect of drought on physiological functions, and 2) the effect of elevated temperature and increased [CO$_2$] on the source-sink relationship in cotton. The two experiments were conducted in the environmentally controlled glasshouse facility at Western Sydney University (Hawkesbury Campus), provided with different conditions to suit the purpose of each study. Ultimately, my thesis aimed to address the following questions.

The research questions for the first experiment are:

1) Does soil water deficit affect the relationship between VPD components (i.e. temperature and RH)?
2) Which VPD components, either rising temperature or lowering RH, will generate more effect on cotton physiology?
3) Does soil water deficit affect the response of cotton to increasing VPD? If so, which factor generates more effect on stomatal response in cotton?

As for the second experiment, the research questions are:

1) Will $T_E$ promote vegetative and reproductive growth in no fruit removed (control) and 50% fruit removed (FR) plants at a faster rate than under $T_A$ conditions?
2) Will $C_E$ increase the growth of vegetative and reproductive components (including existing parts and new fruits) in control and FR plants?
3) Will $T_E$ increase $A$ and $g_s$ in control and FR plants?
4) Will $C_E$ increase $A$ and decrease $g_s$ in control and FR plants?

Thesis outline

Chapter 1 provides a general introduction to my thesis.

Chapter 2 aims to investigate the main and interactive effects of vapour pressure deficit, SWD and elevated air temperature on cotton (*Gossypium hirsutum*). This study compared the effect of low VPD and high VPD (i.e. 1.5 to 4.0 kPa) on cotton physiology at different soil water levels (50% and 100% field capacity). Short-term changes in air temperature in the growth bay to 22, 26, 30 and 34°C and changes to RH in the leaf cuvette of the LI-6800 portable photosynthesis system (LI-COR, Lincoln, USA) were used to generate the selected
This method allowed us to determine the relative contribution of temperature and RH at a given VPD on stomatal physiology. Our results indicated that RH was the most important component of VPD that regulated stomatal closure. SWD primarily reduced $g_s$ and rising VPD intensified the reduction. We found that $A$ did not change in response to SWD treatment, but it changed in response to the change in temperature, RH and VPD. The results also indicated that stomatal closure may have influenced the reduction in $E$ as a response to rising VPD and SWD. Finally, our results suggested that whole-plant water usage in cotton will increase under warmer and drier climates, regardless of the low $g_s$ as a response to future climates.

Chapter 3 aims to examine the main and interactive effect of warmer temperature and elevated CO$_2$ on the source-sink relationship in cotton (Gossypium hirsutum). We grew cotton under the following conditions: (1) ambient [CO$_2$] (420 ppm) and ambient temperature (32/21°C); (2) elevated [CO$_2$] (640 ppm) and ambient temperature (32/21°C); (3) ambient [CO$_2$] (420 ppm) and elevated temperature (36/25°C); and (4) elevated [CO$_2$] (640 ppm) and elevated temperature (36/25°C). We altered sink strength by removing half of the fruit load to assess the potential for changes in biomass allocation under climate change. Cotton produced more vegetative biomass in fruit removed (FR) plants and $T_E$ deceased vegetative biomass and reproductive biomass. Additionally, $C_E$ marginally increased the height of control and FR plants. Fruit removal did not affect the total plant biomass across the treatments, suggesting a shift in biomass partitioning towards vegetative growth and reproductive growth (i.e. new growth and existing components). There was no interactive effect between the climate factors and the reduction of sink strength on vegetative, reproductive or leaf gas exchange traits. $C_E$ was the main factor that affected $A$ as days after planting progressed. $g_s$ was increased under $T_E$ and decreased under $C_E$ as DAP progressed. Neither $A$ nor $g_s$ was affected by the fruit removal. There was no correlation between $A$ and the production of vegetative, reproductive or total plant biomass. The overall results suggested that the source-sink relationship can be altered under elevated climate, and it is unlikely that higher $A$ will promote growth or plants will immediately allocate biomass to reproductive components.
Chapter 2

The effects of vapour pressure deficit and soil water deficit on leaf gas exchange parameters in cotton (*Gossypium hirsutum* L.)

Introduction

In recent years, cotton growers in Australia have experienced warmer air temperature and decreased rainfall (BOM 2018) which results in a drier atmosphere (vapour pressure deficit (VPD)) (Ficklin & Novick 2017) that downgrades crop physiological functions (Duursma et al. 2013; Lobell et al. 2015). VPD represents the difference between the maximum air capacity to hold moisture and the amount of moisture present in the air (Anderson 1936). Previous studies have shown that high VPD (i.e. dry atmospheric conditions) reduces $g_s$, decreases leaf carbon intake (Ko & Piccini 2009), decreases leaf water potential ($\Psi_{\text{leaf}}$) (Conaty et al. 2014; Pettigrew et al. 1990) and increases transpiration rate ($E$) in cotton (Duursma et al. 2013). However, cotton has been found to be less sensitive to increased VPD compared to soybeans because the extensive root system of cotton can access water in deeper soil (Pettigrew et al. 1990).

VPD is a function of air temperature and RH, so the same VPD can be generated by changing air temperature and maintaining a constant RH, or by changing RH and maintaining a constant temperature (Anderson 1936; Duursma et al. 2014) (Figure 2.1); however, warmer temperature and reduced RH have been shown to generate different effects on plant physiology. For warmer temperature, Duursma et al. (2013) found that the photosynthetic rate was decreased in high VPD, which is consistent with Ko & Piccinni (2009). It was noted that the photosynthetic rate was less sensitive to VPD variation compared to $g_s$ and $E$ in cotton.
Duursma et al. (2013) also observed that the combined effects of high VPD and $T_e$ increased $E$ and decreased $g_s$. That study suggested that the photosynthetic rate was less sensitive to changes in air temperature and VPD (without the soil water deficit effect) because it depends on the availability of intercellular [CO$_2$] ($C_i$). On the other hand, the change in RH mainly affected stomatal apertures; the reduction in RH induced stomatal closure (Li et al. 2018).

Furthermore, warmer climate and decreased rainfall have led to lower soil recharge and therefore, lower soil water content (SWC) and reductions in plant physiological processes. Lower SWC has been shown to reduce plant physiological function due to greater plant water stress (lower $\Psi_{leaf}$) and stomatal closure which reduces $C_i$ availability (Ennahli & Earl 2005). However, cotton grown at low soil moisture availability may become more adaptive to new climates by regulating its leaf turgor pressure through leaf osmotic adjustment regardless of the reduction of $\Psi_{leaf}$ (Pettigrew 2004). In a recent study on plant hydraulics in cotton, Li et al. (2019) showed that leaves are more vulnerable than roots and stems to hydraulic failure in soil drought conditions. Li et al. (2019) demonstrated that reductions in hydraulic function led to decreases in $A$ and lower plant water status as more negative $\Psi_{leaf}$ was observed. As the drought progressed, $g_s$ was reduced, eventually resulting in closed stomata, the beginning of embolism formation, and reduced hydraulic function. Increased embolisms contribute to leaf shedding and may lead to plant mortality (Hochberg et al. 2017). Li et al. (2019) was the basis for the present study by showing that soil drought can disrupt physiological function, including carbon assimilation and hydraulic function. The present study aimed to expand on this work by investigating the main and interactive effects of atmospheric drought and soil drought in regulating plant physiological performance.

On the other hand, the interactive effects of low SWC and high VPD have been shown to reduce $A$ and $g_s$ in cotton (Conaty et al. 2014). The $g_s$ of plants grown in a dry soil, and ambient [CO$_2$], is sensitive to high VPD suggesting that higher VPD may lower $C_i$ and reduce the CO$_2$ exchange rate (Pettigrew et al. 1990). Additionally, Pettigrew et al. (1990) demonstrated that high evapotranspiration might not be mitigated by irrigation because stomatal closure will continue to increase as a response to high VPD. Although we have knowledge of some interactive effects of SWC and VPD on cotton physiology, only a limited number of studies have investigated the individual effects of VPD components (i.e.
temperature and RH), which may generate different effects on cotton gas exchange activity when interacting with SWC.

**Figure 2.1** In combination, air temperature ($T_{air}$) and relative humidity (RH) are the environmental factors that determine vapour pressure deficit (VPD). VPD can be changed by altering $T_{air}$ and keeping RH constant or keeping $T_{air}$ constant and varying RH. The empirical fit of the function ($VPD=0.000605 \times T_{air}^{2.39}$) and the estimated VPD at constant RH are represented by the single continuous line and multiple dashed lines, respectively. Figure adapted from Duursma et al. (2014).

Previous studies have shown that SWD reduces physiological function and development and that elevated temperature (not as a component of VPD) can intensify the drought effect. For example, elevated temperature has been shown to reduce cotton water use efficiency (WUE), increase plant water use and decrease $C_i$ (Broughton et al. 2017). Broughton et al. (2017) demonstrated that $T_E$ amplified the negative effect of soil drought on cotton physiology by reducing $g_s$ and $A$, thereby leading to further reduction in $C_i$ and WUE. Cotton grown in warm and dry conditions may also produce a smaller leaf area for capturing light and carbon.
assimilation (Conaty et al. 2014; Pettigrew 2004). Therefore, this study investigated the main and interactive effects of temperature and RH on plant physiology under SWD conditions.

This study used the LI-6800 portable photosynthesis system (LI-COR, Lincoln, USA) to adjust RH in the leaf cuvette chamber to generate the selected VPD range. The temperature was increased by 4°C and remained constant while RH was decreased in order to generate higher VPD levels, rising by 0.5 kPa increments. Therefore, it was possible to examine the relative contribution of each VPD component, both individually and combined, on plant physiology. This study used the unified stomatal model (see Eq. 1) (Medlyn et al. 2011) to further investigate plant WUE. Subsequently, the novel approach of this study was to separate the contributing factors (RH and temperature) to VPD to determine the relative contribution of each factor at different levels of VPD, and whether SWC affected this relationship.

Given that increasing air temperatures, longer warm seasons and reduction in soil moisture are now common throughout Australia (BOM 2018), it is important to identify the main factors that impact the physiological performance of cotton. The objectives of this study were to 1) determine the gas exchange response of cotton to VPD; 2) identify whether increasing temperature or lowering RH generated more impact on plant physiological performance at different VPD levels; and 3) determine the effect of SWD on plant response to VPD. Specifically, the present study addressed the following hypotheses: 1) under well-watered conditions, gs and A would decrease at increasing VPD, 2) temperature will have a more significant effect on A and gs than relative humidity, at any given VPD and soil water condition and 3) A and gs in plants grown in SWD will decrease more strongly with increasing VPD compared to plants grown in well-watered soil.

Methods

Material and growth conditions

During the first four weeks of this study, plants were germinated and maintained at optimal environmental conditions for growth and development. Forty seeds of the most recently developed cotton variety (Gossypium hirsutum Sicot 746B3F) used in commercial growing fields were sown in separate 10 L pots in the environmentally controlled glasshouse facility at
Western Sydney University (Richmond, NSW, Australia). Each pot contained a soil mixture of coco peat, coarse sand, composted pine bark, and composted sawdust purchased from Australian Native Landscapes (NSW, Australia). Nutrients were provided by adding 16 g of Multi-Grow fertiliser (ingredients include 10.1% N, 3.5% P, 5.5% K, 16.3% S and 7.8% Ca) to each pot. All pots were re-watered to full capacity daily. The optimum growth temperature for cotton is 32°C/21°C for day/night (Reddy et al. 2017). These conditions were maintained for four weeks, allowing the plants to develop to the appropriate size for leaf gas exchange measurements. During this period, the holes at the bottom of the pots remained unblocked to avoid soil waterlogging. Height and nodes were measured on a weekly basis during weeks 5 to 8. This study monitored plant growth and developmental stages to determine the effect on cotton associated with SWD.

**Water treatments**

Water treatment started at the beginning of week 5. Plants were divided into two groups, each group receiving different water treatments; one group of pots was watered at 100% field capacity (14 pots of well-watered treatment), while soil water in the other group was maintained at 50% field capacity (14 pots of drought treatment). We randomly allocated seven pots of well-watered soil and seven pots of drought soil in each glasshouse; pots were positioned randomly between water treatments. Both groups contained equivalent numbers of short and tall plants to ensure an equal distribution of similar plant sizes prior to the drought treatment. For the well-watered treatment, water loss between days was replenished daily for plants grown at 100% field capacity. For the drought effect treatment, water was withheld until soil water content dropped below 50% field capacity, and then water was added to maintain 50% field capacity. Plants were maintained at these conditions for two weeks prior to the physiological measurements. Figure 2.2 shows the soil water content measured at the beginning of the day. The study assumed that after re-watering, water did not become available immediately and that gas exchange was measured at the soil water content presented in this figure. During the experiment, there were cloudy days which may have reduced plant water usage under both water treatments.

The amount of water required to replenish the water loss was calculated by weighing pots daily. Prior to placing cotton seed into the pots, soil pots were dried and weighed, and then fully saturated with water and weighed, and these data were used to subsequently calculate
water loss from the pots on a daily basis. A measuring cylinder was used to measure the water for re-watering. Round plastic saucers for 10 L pots were used to keep water in the pots because the soil was dry in the drought treatments and did not completely hold water after the re-watering started. In addition, pots with soil (but no plant) were placed into the glasshouse and used to calculate soil evaporative water loss.

Figure 2.2 Soil water content (SWC, % of Field Capacity) under well-watered treatment and soil water deficit treatment. DAT represents days after water treatment commenced. Values represent mean ± SE of pots weighted each day. For well-watered treatment: 1 to 17 DAT n=22, 18 DAT n=20, 19 to 21 DAT n=18, 22 DAT n=16, 23 DAT n=10, 24 DAT n=7 and 25 DAT n=4. For soil water deficit treatment: 1 to 17 DAT n=21, 18 DAT n=19, 19 to 21 DAT n=17, 22 DAT n=15, 23 DAT n=9, 24 DAT n=7 and 25 DAT n=4.

Physiological measurements

The physiological measurements were conducted in weeks 7 and 8, prior to the early bloom stage. The newly expanded leaf (third or fourth leaf from the apical meristem) was selected for physiological measurements to minimise the effect of leaf age. Leaf gas exchange parameters including $A$, $g_s$ and $E$ were measured at 50% and 100% field capacity over a range
of VPD. The novel approach of this study was separating the individual effects of temperature and RH on VPD and their effects on plant physiological performance. In this study, plants from each water treatment were placed in the glasshouse chamber at 22°C for 30 minutes to acclimate prior to physiology measurements. The LI-6800 portable photosynthesis system (LI-COR, Lincoln, USA) was used to measure the leaf gas exchange parameters at ambient CO₂ of 420 µmol mol⁻¹, saturating PAR of 1500 µmol m⁻² s⁻¹, and the prevailing air temperature of the glasshouse chamber.

VPD can be altered by changing air temperature and maintaining a constant RH, or by changing RH and maintaining a constant temperature (Anderson 1936; Duursma et al. 2014); see Figure 2.1. In this study, the temperatures were set constant and the relative contribution of humidity to VPD on plant performance was investigated. To measure the VPD response, the LI-6800 (LI-COR, Lincoln, USA) leaf cuvette chamber adjusted the relative humidity to generate a range of VPD (approximately 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 kPa) prior to leaf physiological measurements. The Drierite desiccant was checked and replaced regularly; it absorbed high air moisture as VPD increased. High VPD levels were slightly difficult to generate for well-watered pots. The measurement interval was 5 minutes between each VPD increment, and the measurement time was 30 minutes for each plant at each temperature. Measurements began when the room temperature was 22°C, and when measurements were completed, the room temperature was changed to 26°C and plants acclimated to the new temperature for 30 minutes. The same procedures were repeated for 26°C, 30°C and 34°C, representing the range of temperatures commonly observed in cotton fields (Bange et al. 2016). RH in the glasshouse was not controlled in this study and ranged between 50% and 80% throughout the measurement period. Leaves on plants that were used for gas exchange measurements were marked, and then the leaf was removed from the plant for Ψleaf measurement at noon (or before 1 pm) of the following day. A Scholander pressure bomb was used to measure the midday Ψleaf.
Model description

This study used the unified stomatal model (Medlyn et al. 2011) to explore the response of gas exchange parameters to different environmental conditions. These models were used to determine WUE in cotton when exposed to a range of increasing VPD based on the measurements of each parameter under well-watered and SWD conditions. The unified stomatal model (Medlyn et al. 2011) is shown in Eq. (1),

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

where \( g_0 \) and \( g_1 \) are fitted stomatal conductance parameters. Vapour pressure deficit at the leaf surface is indicated by \( D_s \), leaf-to-air atmospheric CO\(_2\) concentration \( C_a \), and net photosynthesis \( A \) (Medlyn et al. 2011). This study used Eq. (1) to estimate \( g_1 \) or leaf diffusive behaviour; the slope that indicates the minimal water loss for a unit of carbon gain. It was noted that the net CO\(_2\) point is affected by temperature and therefore, \( g_1 \) is temperature dependent (Medlyn et al. 2011). Hence, the slope \( g_1 \) was used to compare plant WUE at different temperatures under different water treatments. Medlyn et al. (2011) suggested that an increase in \( g_1 \) will result in decreasing WUE. Thus, this stomatal model was used to examine the effect of different levels of VPD on plant WUE, and also to determine whether SWD has an effect on the model predictions. The results from this model were used to identify the environmental factor (atmospheric drought or soil drought) that had more effect on cotton plant physiological performance.

Statistical analyses

For statistical tests, the effect of SWD on plant heights and number of nodes were repeated measurements, while the effect of water treatment on \( \Psi_{\text{leaf}} \) was not a repeated measurement. Heights, the number of nodes and \( \Psi_{\text{leaf}} \) were compared between water treatments and days after treatment (DAT) by Tukey’s HSD test in RStudio (ver. 3.5.3, RStudio Team 2016). For the correlation between \( \Psi_{\text{leaf}} \) and growth, the linear coefficient \( R^2 \) was used to determine the relationship between \( \Psi_{\text{leaf}} \) and height and between \( \Psi_{\text{leaf}} \) and number of nodes. Outliers were removed as they likely occurred due to errors in measurement techniques and did not represent plant responses. Each measurement was considered to be a replicate.
The physiological data were analysed with RStudio (ver. 3.5.3, RStudio Team 2016), and the car package was used to analyse the analysis of variance (ANOVA). Three-way ANOVA of mixed linear model was performed to test the significance of interactive effects of SWD (as water treatment) and temperature and relative humidity (as VPD components) on gas exchange, including A, gs and E. Water treatment, RH and temperature were treated as independent variables; water treatment was treated as fixed-effect. Plants were used for a random effect. The data were logged where appropriate. The effects of treatments (atmospheric drought or soil drought or combined) on Tleaf-Tair, growth, leaf gas exchange and Ψleaf were significant when the P-value was less than 0.05 (P<0.05). The package ‘Plantecophys’ in RStudio was used to estimate g1 in the unified stomatal model (Eq. (1)) (Duursma 2019). This study used 95% confidence intervals to determine the significance of SWD and temperature effects on the fitted parameter g1. The effect was considered significant when the confidence interval of each g1 value did not overlap with other g1 values within the same temperature or water treatment.

Results

Effects of vapour pressure deficit and soil water deficit on leaf gas exchange parameters

As expected, gs was lower in the SWD treatment, compared to the well-watered treatment, and increasing VPD further reduced gs. The ANOVA results (Table 2.1) indicated that water treatment had a significant effect on gs (P<0.001). For atmospheric factors, VPD (or the reduction in RH) and temperature, either individual or combined, affected gs significantly (P<0.001). Consequently, the effect of water treatment appeared to have a significant role in altering the response of gs to VPD and temperature; there was a two-way interactive effect between water treatment and VPD (P<0.001) or temperature (P=0.035). To demonstrate, Figure 2.4 clearly shows that gs in the SWD treatment was significantly lower than gs in the well-watered treatment. Furthermore, gs was reduced with increasing VPD and gs was lowest with increasing VPD in the SWD treatment (Figure 2.4). In general, gs at low VPD was higher under well-watered treatment, even though there was a large variation among individual plants (Figure 2.3). This variation started to decrease as the VPD increased across all temperatures. Nevertheless, gs did not shut completely at high VPD under either water treatment.
Figure 2.3 Response of stomatal conductance ($g_s$) to vapour pressure deficit (VPD$_{leaf}$) in different water treatments at different measurement temperatures (22, 26, 30 and 34°C). Each line represents repeated measurements of $g_s$ on an individual leaf with varying VPD.
Figure 2.4 Effects of vapour pressure deficit and temperatures (22, 26, 30 and 34°C) on stomatal conductance \( (g_s) \) in well-watered (left) and soil water deficit (right) treatments. Points and bars are means and standard errors, respectively, of 14 plants for each water treatment.

There was a significant increase in \( E \) at lower VPD and \( E \) was lower under SWD treatment at all temperatures (Figure 2.5; Table 2.1). Higher temperatures induced higher \( E \), such that the highest \( E \) among plants was observed at 34°C under well-watered treatment, as well as the highest variation in \( E \) (Figure 2.6). The results in Figures 2.5 and 2.6 also show that the reduction in \( E \) was not obvious when VPD levels exceeded 2.0 kPa or 2.5 kPa; \( E \) was still high after the maximum point was reached. Nevertheless, \( E \) under SWD treatment did not differ at each temperature, indicating that there was an interactive effect between water treatment and VPD (\( P=0.008 \)) or temperature on \( E \) (\( P<0.001 \)).

Figures 2.4 and 2.6 demonstrate that the reduction in \( g_s \) may be associated with lower \( E \). The \( g_s \) was decreasing in response to increasing VPD while \( E \) continued to increase and then decrease relatively. In general, the effect of SWD altered the response of leaf gas exchange parameters to increasing VPD. The summary of ANOVA results in Table 2.1 showed that there were no three-way interactive effects between temperature, VPD and water treatment on \( g_s \) (\( P=0.151 \)) and \( E \) (\( P=0.146 \)).
Figure 2.5 Transpiration ($E$) response to vapour pressure deficit (VPD$_{leaf}$) and water treatments at different measurement temperatures (22, 26, 30 and 34°C). Each line represents repeated measurements of $E$ on an individual leaf as VPD varies.
Figure 2.6 Effects of vapour pressure deficit and air temperature (22, 26, 30 and 34°C) on transpiration (E) in well-watered (left) and soil water deficit (right) treatments. Points and bars are means and standard errors, respectively, of 14 plants for each water treatment.

Temperature and VPD had a significant effect on A (P=0.004 and P<0.001, respectively). The results in Figure 2.7 demonstrate that there was a significant effect of VPD on A, but there was no significant effect of soil water treatment on A (P=0.116). This figure also shows that there was a large variation of A amongst plants. It was also expected that A at low VPD (1.5 and 2 kPa) would be higher at 34°C under well-watered treatment, but Figure 2.8 shows that the highest A at most VPD levels was observed at 30°C. We observed that there were no interactive effects between water treatment and VPD or temperature on A (P=0.439).
Figure 2.7 Photosynthesis (A) response to vapour pressure deficit (VPDleaf) and water treatments at different measurement temperatures (22, 26, 30 and 34°C). Each line represents repeated measurements of A on an individual leaf as VPD varies.
Figure 2.8 Effects of vapour pressure deficit and measurement temperature (22, 26, 30 and 34°C) on photosynthesis (A) in well-watered (left) and soil water deficit (right) treatments. Points and bars are means and standard errors, respectively, of 14 plants for each water treatment.

Table 2.1 Analysis of variance (ANOVA) for the interactive effects of drought, vapour pressure deficit (VPD) and temperature on stomatal conductance ($g_s$), photosynthesis (A), transpiration (E), intercellular CO$_2$ ($C_i$), ratio of intercellular CO$_2$ ($C_i$) to ambient CO$_2$ ($C_a$) and intrinsic water use efficiency (WUE$_i$) in cotton. Values in the table represent P-values; significant results (P<0.05) are indicated in bold type and italics indicate marginal significance. The results are based on 14 samples for each water treatment.

<table>
<thead>
<tr>
<th>Factors</th>
<th>$g_s$</th>
<th>A</th>
<th>E</th>
<th>$C_i/C_a$</th>
<th>WUE$_i$</th>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
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<td>&lt;0.001</td>
<td>0.238</td>
<td>0.143</td>
</tr>
<tr>
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<td>0.250</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VPD × Water treatment</td>
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<td>0.008</td>
<td>0.008</td>
<td>0.006</td>
</tr>
<tr>
<td>Temperature × Water treatment</td>
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<td>0.789</td>
<td>0.003</td>
<td>0.105</td>
<td>0.070</td>
</tr>
<tr>
<td>Temperature × VPD × Water treatment</td>
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<td>0.439</td>
<td>0.146</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
The effect of soil water deficit on growth and leaf water potential

SWD treatment, either individually or combined with treatment duration, reduced height and number of nodes significantly (P=0.043 and P=0.031, respectively). The plants grown under SWD treatment were significantly shorter than those grown under well-watered treatment by ca. 8% and 13% at 14 and 21 days after treatment (DAT), respectively (Figure 2.9). The SWD treatment significantly decreased the number of nodes at 7, 14 and 21 DAT (Figure 2.9).

Figure 2.9 The effect of soil water deficit treatment on growth, height (left) and the number of nodes (right), measured on a weekly basis. Plots and bars are means and standard errors, respectively. DAT is days after water treatment commenced. Number of samples for height and number of nodes measured on days 0, 7, 14 and 21 were 22, 22, 22 and 18 plants for well-watered treatment and 21, 21, 21 and 17 for soil water deficit treatment, respectively. Significant effects with P-values <0.05, <0.01 and <0.001 are represented by *, ** and $$$, respectively.

Although SWD reduced growth significantly, midday $\Psi_{\text{leaf}}$ was unaffected by SWD treatment at any DAT (P=0.827), see Figure 2.10. There was no interactive effect between water treatment and its duration on midday $\Psi_{\text{leaf}}$ (P=0.355). Moreover, the results suggested that the growth rate may be associated with the variation in $\Psi_{\text{leaf}}$. There was a weak relationship between $\Psi_{\text{leaf}}$ and height ($R^2 = 0.20$) and between $\Psi_{\text{leaf}}$ and number of nodes ($R^2 = 0.14$) (Figure 2.11). The number of nodes did not affect $\Psi_{\text{leaf}}$ (P=0.160), whereas height was significantly affected by $\Psi_{\text{leaf}}$ (P=0.012). There was a marginal interactive effect between
water treatment and the number of nodes on $\Psi_{\text{leaf}}$ ($P=0.055$), but not between water treatment and height ($P=0.254$).

Figure 2.10 Midday leaf water potential, under well-watered treatment and soil water deficit treatment, measured daily. DAT is days after water treatment commenced. Plots and bars are means and standard errors, respectively, of number of samples measured each day. For well-watered treatment, 17, 18 and 21 DAT $n=2$, 22 DAT $n=6$, 22 and 24 DAT $n=3$ and 25 DAT $n=4$. For soil water deficit treatment, 17, 18, 21 and 23 DAT $n=2$, 22 DAT $n=6$, 24 DAT $n=3$ and 25 DAT $n=4$.

Figure 2.11 The effect of height (left) and number of nodes (right) on midday leaf water potential ($\Psi_{\text{leaf}}$) in well-watered and soil water deficit treatments. Number of samples for well-watered and soil water deficit treatments were 21 and 20 plants, respectively.
Effect of soil water deficit on the relationship of vapour pressure deficit components

The difference between $T_{\text{leaf}}$ and $T_{\text{air}}$ was found to be significantly affected by the reduction in RH (Figure 2.12) with the temperature difference decreasing at increasing VPD ($P=0.047$). At most VPD levels, $T_{\text{leaf}}$ of plants grown under well-watered treatment was lower compared to $T_{\text{leaf}}$ of plants grown under SWD treatment (Figure 2.12). However, it was unlikely due to soil water content as there was no significant effect of water treatment on the temperature difference ($P=0.376$). The effect of temperature on the $T_{\text{leaf}}$-$T_{\text{air}}$ difference was not significant ($P=0.827$). There were no two-way or three-way interactive effects of SWD and VPD or temperature on $T_{\text{leaf}}$-$T_{\text{air}}$.

Soil water content had some effect on VPD$_{\text{leaf}}$ generation, while it had no effect on VPD$_{\text{air}}$ generation. We observed that VPD$_{\text{air}}$ was not clearly separated between each VPD increment under the well-watered (Figure 2.13a) and SWD treatments (Figure 2.13b) as no gap was observed between VPD levels. On the other hand, our results showed that the increment of VPD at leaf surface under SWD treatment (Figure 2.13d) was noticeably more separated compared to VPD$_{\text{leaf}}$ under the well-watered treatment (Figure 2.13c). Due to technical limitations of the LI-6800 (LI-COR, Lincoln, USA), it was not possible to generate high VPD (e.g. 3.5 and 4.0 kPa) at 30°C and 34°C for cotton grown under well-watered treatment compared to SWD treatment (see Figure 2.11).
Figure 2.12 The difference between leaf surface temperature ($T_{leaf}$) and air temperature ($T_{air}$) at different temperatures (shown above graphs) and soil water levels across vapour pressure deficit (VPD) levels. Points and bars are means and standard errors, respectively, of 14 plants for each water treatment.
Figure 2.13 The relationship between air temperature ($T_{air}$), vapour pressure deficit ($VPD_{leaf}$) and relative humidity (RH) at different soil water treatments; well-watered treatment (a and c) and drought treatment (b and d).
Effect of soil water deficit on intrinsic water use efficiency at different vapour pressure deficit levels

The effect of temperature and VPD on WUE$_i$ was more significant compared to the individual effect of water treatment (Table 2.1). There was a significant effect of VPD and temperature, either individually or combined, on WUE$_i$ (Table 2.1). WUE$_i$ increased significantly at increasing VPD under both water treatments (Figure 2.14). Moreover, elevated temperature reduced WUE$_i$; for example, the highest and lowest WUE$_i$ were observed at 22°C and 34°C, respectively, under both water treatments (Figure 2.14). We observed that the individual effect of water treatment on WUE$_i$ was not significant (P=0.143; Figure 2.14). There was a significant interactive effect of VPD, temperature and SWD on WUE$_i$ (Table 2.1). Moreover, the interactive effect of water treatment and temperature on WUE$_i$ was marginally significant (P=0.070), whereas the interactive effect of water treatment and VPD on WUE$_i$ was significant (P=0.006).

![Figure 2.14](image-url) Effects of vapour pressure deficit and measurement temperature (22, 26, 30 and 34°C) on intrinsic water use efficiency (WUE$_i$) in well-watered (left) and soil water deficit treatments (right). Points and bars are means and standard errors, respectively, of 14 plants for each water treatment.
Effects of soil water deficit and vapour pressure deficit on intercellular CO₂ and ambient CO₂ ratio at different temperatures

The reduction in the $C_i/C_a$ ratio was due to the increase in VPD under both water treatments, indicating that the $C_i/C_a$ ratio was not affected by water treatment, either alone or combined with temperature (Figure 2.15). In contrast, there was a significant effect of VPD and temperature, either separated or in concert, on the $C_i/C_a$ ratio (Table 2.1). We observed that elevated temperature increased the $C_i/C_a$ ratio and that increasing VPD decreased the $C_i/C_a$ ratio (Figure 2.15).

Figure 2.15 Response of intercellular CO₂ ($C_i$) to ambient CO₂ ($C_a$) ratio to vapour pressure deficit across different measurement temperatures (22, 26, 30 and 34°C) under well-watered (left) and soil water deficit (right) treatments. Points and bars are means and standard errors, respectively, of 14 plants for each water treatment.
Effects of vapour pressure deficit and soil water deficit on the unified stomatal model and leaf diffusive control

**Figure 2.16** Measurements of stomatal conductance plotted against stomatal predictability under both water treatments, separated by temperature. The black line is used for illustration purposes to represent a fitted linear regression $g_s = A/(C_a \sqrt{D_s})$ to all data from four temperatures and both water treatments combined.

The present study found that SWD moderately affected the predictability of the unified stomatal model (Eq. (1)). The blue and red lines representing repeated measurements of stomatal conductance plotted against the stomatal index on an individual leaf did not align
with the fitted linear regression $g_s = A/(C_a\sqrt{D_s})$ line and model predictability was affected by temperature (Figure 2.16). The most obvious difference was observed at 34°C where the blue line was steep, so that it occurred above the fitted linear regression line. On the other hand, soil water content may also have an effect on model predictability as both the blue and red lines appear to diverge from the fitted linear regression line (Figure 2.16).

The $g_1$ in cotton grown under SWD treatment was significantly lower than cotton grown in well-watered treatment at 22°C, 26°C and 34°C and marginally lower at 30°C (Figure 2.17). For the well-watered treatment, there was no significant effect of temperature on $g_1$ at 22, 26 and 30°C while $g_1$ increased significantly at 34°C. For the SWD treatment, the temperature effect on $g_1$ between 26°C and 30°C was not significant. The significant effect of temperature on $g_1$ in SWD treatment was observed between 22°C and 26°C and between 30°C and 34°C.

**Figure 2.17** The fitted parameter $g_1$ or leaf diffusive behaviour obtained from the unified stomatal model, see Eq. (1). Points and bars represent means and 95% CI, respectively. Data are significantly different when 95% CIs do not overlap.
Discussion

Rising VPD reduced $g_s$, $A$ and $E$ whereas SWD reduced $g_s$ and $E$. The interactive effect of elevated VPD and SWD reduced $g_s$ and $E$, but this interactive effect did not affect $A$. The SWD treatment reduced cotton growth but did not affect the relationship of VPD components and the midday $\Psi_{\text{leaf}}$. We found that RH was the key factor that affected $g_s$ and temperature affected $A$. In addition, $A$ was less sensitive to environmental adjustment compared to $E$, which was relatively responsive to the change in atmospheric condition. The key findings of this study are that SWD decreased $g_s$ and rising VPD intensified this reduction and VPD had more impact on leaf gas exchange than soil water content. These findings allow us to conclude that whole-plant WUE in cotton plants (determined by $g_1$) is likely to decrease under the projected drier and warmer future climate.

Effects of vapour pressure deficit on gas exchange parameters

Our first objective was to determine the gas exchange response of cotton to VPD and we found that the response of stomata to changing environmental conditions was the key factor in controlling $A$ and $E$. In this study, the LI-6800 (LI-COR, Lincoln, USA) was used to generate a range of VPD levels, beginning at low VPD and ending at high VPD, by reducing RH to generate each increment of VPD at each measurement temperature. Therefore, although $g_s$ was affected by elevated temperatures, increased VPD (or decreased RH) appeared to induce stomatal closure more significantly. The temperature effect on $g_s$ was inconsistent with a study that found no significant effect of temperature on $g_s$ but a significant effect of VPD on $g_s$ (Li et al. 2018). However, our results are similar to other studies that found the response of $g_s$ was significantly correlated with VPD (Baker et al. 2007) and that increasing VPD decreased $g_s$ under different temperatures (Duursma et al. 2013). Another study suggested that reduced $g_s$ in response to increasing VPD was a function of stomatal control of $\Psi_{\text{leaf}}$ when exposed to atmospheric drought (Oren et al. 1999).

We further demonstrated that stomatal closure was more likely to be regulated by the change in RH rather than the change in temperature, with rising VPD. Our results showed that the high variation among individual plants began to decrease as the VPD increased across all temperatures, which is consistent with Duursma et al. (2013), indicating that rising VPD generated more significant effects on $g_s$ than temperature. For example, we found that rising VPD reduced $g_s$ whether the temperature was high or low (Figure 2.4), suggesting that $g_s$ did
not directly respond to VPD, but more likely to the reduction in RH. Similar observations were made by Ball, Woodrow & Berry (1987), suggesting that a reduction in air moisture had more impact on $g_s$ than temperature variation. Therefore, we reject our second hypothesis that temperature would generate a greater reduction in $g_s$ than RH.

We found that $g_s$ and $E$ decreased at increasing VPD, whereby $E$ and $g_s$ were considerably reduced when VPD rose to 2.0 kPa or 2.5 kPa, indicating that there was an effect of stomatal closure on $E$ reduction (Figures 2.4 and 2.6). These results were consistent with other studies (e.g. Ambrose et al. 2010; Devi & Reddy 2018; Marchin et al. 2016) which also found that the reduction in $E$ was associated with stomatal closure. Although we did not find a clear dissociation between $g_s$ and $E$ at any given VPD, Marchin at al. (2016) found that the correlation between these two parameters may be reduced if $g_s$ becomes acclimated to rising VPD. Marchin et al. (2016) demonstrated that $E$ measured after the stomata acclimatised to the increasing VPD was two-fold higher than the measurement of $E$ made before stomatal acclimatisation. Future research should investigate the response of $E$ to increasing VPD as a result of stomatal acclimatisation to better understand the gas exchange response of cotton to drier atmospheres.

For VPD components, we found that lower temperature and moist air decreased $E$. Our results showed that immediate reduction in $E$ was mainly observed at 22°C and rising VPD; this condition may have restricted $g_s$ and resulted in increased leaf diffusion resistance (Drake and Salisbury 1972). On the other hand, we observed that the difference in $E$ between the lowest and highest VPD levels at 26, 30 and 34°C was lower than at 22°C, while $E$ measured at 30 and 34°C and VPD of 4.0 kPa was higher than $E$ measured at 1.5 kPa. Since there was an interactive effect between temperature and VPD on $E$, this finding suggests that rising air temperature and drier air reduced leaf diffusion resistance (Drake, Raschke & Salisbury 1970) in cotton which induced higher $E$. Moreover, $E$ did not reduce further, regardless of the rising VPD at 26, 30 and 34°C, which may be due to stomata not fully closing so that leaves transpire at a higher rate under high temperatures and high VPD levels.

Increasing VPD generally reduced $A$ and the variation among individuals was significantly large. The reduction in $A$ was likely due to the reduction in $C_i$ as a result of stomatal closure (Duursma et al. 2013). Duursma et al. (2013) also found that $A$ may be relatively insensitive to atmospheric drought, compared to $g_s$ and $E$, which explains why the variation among
individuals was significantly large. Nevertheless, we found support for our hypothesis that $A$ would decrease with increasing VPD under well-watered conditions.

On the other hand, we observed that both increased temperature and reduced RH contributed to a reduction in $A$ across VPD. Our results demonstrated that $A$ increased at temperatures from 22°C to 30°C then decreased at 34°C, and that $A$ did not respond correspondingly to increasing VPD levels at 34°C. This finding suggested that $A$ may be dependent on $C_i$ availability at lower temperatures (and increasing VPD) as a response to the reduction in $g_s$. Then, $A$ would decrease when the temperature exceeded the optimum air temperature, which may be due to restrictions in Rubisco carboxylation (Li et al. 2018). In addition, Li et al. (2018) also observed that there was a moderate relationship between Rubisco carboxylation rate and $A$ (temperature dependent) and between $g_s$ and $A$ (RH dependent).

**Effects of soil water deficit on cotton growth**

SWD significantly decreased plant growth, particularly during the later period of the experiment, which is similar to the results of Broughton et al. (2017) and Hejnák et al. (2015). However, these findings are inconsistent with Pettigrew (2004) who found that the effect of SWD on cotton plants at an early growth stage was insignificant for height and number of nodes. This variation may be due to the difference in sample numbers between studies, given that the sample number was substantively larger in our study.

The SWD treatment of soil water content at 50% field capacity affected growth significantly; however, the soil water content inadvertently reached nearly zero for a few days during commencement of the water treatment. This short severe drought condition may have generated some impacts on the number of nodes, which was significantly different between water treatments only after 7 DAT. Nevertheless, the general findings of the present study on the effect of SWD on growth is similar to wheat (Qiao et al. 2010); the number of spikes in wheat growth was decreased by SWD, except that height was not affected. In contrast to the treatment duration effect, another study has shown that maize growth was also significantly reduced by SWD, but the difference in growth between water treatments was more significant during later growth stages (Wen, Li & Li 2015).
**Effect of soil water deficit on the relationship of VPD components**

Although SWD may have marginally decreased $T_{leaf}$, it did not affect $T_{leaf}-T_{air}$ at increasing VPD. This finding suggests that $T_{leaf}$ was controlled by atmospheric factors rather than the availability of soil water content. In this case, RH appeared to be the main contributing factor to the temperature difference. However, Elfeel (2017) observed a significant effect of SWD on $T_{leaf}-T_{air}$ (Elfeel 2017) and suggested that the higher $T_{leaf}$ under SWD treatment was caused by increasing VPD and soil water shortage (Elfeel 2017). Nevertheless, the variation between the previous study and the present study may be due to the difference in the amount of water provided to plants.

There was no significant effect of water treatment on the temperature difference, which highlighted that soil water content might not have affected the atmospheric drought. We did not observe a clear difference in VPD generation under both water treatments at leaf and atmospheric scales. However, the LI-6800 (LI-COR, Lincoln, USA) was unable to generate high VPD levels efficiently under well-watered conditions because the plants were well hydrated and released water via transpiration, which increased air moisture. Cotton under well-watered conditions may not control water usage and, therefore, has high rates of transpiration, particularly in high VPD environments (Vadez et al. 2012). Overall, RH was the most important factor that regulated plant response to variable VPD.

**Effect of soil water deficit on leaf gas exchange**

SWD decreased $g_s$ and $E$ but not $A$, which is similar to findings by Broughton et al. (2017) which demonstrated that SWD significantly reduced $g_s$. We did not observe a significant reduction in $A$, although we expected SWD to reduce $A$ as a result of stomatal closure (Chastain et al. 2014) which decreases $C_i$ availability (Ennahli & Earl 2005). On the other hand, Chastain et al. (2014) found that $A$ in dry-land cotton was significantly lower compared to $A$ in irrigated cotton, though the soil water content may have been much lower in their study. Ennahli and Earl (2005) found that relative soil water contents at 25% and 15% can drastically reduce $A$. Hence, a reduction in $A$ under severe drought conditions may also cause embolism formation in xylem (Li et al. 2019), increased dark respiration (Chastain et al. 2014) or reduced amounts of ribulose bisphosphate (RuBP) (Flexas & Medrano 2002).
Effects of soil water deficit on leaf hydraulic status

SWD reduced plant growth, but did not change $\Psi_{\text{leaf}}$, indicating some adjustment at the whole-plant scale. Ennahli and Earl (2005) found that decreasing soil water content led to a significant reduction in $\Psi_{\text{leaf}}$ but the experimental SWD was much more severe in that study compared to our study. As for the present study, the lack of change in $\Psi_{\text{leaf}}$ could be due to leaf osmotic adjustment during the SWD treatment and therefore, maintenance of leaf turgor (Pettigrew 2004). Young leaves are more tolerant to hydraulic stress than older leaves (Chastain et al. 2016), so our newly expanded leaves that were selected for physiological measurements may have been more resilient than older leaves on the plant. Most likely, soil water content at 50% field capacity was sufficient to reduce growth, but insufficient to substantially alter $g_s$ (compared to $g_s$ under prolonged and severe drought exposure) and subsequently alter midday hydraulic status.

Domec et al. (2004) and Li et al. (2019) observed that stomatal closure occurred before increased xylem tension and lower $\Psi_{\text{leaf}}$ under decreasing soil water content. Both studies found that the xylem tension started to increase significantly after the stomata were fully closed. In this case, the incomplete stomatal closure observed in the present study may not have allowed the tension in xylem to increase when soil water content was at 50% field capacity. Therefore, the $\Psi_{\text{leaf}}$ between water treatments across the measured days remained similar.

Chastain et al. (2014) suggested that predawn $\Psi_{\text{leaf}}$ was the more suitable measure in determining plant water status under SWD conditions. Additionally, a study also showed that predawn $\Psi_{\text{leaf}}$ was well correlated with $A$ and $g_s$ (Williams & Araujo 2002). However, predawn $\Psi_{\text{leaf}}$ was not recommended for examining hydraulic stress in plants grown under well-watered conditions due to the abundant availability of water (Ameglio et al. 1999). Both predawn $\Psi_{\text{leaf}}$ and midday $\Psi_{\text{leaf}}$ are affected by soil water content; however, predawn $\Psi_{\text{leaf}}$ appeared to be less associated with environmental factors (Correia & Martins-Loucao 1995). The same study also indicated that midday $\Psi_{\text{leaf}}$ was affected by temperature and $E$ and midday $\Psi_{\text{leaf}}$ fluctuated more between seasons compared to predawn $\Psi_{\text{leaf}}$. Therefore, future research should observe predawn $\Psi_{\text{leaf}}$ and midday $\Psi_{\text{leaf}}$ for comparison when examining the relative effect of soil drought and atmospheric drought (VPD) on plant physiology.
Inter
teractive effects of vapour pressure deficit and soil water deficit on leaf gas exchange

We observed that SWD generally induced stomatal closure (Fasehun 1979) and the response of $g_s$ to increasing VPD was less obvious under this soil water level. This result suggested that $g_s$ might be more adaptive to dry conditions and that $g_s$ in plants grown under SWD were less sensitive to increasing VPD compared to $g_s$ in plants grown under the well-watered treatment. Oren et al. (1999) found that plants that exhibit low $g_s$ at low VPD are less sensitive to increasing VPD. Our result is also consistent with Domec et al. (2009), which suggested that the effect of SWD should lower $g_s$. Furthermore, Ambrose et al. (2010) demonstrated a significant reduction in $g_s$ at increasing VPD and lower soil water content conditions, which is the same as in the present study. The overall results supported our hypothesis that $g_s$ in plants grown under SWD treatment would decrease with rising VPD to a greater degree than $g_s$ in plants grown under well-watered conditions.

Our results demonstrate that the change in $E$ under increasing VPD and SWD treatment was less varied than $E$ under well-watered conditions, suggesting that an interactive effect of SWD and atmospheric drought decreased $E$. We observed that the maximum $E$ under SWD at each temperature is lower than well-watered $E$ but occurred at higher VPD levels. This finding suggested that SWD and rising VPD may contribute to lowering water loss through transpiration in cotton. Our results also showed that this cotton variety (*Gossypium hirsutum* Sicot 746B3F) does not exhibit a clear threshold for the response of $E$ to rising VPD, as $E$ reached the maximum point and started to decrease, particularly at 30°C and 34°C, under both water treatments. Therefore, $E$ of this cotton variety is classified as the non-limiting type (Devi and Reddy 2018), and it might instead lose more water under a warmer and drier environment. Moreover, losing water through transpiration is an indication that this cotton variety may not be efficient in conserving water (Belko et al. 2013) at high temperatures.

We did not observe an interactive effect of rising VPD and SWD on $A$. The insensitivity of $A$ to drought may indicate that cotton has the ability to maintain its photosynthetic capacity in SWD conditions after a certain period by reducing $g_s$ to control $C_i$ (Rowland et al. 2015). Similarly, the overall trend of $A$ under SWD conditions was slightly decreased across VPD levels, which was also possibly correlated with the stomatal response to increasing VPD and resulted in the decrease in $C_i$ (Duursma et al. 2013). The results, however, did not support the
third hypothesis of our study because we did not observe a significant reduction in SWD A under rising VPD compared to well-watered A.

WUE$_i$ was inversely correlated with $g_s$ in that with increasing VPD, we observed that $g_s$ was decreasing and WUE$_i$ was increasing. As aforementioned, we observed that $g_s$ was significantly decreased at increasing VPD under both water treatments, but A was not affected by water treatment. The similarity in WUE$_i$ between water treatments may be, therefore, due to the insensitivity of A. Our results also indicated that elevated temperatures reduced WUE$_i$ significantly, whereas WUE$_i$ remained almost the same under both water treatments. WUE$_i$ in cotton is expected to decrease under a warmer and drier climate. With no effect of SWD on WUE$_i$, Zhang et al. (2019) suggested that rising VPD may have more control on WUE$_i$ than soil water content.

As expected, we observed that the $C_i/C_a$ ratio was correlated with WUE$_i$. For example, the lowest $C_i/C_a$ ratio was observed at 22°C and VPD 3 kPa under both water treatments, while the highest WUE$_i$ was observed under the same condition. This result is in line with the findings from Brodribb (1996) which described that the minimum $C_i/C_a$ ratio is proportional to WUE$_i$. Thus, the $C_i/C_a$ ratio may be used to determine the maximum WUE$_i$ in plants under different water treatments (Brodribb 1996).

Next, we investigated change in the $C_i/C_a$ ratio and found that this ratio was dependent on $g_s$ rather than carbon assimilation. This change demonstrated that a reduction in the $C_i/C_a$ ratio corresponded with a reduction in $g_s$ at increasing VPD under both water treatments (Figures 2.4 and 2.15). This finding is in line with Broughton et al. (2017), which found an association between the $C_i/C_a$ ratio and stomatal closure (without VPD effects). Duursma et al. (2013) also suggested that $C_i$ decreased as a response of $g_s$ exposed to increasing VPD. Moreover, an interactive effect of SWD and VPD significantly affected the $C_i/C_a$ ratio and $g_s$. The $C_i/C_a$ ratio was correlated with $g_s$ as stomatal closure reduced the $C_i/C_a$ ratio (Tan et al. 2017). Therefore, a reduction in RH can generally result in a lower $C_i/C_a$ ratio.

In contrast, we also observed inconsistencies between the response of the $C_i/C_a$ ratio and $g_s$. For instance, the results indicated that SWD treatment reduced $g_s$ significantly, whereas the $C_i/C_a$ ratio did not differ significantly between water treatments. Nevertheless, this finding is similar to another study which indicated that the $C_i/C_a$ ratio was marginally reduced under
low soil water content (Brodribb 1996). Moreover, $g_s$ was significantly affected by temperature, which was opposite to the response of the $C_i/C_a$ ratio. This variation, and the fact that both $g_s$ and the $C_i/C_a$ ratio did not reach zero under all treatments, may support the unusual increase of $A$ at 30°C and 34°C under the well-watered treatment (see Figure 2.8). Additionally, $A$ may have become non-stomatal limited, particularly in the late afternoon (Tan et al. 2017).

**Effect of vapour pressure deficit and soil water deficit on stomatal diffusive behaviour**

Previously, we indicated that the plants acclimatised to the treatment; however, the stomatal model appeared to disagree and $g_s$ under both water treatments did not acclimatised to elevated temperature and VPD. Although the ANOVA results indicated that there was a significant effect of temperature on $A$, it cannot be concluded that higher temperature was the individual factor that contributed to higher $A$. Our results showed that high temperature and soil water availability might have stimulated $A$ and $g_s$, which resulted in an increase in stomatal diffusive response (i.e. $g_s = A/(C_a \sqrt{D_s})$) and an equivalent increase in $g_s$, respectively. Next, we observed the unusual response of $g_s$ and the stomatal diffusive response at 34°C; such responses may be due to the relative effects of high temperature and high RH on $g_s$ under well-watered treatment. Additionally, the variation in this relationship could be caused by the effect of the large variation in $A$ amongst individuals. Lastly, the result also showed that well-watered $A$ was slightly lower at 30°C and 34°C compared to 22°C and 26°C; this finding was inconsistent with Duursma et al. (2013), which showed that higher temperature increased $A$.

SWD may have limited or controlled WUE in cotton at lower temperatures, whereas high temperatures can reduce the efficiency in plant water usage for both water treatments. To demonstrate, $g_t$ under SWD treatment was lower than $g_t$ under well-watered treatment; however, $g_t$ under both water treatments clearly increased at 34°C (Figure 2.17). Under SWD treatment, stomata responded to conserve water as a result of water limitation. In contrast, the higher $g_t$ under the well-watered treatment suggested that cotton loses more water as soil water content becomes abundant. This finding is consistent with Lin et al. (2015), which found that the increase in $g_t$ represented lower WUE. Since cotton is classified as a C3 crop, $g_t$ was expected to be higher under well-watered conditions (Lin et al. 2015).
On the other hand, the significant elevation of $g_1$ at 34°C may be due to the temperature being slightly over the optimum growth temperature for cotton (Bange et al. 2016). Additionally, it could be that $g_1$ is temperature dependent as it is equivalent to the amount of water required for a unit of carbon gain and net CO$_2$ exchange point. The net CO$_2$ exchange point is affected by temperature and therefore, $g_1$ is temperature dependent (Medlyn et al. 2011; Lin et al. 2015). Consequently, stomatal behaviour under high soil water content and high temperatures will likely lead plants to lose more water. Both atmospheric factors (i.e. temperature and RH) and soil drought appeared to have significant roles in regulating water use in cotton, depending on which factor has a greater effect on the physiological function. Nevertheless, another study has suggested that atmospheric factors seem to have a greater effect on plant physiological response than soil water content (Correia and Martins-Loucao 1995). To conclude, $g_1$ indicated that plant water usage was temperature dependent and the response was negatively affected. The overall results of $g_1$ strongly suggested that drier and higher temperature atmospheric conditions could lead to higher whole-plant water use.

Future research

Drought is a major problem for the agricultural industry. Future research should investigate the effect of soil water levels below 50% field capacity, which will allow us to observe the interactive effects of SWD and VPD on cotton under more severe drought environments, which are expected in future climates. Greater numbers of plants are required so that $\Psi_{\text{leaf}}$ can be destructively measured at predawn and midday, on plants that are exposed to variable VPD and temperature conditions during physiology measurements.

Future research should observe the response of $\Psi_{\text{leaf}}$ to long-term exposure to atmospheric drought and soil drought conditions to determine which is the main effect on plant hydraulic status and gas exchange. A previous study has shown that $\Psi_{\text{leaf}}$ was significantly lower under SWD treatment and may have an effect on gas exchange parameters (Fasehun 1979). Another study indicated that atmospheric drought might have been the main contributor to the change in midday $\Psi_{\text{leaf}}$ compared to soil drought (Correia and Martins-Loucao 1995). Li et al. (2019) found that a reduction in $\Psi_{\text{leaf}}$ can generate a negative effect on stomatal apertures and the reduction of $g_s$ occurred before the decrease in $\Psi_{\text{leaf}}$. 
Future research should include plants grown at low and high VPD and measure the physiological functions at these conditions to compare the adaptability of cotton between moist air and dry air conditions under different soil water levels. Additionally, the measurement time should be longer to allow plant acclimation to each incremental level of VPD. It is important to allow stomata to adjust to each VPD increment because $g_s$ in cotton has different sensitivity responses to increasing VPD under different soil water treatments. The results also indicated that there may be a measurement time effect on gas exchange measurements (Tan et al. 2017) and therefore, future research should ensure that all leaf gas exchange is completed within the same time-frame; e.g. measuring leaf gas exchange between 9 am and 1 pm for all treatments. Future research should also observe these effects during the productive stage. Lastly, the LICOR-6800 is a newly developed photosynthesis system, and it is important that operators are well-trained to ensure that all data are collected accurately.

**Conclusion**

Our study expands the understanding of how VPD affected plant physiology by separating temperature and RH and whether SWD has a role in altering this relationship. We found that rising VPD reduced $g_s$, $A$ and $E$. However, the key finding of this study was that SWD decreased $g_s$ and rising VPD exacerbated this reduction. We also found that SWD did not affect the relationship between VPD components and that each VPD component can affect plant physiology, either individually or combined. The results further indicated that the reduction in RH had a stronger effect on $g_s$ than temperature, regardless of soil water content. In addition, the stomatal model indicated that WUE in well-watered cotton was reduced at high temperature at given VPD levels. The SWD treatment did not alter plant hydraulic status, but significantly reduced height and number of nodes. This type of study should be further investigated as it has the potential to further our understanding of crop adaptation to drier and warmer climates, particularly in dry-land irrigation cotton farming systems.
Acknowledgement

This study was supported by CRDC grant (CSP-1804; CIs David Tissue and Katrina Broughton) and Western Sydney University as part of the Master of Research thesis. The LICOR-6800 photosynthesis systems were provided by CSIRO and ANU. We thank Dr Russell Thomas for RStudio consultation.
Chapter 3

Investigating the source-sink relationship in cotton (*Gossypium hirsutum* L.) under elevated CO2 and elevated temperature

Introduction

The source-sink relationship can determine the response of plant biomass partitioning to environmental conditions, and reproductive part removal is one of the methods that is widely used to investigate this partitioning. Specifically, sink manipulation has been investigated in crops such as coffee (DaMatta et al. 2008), wheat and triticale grains (Ballesteros-Rodriguez et al. 2019) under current environmental conditions at field scale. These studies found that there was a significant change in biomass partitioning between vegetative and reproductive components. DaMatta et al. (2008) investigated the effect of sink manipulation on biomass partitioning in coffee trees and found that coffee trees with half of their fruit load removed grew longer branches, increased fruit biomass and reduced their fruit shedding rate. Their findings are in line with Wardlaw (1990) who summarised that plants prioritise the growth of reproductive parts over vegetative parts under ambient conditions. Moreover, Ballesteros-Rodriguez et al. (2019) found that grain removal (approximately 50% of spikelets were removed) increased the weight of individual grains in triticale grain but not in wheat. DaMatta et al. (2008) also indicated that fruit removal had no effect on leaf gas exchange activity. Although Lemoine et al. (2013) indicated that the source-sink relationship can be affected under changes in environmental conditions, only a limited number of studies have investigated the effect of climate change on the source-sink relationship in cotton. Therefore, this study aimed to explore the relative effects of elevated [CO2] and air temperature on
source-sink relationships in cotton grown by experimentally controlling sink strength and activity.

Rising atmospheric [CO$_2$] (C$_E$) has been shown to affect plant growth (de Graaff et al. 2006) and physiological activity (Dusenge, Duarte & Way 2019). Specifically, these changes were primarily caused by increased photosynthesis ($A$) in cotton (Harley et al. 1992) despite reductions in stomatal conductance ($g_s$) (Ko & Piccinni 2009). Cotton has very strong sink strength because it is an indeterminate plant and can use extra carbon produced in elevated CO$_2$, which may explain why Harley et al. (1992) found that C$_E$ did not reduce Rubisco activity or cotton carbon assimilation. Cotton grown under C$_E$ has been shown to reduce $g_s$, increase $A$ and decrease transpiration ($E$) and thus improve whole-plant water use efficiency (Broughton et al. 2017; Ko & Piccinni 2009). The study conducted by Broughton et al. (2017) found that cotton produced larger leaf area when grown under C$_E$ due to higher A. C$_E$ has been shown to increase the number of small bolls per plant, which results in lower seed cotton yields (Osanai, Tissue, Bange, Anderson, et al. 2017). The increased leaf area and greater fruit production observed in C$_E$ (Broughton et al. 2017) suggested an increase in the strength of source and sink where C$_E$ promotes photosynthesis per unit leaf area to balance with sink capacity (Barrett & Gifford 1995). However, it is important to note that larger leaves, as a result of long C$_E$ exposure, may also lead to downregulation of photosynthesis because the plant will balance carbon source-sink capacity (Barrett & Gifford 1995) through changes or shifts in biomass allocation.

Elevated air temperature (T$_E$) can promote both reproductive and vegetative growth and plant physiology. In a recent glasshouse experiment, Osanai, Tissue, Bange, Anderson, et al. (2017) found that temperature elevated from 28°C to 32°C increased seed cotton yield, boll number and boll size compared to cotton grown in ambient air temperature (T$_A$). Their findings indicated that warmer temperatures may increase sink strength and thereby, an increase in sink strength at warmer temperatures generally increases growth. These findings are also similar to Broughton et al. (2017) who found that the same temperature range increased total vegetative biomass and reproductive biomass. Additionally, Osanai, Tissue, Bange, Anderson, et al. (2017) observed that a temperature of 32°C accelerated the growth rate as the height and number of nodes developed faster than in cotton grown under 28°C. Thus, we expected a change in the source-sink relationship in cotton grown under T$_E$ conditions combined with the effect of fruit reduction.

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Moreover, another study conducted in controlled environment growth chambers found that temperature elevated from 26°C to 36°C increased photosynthesis (Reddy, Reddy & Hodges 1998), indicating an increase in source strength. However, a different study also conducted in growth chambers but with air temperature elevated from 22°C to 29°C found that $T_E$ decreased photosynthesis with a reduction of fruit biomass in potatoes (Hastilestari et al. 2018). Hastilestari et al. (2018) indicated that the decrease in $A$ was associated with a reduction in ribulose-1,5-bisphosphate (RuBP) content while reproductive growth was related to a reduction in starch, indicating reduced sink strength. Conversely, $T_E$ (from 27°C to 34°C) can reduce starch in cotton (Zhang et al. 2017). This finding may not be conclusive that source strength will also be reduced because, as mentioned, Broughton et al. (2017) found a significant increase in total biomass production under a similar temperature range. Thus, it is unclear that there will be a shift in biomass allocation under $T_E$ conditions as compensatory growth was observed when fruit branches of cotton grown at average temperatures approximated between 31°C and 35°C were removed (Saleem et al. 2018). Saleem et al. (2018) showed that, after fruit removal, plants subsequently increased numbers of bolls and branches, and extended the boll retention period which increased the number of open bolls on plants in their field experiment. Therefore, our objective was to investigate the effect of $T_E$ on the source-sink relationship in cotton with sink strength manipulation (fruit removal).

Our study investigated the effect of elevated temperature on plant growth at 36°C for $T_E$ and 32°C for $T_A$ as Reddy, Hodges & McKinion (1995) found that temperatures over 35°C can restrict cotton growth. Reddy, Hodges & McKinion (1995) observed that vegetative biomass, including leaves and stems, was significantly increased from 19°C to 31°C then decreased at 35°C. Similarly, another study found that a temperature of 32°C promoted growth (Broughton et al. 2017). Meanwhile, recent temperature data recorded by BOM (2018) showed that the average daytime temperature in cotton growing areas, such as the Narrabri and Moree areas, is approximately 32°C during the growing season; see Figure 3.1. Additionally, the climate change report presented by CSIRO and BOM (2015) indicated that the annual average temperature in Australia is projected to increase by up to 1.3°C in coastal areas and may increase by more than 1.3°C in inland areas by 2030. Therefore, we investigated the effect of a higher temperature at 36°C on cotton; note that this temperature is beyond the optimum growth temperature, based on the results from Reddy, Hodges & McKinion (1995).
Figure 3.1 Monthly mean temperature of cotton growing area in North-West New South Wales, Australia, including a) Narrabri and b) Moree. Temperature data was obtained from the Bureau of Meteorology (BOM 2018).

This study aimed to examine the interactive effects of $C_E$ and $T_E$ on the source-sink relationship in cotton growth and physiology by removing fruits from the plant. The dry biomass of vegetative and reproductive parts was used to investigate the source strength and sink strength, respectively (similar to Marcelis et al. 2004). Broughton et al. (2017) showed that $C_E$ and $T_E$ increased total vegetative biomass and net photosynthesis and that $T_E$ significantly increased total fruit biomass. In this study, we used a higher temperature to investigate the response of the source-sink relationship to warmer temperatures. Based on previous and recent literature, we expected that cotton would increase its growth rate and biomass production as the temperature increased. As for gas exchange activity, we expected to observe increases in $A$ and $g_s$ under $T_E$. We hypothesise that source strength will be increased by $C_E$ and $T_E$ treatments by increasing leaf biomass and photosynthesis; sink strength will be increased by the $T_E$ treatment by increasing fruit production. As for experimentally manipulating sink strength and activity, the fruit removal will reduce sink strength, and in combination with other environmental treatments, this approach should allow us to better understand source-sink dynamics under elevated [CO$_2$] and warmer air temperature. We expected that $C_E$ would not alleviate the effect of $T_E$ on plant growth and physiological function in plants with and without fruit removal.
This study addressed the following research questions: 1) will Te promote vegetative and reproductive growth in 50% fruit removed (FR) plants at a faster rate than at Ta condition?; 2) will Ce increase the growth of vegetative and reproductive components (including existing parts and new fruits) in FR plants?; 3) will Te increase A and gs in FR plants?; and 4) will Ce increase A and decrease gs in FR plants? This study tested the following hypotheses: 1) Te (36°C) will increase the source strength and sink strength by promoting the growth of leaf biomass and fruit, respectively, in no fruit removal (control) and FR plants at a faster rate than at Ta (32°C); 2) Ce will increase the source strength and sink strength by allocating biomass to vegetative and reproductive components, respectively, in control and FR plants under Ta or Te conditions; 3) Te will increase leaf photosynthesis, as a result of increased source strength, and increase gs in control and FR plants under Ca and Ce; and 4) Ce will increase leaf photosynthesis and decrease gs in control and FR plants.

Methods

Material and growth conditions

Cotton seeds (Gossypium hirsutum Sicot 746B3F) were sown separately in 10 L pots in a soil mixture of coco peat, coarse sand, composted pine bark and composted sawdust purchased from Australian Native Landscapes (NSW, Australia) to produce 40 experimental plants. The plants were grown in the glasshouse under natural sunlight and supplied with non-limiting water and nutrients (two teaspoons of Multi-Grow fertiliser consisting of 10.1% N, 3.5% P, 5.5% K, 16.3% S and 7.8% Ca) and grown at 32/21°C (day/night) and ambient [CO2] (420 ppm) for 65 days (or until the flowering period began). After plants were sufficiently developed at 66 days after planting (DAP), ten plants were moved into each of four glasshouse bays with the following treatments: (1) ambient [CO2] (420 ppm) and ambient temperature (32/21°C); (2) elevated [CO2] (640 ppm) and ambient temperature (32/21°C); (3) ambient [CO2] (420 ppm) and elevated temperature (36/25°C); and (4) elevated [CO2] (640 ppm) and elevated temperature (36/25°C).
**Fruit removal**

Fruit removal treatment commenced after allocating plants to different [CO$_2$] and temperature treatments at 70 DAP. By this DAP, all types of reproductive components had developed including squares, flowers and green bolls (number was not recorded) which were suitable for the fruit removal treatment. For each treatment combination, five plants had no fruits removed (control) and five plants had 50% of their fruit removed (FR). We removed every second fruit by classifying flowers and green bolls as fruits. The first fruit to be removed was located on the lowest branch and closest to the main stem. For example, Figure 3.2 shows that the fruit to be removed was fruit [1] which was located on the 5$_{th}$ node on the first branch. Fruit [2] was retained, and the next fruit to be removed was fruit [1] on the 6$_{th}$ node, with the pattern repeated for the whole plant. Consequently, twenty plants had 50% of the fruit removed to adjust sink capacity, and twenty plants retained all fruit. Plants were grown for four weeks following fruit removal at the four different CO$_2$ and temperature treatments to assess impacts on physiology and growth.

**Plant mapping**

Plant mapping quantifies plant growth and development, noting fruit position, number of nodes, height, number of squares and number of bolls (Albers 1993), and was used to assess the impact of source-sink allocation under different environmental treatments. Growth measurements were conducted and recorded by measuring height and number of nodes on a weekly basis for four weeks. Reproductive measurements used for the plant map were the number of squares (developing flower buds), green bolls and open bolls. Squares were counted when the bud was at least 7 mm in length; candle-shaped squares were noted. White blooms and bolls were counted as green bolls, and bolls with at least two cracks were counted as opened bolls. The plant map also noted the type of branches, i.e. fruit branches and vegetative branches; see Figure 3.2. Experimentally removed fruits and naturally shed fruits were counted as shed fruits. Fruiting position was very important because it helped us determine allocation patterns of resources within a plant. Cotton bolls, flowers and squares were removed and counted at the harvest stage (discussed later).
Figure 3.2 The position of cotton bolls on fruiting branches and vegetative branches. Boll number including the first bloom, green bolls and open bolls. This figure is a recreation of the diagram from Ritchie et al. (2007).

Physiological measurements

A portable photosynthesis system (LI-6400XT) was used to measure photosynthesis, stomatal conductance, and transpiration rate before and after the fruit removal treatment at 67 DAP and 77 DAP, respectively. Leaf gas exchange spot measurements were conducted on a weekly basis after the fruit removal treatment (from week 7 onwards). The measurement conditions in the LI-6400XT were set according to the glasshouse chamber conditions: 1) ambient [CO₂] (420 ppm) and ambient temperature (32°C); 2) elevated [CO₂] (640 ppm) and ambient temperature (32°C); 3) ambient [CO₂] (420 ppm) and elevated temperature (36°C); and 4) elevated [CO₂] (640 ppm) and elevated temperature (36°C). The light intensity was saturating for photosynthesis (1500 µmol m⁻² s⁻¹) and the average VPD levels during the measurement were ca. 1.50±0.02 and 1.48±0.02 kPa for ambient and elevated temperature treatment, respectively. The measurement time was between 9:00 am and 3:00 pm for all treatments.

Harvesting and dry biomass

Squares, green bolls and opened bolls were removed from the plants and put in separate paper bags. The green bolls, both fully closed and slightly opened, were cut open to accelerate the
drying process. Reproductive structures were counted prior to placing the removed material in the oven at 70°C for ten days. The leaves were collected to measure the leaf area and biomass. Sub-samples of leaves were randomly selected to be measured using the LI-3100 leaf area meter. The remaining plant parts (i.e. stem and branches) were segregated and analysed with the leaf biomass to determine the total vegetative aboveground biomass. After ten days, bags were removed from the oven to measure dry mass.

**Statistical analyses**

The ANOVA results were analysed with RStudio (ver. 3.5.3, RStudio Team 2016), car package. The treatment effects on harvest data, including height, number of nodes, vegetative biomass, and reproductive biomass, was analysed with three-way ANOVA with no repeated measures. The effect size was used to compare the treatment effects by calculating percentage differences between the control and treatment groups. In this study, the differences in reproductive biomass did not account for shed fruits. For leaf gas exchange measurements, three-way ANOVA (with repeated measures) was used to analyse the interactive effects of temperature, [CO₂] and fruit removal treatment on A and gₛ as all data from all measurement dates were included. The treatment effect on each parameter was considered significant when P<0.05. Each treatment included five plants, with each plant considered as the replicate. The normality of all data was tested and log-transformed, where applicable.

**Results**

*Effects of fruit removal on vegetative, reproductive growth and total biomass*

Fruit removal has a significant effect on vegetative growth. Our results show that the total vegetative biomass of FR plants was higher than in control plants by nearly 10% (P=0.007; Figure 3.5). Height was marginally increased by fruit removal (P=0.066); the height of FR plants was increased by nearly 4% (Figure 3.4). There was no significant effect of fruit removal on the number of nodes (P=0.348) and SLA (P=0.203), see Table 3.1. Moreover, the total biomass was not affected by the fruit removal treatment (P>0.876; Table 3.1). We expected that the FR plants would exhibit lower reproductive biomass than the control plants.
as half of the bolls were removed. However, the total biomass of both control and FR plants was not significantly different (Figure 3.11).

For reproductive growth, we observed that fruit removal mainly affected overall reproductive biomass and the number of squares. As expected, there was a significant decrease in reproductive biomass when half the fruit load was removed from the plants (P<0.001), see Table 3.1. Our results showed that there was likely a compensatory growth in FR plants as the reproductive biomass of FR plants was higher than half of the reproductive biomass produced by the control plants (Figure 3.7). The fruit removal significantly increased the biomass of squares (P<0.001; Figure 3.8) and green bolls (P<0.001; Figure 3.9). There was no effect of fruit removal on the biomass of open bolls (P=0.351). Additionally, the number of squares on FR plants was increased drastically by 136% (P<0.001; data not shown). We did not observe a significant effect of fruit removal on the number of green bolls (P=0.324) or open bolls (P=0.902), see Table 3.1.

Effects of increased temperature and elevated [CO\textsubscript{2}] on vegetative growth, reproductive growth and total biomass

There were no significant effects of C\textsubscript{E} and T\textsubscript{E} on total vegetative growth. Amongst the vegetative parameters, only height and specific leaf area (SLA) were affected by C\textsubscript{E} and T\textsubscript{E}, respectively (Table 3.1). Our results indicated that C\textsubscript{E} increased plant height marginally (P=0.094); plant height increased by approximately 3% as per the last measurement at 99 and 100 DAP, see Figure 3.4. We did not observe a significant effect of T\textsubscript{E} on height (P=0.883). Next, SLA was mainly affected by T\textsubscript{E} (P<0.001); this factor increased SLA by 7% under ambient [CO\textsubscript{2}] (Figure 3.6). There was no significant effect of C\textsubscript{E} on SLA (P=0.559). The number of nodes was not affected by either T\textsubscript{E} (P=0.146) or C\textsubscript{E} (P=0.260), see Figure 3.3. Similarly, there was no individual effect of T\textsubscript{E} (P=0.621) or C\textsubscript{E} (P=0.587) on the total vegetative biomass (Figure 3.5). Also, there was no interactive effect between T\textsubscript{E} and C\textsubscript{E} on any vegetative parameters: vegetative biomass (P=0.227), height (P=0. 969), the number of nodes (P=0.774) or SLA (P=0.647), see Table 3.1.

T\textsubscript{E} decreased reproductive growth significantly, and C\textsubscript{E} may have alleviated this reduction. Our results indicated that there was a significant interactive effect of T\textsubscript{E} and C\textsubscript{E} on overall reproductive biomass. T\textsubscript{E} reduced the reproductive growth by ca. 5% and 17% under C\textsubscript{E} and
CA conditions, respectively. The main reproductive component that contributed to the significant reduction in reproductive growth under TE and CA conditions was the green boll, see Figure 3.1. CE did not affect total reproductive biomass at Ta (P=0.243). We observed that TE significantly decreased green boll biomass (P<0.001) but increased open boll biomass (P<0.001). On the other hand, there was no effect of TE or CE on square biomass. We did not observe the combined effects of TE and CE on any reproductive parameters including squares, green bolls or open bolls (Table 3.1).

Our results also show that the number of open bolls remaining on the plants increased by an average of one fruit per plant under elevated temperature (P<0.001). There was no significant effect of warmer temperature on the number of squares (P=0.896) or green bolls (P=0.853). Additionally, there was no significant effect of elevated [CO2] on reproductive biomass (P=0.243), the number of squares (P=0.106), green bolls (P=0.618) or open bolls (P=0.953). We did not observe any interactive effects of TE and CE on the number of squares (P=0.865), green bolls (P=0.414) or open bolls (P=0.412).

The interactive effect of TE and CE marginally affected the total biomass (P=0.056; Figure 3.11). The highest and lowest total biomass was observed under TA CA and TE CA conditions, respectively. TE reduced the total biomass at TE CA by ca. 18% in control plants and by 12% in FR plants compared to total biomass of cotton plants grown under TA CA conditions. TE increased the total biomass of control and FR plants at CE more than the total biomass observed under TE CA conditions.

Interactive effects of temperature, atmospheric [CO2] and fruit removal on total biomass, vegetative and reproductive growth

We did not find a three-way interactive effect of warmer temperature, elevated [CO2] and fruit removal on any vegetative parameters, reproductive parameters or total biomass (Table 3.1). The total biomass between control and FR plants under all treatments was not significantly different (Figure 3.11). On the other hand, plants with lower reproductive biomass generally had higher vegetative biomass (Figure 3.12). Figure 3.12 shows that there was no significant relationship between vegetative biomass and reproductive biomass; however, the vegetative biomass in FR plants under all treatments was higher than vegetative biomass in control plants. Control plants grown under TA CA conditions had the highest
vegetative biomass and reproductive biomass among the control plants. Thus, there was no trade-off between vegetative and reproductive biomass production under T\textsubscript{A}C\textsubscript{A} conditions. For the T\textsubscript{E}C\textsubscript{A} condition, this condition induced vegetative biomass and reproductive biomass at the slowest rate across both control and FR plants. Our results showed that the response of vegetative biomass in FR plants was less varied among treatments than control plants. The average vegetative biomass of FR plants ranged from 66 to 68 g per plant and from 54 to 62 g per plant for control plants. Lastly, there was no relationship between vegetative biomass and reproductive biomass (Figure 3.12).

Figure 3.3 Effect of ambient temperature (a) and elevated temperature (b), CO\textsubscript{2} concentration ([CO\textsubscript{2}]) and fruit removal treatment on number of nodes measured on the day before harvest. No fruit removal and 50% fruit removed plants are shown as control and FR, respectively. Plots and bars are means and standard errors, respectively, of five plants.
Figure 3.4 Effect of ambient temperature (a) and elevated temperature (b), CO₂ concentration ([CO₂]) and fruit removal treatment on height measured on the day before the harvest. No fruit removal and 50% fruit removed plants are shown as control and FR, respectively. Plots and bars are means and standard errors, respectively, of five plants.

Figure 3.5 Effect of ambient temperature (a) and elevated temperature (b), CO₂ concentration ([CO₂]) and fruit removal treatment on total vegetative biomass including leaves and stem. No fruit removal and 50% fruit removed plants are shown as control and FR, respectively. Columns and bars are means and standard errors, respectively, of five plants.
Figure 3.6 Effect of ambient temperature (a) and elevated temperature (b), CO$_2$ concentration ([CO$_2$]) and fruit removal treatment on specific leaf area (SLA). No fruit removal and 50% fruit removed plants are shown as control and FR, respectively. Columns and bars are means and standard errors, respectively, of five plants.

Figure 3.7 Effect of ambient temperature (a) and elevated temperature (b), CO$_2$ concentration ([CO$_2$]) and fruit removal treatment on total reproductive biomass including squares, green bolls and open bolls. No fruit removal and 50% fruit removed plants are shown as control and FR, respectively. Columns and bars are means and standard errors, respectively, of five plants.
Table 3.1 P-values from three-way ANOVA analysis of elevated growth temperature, increased [CO₂] and fruit removal treatment effect on vegetative biomass (included total dry leaf mass and stem), height, number of nodes, specific leaf area, total reproductive biomass (including dry mass of squares, green bolls and open bolls). We also tested the treatment effect on the number of squares, green bolls and open bolls. The treatment effect on each parameter was considered significant when $P<0.05$, shown in bold, and italics when the treatment effect was marginally significant.

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Figure 3.8 Effect of ambient temperature (a) and elevated temperature (b), CO$_2$ concentration ([CO$_2$]) and fruit removal treatment on the square biomass. No fruit removal and 50% fruit removed plants are shown as control and FR, respectively. Columns and bars are means and standard errors, respectively, of five plants.

Figure 3.9 Effect of ambient temperature (a) and elevated temperature (b), CO$_2$ concentration ([CO$_2$]) and fruit removal treatment on green boll biomass. No fruit removal and 50% fruit removed plants are shown as control and FR, respectively. Columns and bars are means and standard errors, respectively, of five plants.
**Figure 3.10** Effect of ambient temperature (a) and elevated temperature (b), CO$_2$ concentration ([CO$_2$]) and fruit removal treatment on open boll biomass. No fruit removal and 50% fruit removed plants are shown as control and FR, respectively. Columns and bars are means and standard errors, respectively, of five plants.

**Figure 3.11** Effect of ambient temperature (a) and elevated temperature (b), CO$_2$ concentration ([CO$_2$]) and fruit removal treatment on total biomass including leaves, stems, squares, green boll and open boll biomass. No fruit removal and 50% fruit removed plants are shown as control and FR, respectively. Each column and each stack represented the average of total biomass and each growth component, respectively.
Figure 3.12 Effect of elevated temperature, increased [CO₂] and fruit removal treatment on average vegetative biomass and reproductive biomass in cotton plants. Plots are means of five plants harvested on 99 and 100 DAP. Vertical bars and horizontal bars are standard errors of vegetative biomass and reproductive biomass, respectively. Ambient temperature (32°C) and elevated temperature (36°C) are represented in blue and red, respectively. Ambient [CO₂] is represented as triangles and circles are used to indicate elevated [CO₂]. The fruit removal treatment is shown as closed and open for control and fruit removal treatment, respectively.

Interactive effects of warmer temperature, elevated atmospheric [CO₂] and fruit removal on leaf gas exchange

There were interactive effects of Cₑ and number of days after planting (DAP) on A (P=0.049) and gₛ (P=0.046), while the interactive effect of Tₑ and DAP was mainly observed on gₛ (P=0.001), see Table 3.2. In general, A was decreasing at increasing DAP; DAP from day 77 to 91 decreased A at Cₐ but the reduction was more obvious under Cₑ conditions (Figure 3.13). On the other hand, increasing DAP moderately increased gₛ at Cₐ and significantly decreased gₛ at Cₑ (Figure 3.14). Our results also showed that increasing DAP decreased gₛ under TₑCₑ conditions faster than under TₑCₐ conditions (Figure 3.14). Tₑ marginally increased A in control and FR plants. We did not observe the effect of fruit removal on A (P=0.870) or gₛ (P=0.781). There were no three-way or four-way interactive effects between Tₑ, Cₑ, fruit removal and DAP on A or gₛ (Table 3.2).
Effect of elevated temperature, [CO₂] and fruit removal on carbon assimilation and dry plant biomass

Plants with higher A did not generate more vegetative biomass, reproductive biomass or total biomass (Figure 3.15). For example, both control and FR plants grown under ambient conditions (T<sub>AC</sub>) had low photosynthetic rates but high vegetative biomass and reproductive biomass, relative to other treatments (Figures 3.15a and 3.15b). On the other hand, both control and FR plants grown under T<sub>EC</sub> conditions had similar photosynthetic rates, but the vegetative biomass in control plants was clearly higher than in FR plants, by 19% (Figure 3.15a). Similar to the reproductive biomass, the photosynthetic rates of control and FR plants grown under T<sub>EC</sub> were almost the same, but the reproductive biomass of control plants was higher than FR plants by almost 24% (Figure 3.15b). Our results also showed that the total biomass did not correspond to the rate of photosynthesis (Figure 3.15c). Therefore, there was no significant relationship between carbon assimilation and dry plant biomass.
Table 3.2 P-values from ANOVA results of the effect of temperature, [CO₂] and fruit removal treatment on photosynthesis and stomatal conductance at 77, 84 and 91 DAP. The treatment effect on each parameter was considered significant when P<0.05, shown in bold, and italics when the treatment effect was marginally significant.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Photosynthesis</td>
</tr>
<tr>
<td>Temperature</td>
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</tr>
<tr>
<td>CO₂</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fruit removal</td>
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</tr>
<tr>
<td>DAP</td>
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<tr>
<td>Temperature × CO₂</td>
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<tr>
<td>Temperature × fruit removal</td>
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<td>CO₂ × fruit removal</td>
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</tr>
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<tr>
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<td>CO₂ × fruit removal × DAP</td>
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</tr>
<tr>
<td>Temperature × CO₂ × fruit removal × DAP</td>
<td>0.303</td>
</tr>
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</table>
Figure 3.13 The effects of elevated temperatures and [CO$_2$] on photosynthesis (A) under the four treatments (C$_A$TA, C$_A$TE, C$_E$TA and C$_E$TE). Plots and bars represent mean and standard errors, respectively, of five plants, except C$_A$TA with full fruit treatment which had four plants at 91 and 98 DAP. No fruit removal and 50% fruit removed plants are shown as close and open symbols, respectively.
Figure 3.14 The effects of elevated temperatures and [CO$_2$] on stomatal conductance ($g_s$) under the four treatments (C$_A$TA, C$_A$TE, C$_E$TA and C$_E$TE). Plots and bars represent mean and standard errors, respectively, of five plants, except C$_A$TA with full fruit treatment which had four plants at 91 and 98 DAP. No fruit removal and 50% fruit removed plants are shown as close and open symbols, respectively.
Figure 3.15 Effect of elevated growth temperature, increased atmospheric [CO$_2$] and fruit removal treatment on a) average vegetative biomass, b) average reproductive biomass and c) average total biomass and average photosynthesis (A) of five plants. No fruit removal and 50% fruit removed plants are shown as control and FR, respectively. The average of A was calculated on the measurements taken at 77, 84 and 91 DAP. Vertical bars and horizontal bars are standard errors of vegetative biomass and reproductive biomass, respectively.


Discussion

The fruit removal treatment did not affect the final total biomass, indicating that the plants compensated for the loss of fruit load. There was a shift in final biomass partitioning towards vegetative growth, but reproductive growth in FR plants must have been faster than in the control plants as the FR plant reproductive biomass was higher than half of the control plant reproductive biomass. Fruit removal treatment also stimulated the initiation of new fruits, as the biomass of squares significantly increased. For the gas exchange activity, fruit removal in cotton did not affect either A or gs, indicating that the reduction in sink strength did not change the source strength. The Ce treatment increased the photosynthetic rate on average, although there was a time factor involved in the response of gas exchange to the change in environmental conditions. A and gs declined over time in all treatments, but the decrease in A and gs over time under Ce was greater than that under Ca conditions. The effect of Te on A was not significant.

Our results indicated that there was no significant relationship between leaf gas exchange activity and the growth of plant biomass. Changes in source strength via changes in A did not relate to higher growth of vegetative biomass, reproductive biomass or total biomass. Although Ce increased A, it only led to an increase in total reproductive biomass under Te. Additionally, while Te did not affect A, it had a stronger effect on reproductive parameters than Ce as it decreased the biomass of green boll and increased open boll biomass under Ca conditions, while Ce had no effect on any reproductive parts. These findings suggested that Te restricted the sink strength and that Ce alleviated this effect to some extent. In summary, above-optimal temperature decreases sink strength, and Ce should not be considered as ameliorating the negative effect of high temperature on sink strength since the alleviation is minimal.

Effect of elevated temperature, [CO2] and fruit removal on vegetative growth

Our results showed that FR plants were taller and produced more vegetative biomass than the control plants, suggesting that each plant component was competing for resources within plants for growth and development (DaMatta et al. 2008; Rosati et al. 2018). Moreover, the results showed that the vegetative biomass of FR plants was highest under ambient conditions (TaCa). This finding is in line with Sadras (1996) and Bustan et al. (2016); they demonstrated that plants with fruits removed, grown under optimal conditions, produced more vegetative
biomass. This result also suggested that vegetative growth had less competing requirement for resources and thereby, the growth rate was increased. Furthermore, our results are also consistent with Rosati et al. (2018), which demonstrated that having more fruits remaining on plants can reduce heights and leaf biomass. Rosati et al. (2018) suggested that, on the other hand, resources allocated to reproductive components may be a plant strategy to maintain the existing fruits and therefore, the stem is shorter when more fruits are retained on the plants. Finally, these results indicated that carbon partitioning in cotton prefers low energy cost components such as stems and leaves.

\( T_e \) increased SLA significantly in control and FR plants, indicating that \( T_e \) may be the main climate factor that promoted the source strength in this study. This finding is consistent with Rosbakh, Römermann & Poschlod (2015) who also found that SLA at species and community levels were increased at increasing temperatures. As SLA responded to elevated temperature, we demonstrated that cotton’s SLA value was over 16 m²kg⁻¹, and this SLA value is considered high, which indicates that cotton might have a high-temperature baseline for leaf expansion (Kumar, Singh & Boote 2012). High SLA in cotton plants also indicated that cotton might generate leaf biomass at a faster rate (Kumar, Singh & Boote 2012; Rosbakh, Römermann & Poschlod 2015) and cotton grown in this study was not nutrient-limited (Poorter & De Jong 1999). The rapid leaf biomass production based on SLA suggested that cotton can be considered as not growing at an efficient rate. Resources such as water and nutrients are required to develop and produce biomass (Poorter and De Jong 1999); the faster growth rate in cotton means faster resource depletion. Our result supported the first hypothesis of this study as \( T_e \) increased the source strength by generating more leaf biomass in control and FR plants.

Our results, however, indicated that \( T_e \) did not affect the number of nodes, height and the total vegetative biomass. These results are inconsistent with previous studies which found that \( T_e \) increased height and number of nodes, which developed at a faster rate (Osanai, Tissue, Bange, Anderson, et al. 2017) and increased total vegetative biomass (Broughton et al. 2017) in cotton. Allen et al. (2018) also observed that \( T_e \) may decrease the rate of reproductive development, but increased photoassimilate demand and vegetative growth in soybeans which then stimulated growth in the number of nodes. Nevertheless, our result showed that there is no indication that cotton partitioned carbon towards vegetative parts more than reproductive parts under \( T_e \) at 36°C since the reproductive growth under this condition was lower than
under other conditions. This finding may also suggest that the temperature at 36°C was extreme for cotton growth.

We observed that $C_E$ marginally increased plant height but did not affect other vegetative parameters. This finding is inconsistent with Osanai, Tissue, Bange, Anderson, et al. (2017) which found that $C_E$ did not affect height but significantly increased the number of nodes. Although $C_E$ may have affected the plant height in this study, we expected $T_E$ to be the main factor that accelerated plant development, as observed in cotton (Osanai, Tissue, Bange, Anderson, et al. 2017) and wheat (Tan et al. 2015). There were no interactive effects of $T_E$ and $C_E$ on any vegetative parameters in this study. However, Osanai, Tissue, Bange, Anderson, et al. (2017) found that there was a significant interactive effect of $T_E$ and $C_E$ on height in cotton plants. The variation between studies may be due to soil type as we used potting mix (described in the result section) which may affect some soil properties such as water-holding capacity, nutrient withholding and bulk density which may have generated confounding effects on growth. However, the climate factors seemed to create a significant shift in carbon partitioning between vegetative and reproductive development within plants (Osanai, Tissue, Bange, Anderson, et al. 2017); we observed that the interactive effect of the climate factors slightly decreased the reproductive biomass (discussed in the later section).

We did not observe a three-way interactive effect of $T_E$, $C_E$ and fruit removal on vegetative biomass, height, the number of nodes and SLA. The vegetative biomass in cotton was expected to increase significantly under $T_E$ and $C_E$ (Broughton et al. 2017); however, our results showed that the vegetative biomass of control and FR plants was similar within the groups across all treatments. The growth temperature used in this study (36°C) may be exceedingly high for cotton and therefore, no interactive effect was observed. Moreover, this result may indicate that climate factors did not induce vegetative growth regardless of the change in sink strength. $T_E$ may have offset the positive effect of $C_E$ on plant development (Broughton et al. 2017; Osanai, Tissue, Bange, Anderson, et al. 2017). Our results, therefore, did not fully support our hypotheses as we did not observe a significant increase in source strength in control and FR plants under $T_E$ and $C_E$ conditions.
We demonstrated that fruit removal can shift the biomass partition towards existing components or new growth. For instance, the fruit removal treatment increased square biomass and decreased green boll biomass. The increase in square biomass or new growth may be considered as compensatory growth for the removed fruits. Moreover, the decrease in green boll biomass may indicate that it was the result of fruit removal that the plants are yet to replenish, or that existing green bolls developed into open bolls. This finding is similar to Saleem et al. (2018) who found more cotton bolls on plants after fruit removal. In general, our results suggested that the reduction in sink strength may move the biomass partition to the existing reproductive part (i.e. green bolls) and then promote new growth (i.e. squares).

Te decreased the overall reproductive biomass under CA and CE conditions, whereas Te mainly increased the open boll biomass which resulted in decreased biomass of green boll on the plants under CA condition. Our results did not agree with the findings from Broughton et al. (2017) who found that Te increased total fruit biomass under CA and CE conditions. Once again, the temperature at 36°C may have exceeded the optimum growth temperature range for cotton. The reduction in green boll biomass and the increase in open boll biomass observed under Te conditions suggested that Te accelerated the growth rate of fruit development. Additionally, we observed an increase in number of one open boll per plant under the elevated temperature, which suggested that the warmer temperature did not reduce fruit retention in cotton; this finding is consistent with Hastilestari et al. (2018). Nevertheless, we accepted part of our first hypothesis as Te increased the sink strength by developing the existing fruit. In contrast, we rejected our second hypothesis as there was an interactive effect of Te and Ce on the total reproductive biomass; Ce did not promote sink strength under TA and Te conditions.

The change in reproductive biomass may be due to the extreme temperature (Allen et al. 2018), soil nutrients (Osanai, Tissue, Bange, Anderson, et al. 2017), pollination failure (Hatfield & Prueger 2015) or change in growth stage of reproductive components. First, we found that control and FR plants grown under Te had lower reproductive biomass than plants in other climate conditions, which is consistent with Allen et al. (2018) who showed that extreme temperatures reduce pod numbers in soybeans significantly. Moreover, our results also showed interactive effects of Te and Ce on reproductive biomass as Te decreased the reproductive biomass at CA and CE. This result suggested that Te may be the main climate
factor that reduced reproductive biomass and that CE did not alleviate the negative effect of TE on cotton productivity (Broughton et al. 2017). Second, Osanai, Tissue, Bange, Anderson, et al. (2017) showed that soil nitrate significantly increased cotton yield during the flowering stage, which may explain why the number of squares in our study increased significantly in FR plants. Third, we observed a significant reduction in square biomass, which was more intense under CA than CE in control plants. Hatfield and Prueger (2015) demonstrated that extreme temperatures could reduce crop productivity drastically due to reduction in the pollination rate. They also found that higher air temperatures, exceeding optimal growth temperatures, can relatively increase vegetative biomass. Lastly, as mentioned, our results showed that the number of squares increased significantly in FR plants; however, this biomass did not contribute substantially to overall reproductive biomass due to the light weight of squares. On the other hand, some squares may have developed into green bolls for the control plants.

Effect of TE, CE and fruit removal on biomass partitioning between vegetative and reproductive components

We are uncertain whether cotton will stop partitioning carbon into leaves and continue to partition carbon into fruit growth, considering the increased vegetative biomass after fruit removal. We expected that cotton would produce more vegetative biomass and reproductive biomass under TE and CE conditions, based on Broughton et al. (2017), who found that TE promoted the growth of vegetation and fruit at CA and CE. However, our results did not completely agree with their findings as we found that reproductive biomass and total biomass were the only parameters that were affected by the interactive effect of TE and CE. Our TE at 36°C may have exceeded the optimum growth temperature for cotton, leading to the reduction in biomass instead. Nevertheless, the FR plants produced similar amounts of total biomass to the control plant total biomass across all treatments (Figure 3.12), suggesting a shift in biomass partition. The significant increase in vegetative biomass, particularly in leaf biomass, may suggest that plants use resources available at the time to increase source strength by promoting leaf growth before shifting the biomass partition towards new growth or existing reproductive parts. In this case, we observed a significant increase in square biomass and decrease in open boll biomass. However, the timeline may need to be investigated further as the results are based on the final harvest.
Therefore, by decreasing reproductive components or sink strength, the allocation of the available resources in cotton plants showed a preference for sink or existing structure. These components, such as leaves, stems and existing cotton bolls, supposedly require lower energy and resources for growth and maintenance. Finally, the significant increase in vegetative biomass in FR plants suggested that cotton tends to induce source strength. This finding does not seem to indicate that cotton plants were able to acclimatise or could balance their source-sink capacity under $T_E$ and $C_E$ conditions.

We did not observe a significant correlation between total vegetative growth and total reproductive components (Figure 3.7). In contrast, Osanai, Tissue, Bange, Anderson, et al. (2017) observed a significant relationship between seed cotton yield and vegetative growth and suggested that there was an alteration in carbohydrate allocation regulated within plants. However, we observed that the shorter plants had higher reproductive biomass, which is in line with Rosati et al. (2018); they suggested that plants prefer to allocate available resources within the plant or when plant height is restricted. Rosati et al. (2018) also indicated that plants are likely to allocate carbohydrates into remaining reproductive components (not new growth).

*Effect of elevated temperature, [$CO_2$] and fruit removal on leaf gas exchange parameters*

We found that $C_E$ increased $A$ significantly while $T_E$ marginally increased $A$ in control and FR plants. Our result for $A$ is similar to Broughton et al. (2017) who demonstrated that $A$ in control plants increased significantly under $C_E$ and that there was no interactive effect between $T_E$ and $C_E$ on $A$. However, Broughton et al. (2017) did not observe an increase in $A$ under $T_E$ conditions. On the other hand, Reddy, Reddy & Hodges (1998) demonstrated that the interactive effect of $T_E$ and $C_E$ induced higher $A$ in cotton. Furthermore, our results are consistent with Osanai, Tissue, Bange, Anderson, et al. (2017) which also found that $A$ was significantly affected by elevated temperature and increased [$CO_2$]. Osanai, Tissue, Bange, Anderson, et al. (2017) also demonstrated that there was an interactive effect between temperature and [$CO_2$] on $A$ which we did not find in the present study. Generally, these findings partially supported our hypotheses which anticipated the increase in leaf photosynthesis under $T_E$ and $C_E$ in control and FR plants. The results did not fully support the second hypothesis because we did not observe interactive effects between [$CO_2$] and temperature on $A$ in control and FR plants.
The highest photosynthetic rate observed in this study may be more dependent on [CO$_2$] than on temperature. Our results indicated that there was no interactive effect between T$_E$ and C$_E$ on $A$. At the same time, we observed that C$_E$ increased $A$ significantly under T$_A$ and T$_E$, while T$_E$ marginally increased $A$ under C$_A$ and C$_E$ from 77 to 91 DAP. Harley et al. (1992) demonstrated that the Rubisco activity of cotton was not affected significantly by C$_E$ and thereby, the increase in $A$ observed during early DAP could be due to cotton assimilating the CO$_2$ that was abundant in the atmosphere. Similarly, C$_E$ has been shown to increase photosynthetic capacity in reed canary grass (Zhou et al. 2011). The reduction in $A$ observed in this study was unlikely to be associated with the C$_i$:C$_a$ ratio as Broughton et al. (2017) found that C$_E$ at 640 ppm did not increase the C$_i$:C$_a$ ratio at T$_A$ and T$_E$ despite the increase in $A$. On the other hand, as the DAP progressed, Zhu et al. (2014) suggested that the reduction in $A$ during the grain development of rice was due to the reduction in Rubisco content and its activity and $A$ became acclimatised to the C$_E$ condition. Furthermore, Zhou et al. (2011) observed that T$_E$ accelerated leaf senescence, which reduced $A$. Although we found that T$_E$ accelerated the growth rate of reproductive components and the lowest $A$ was observed under T$_E$ conditions, we did not particularly investigate the effect of T$_E$ or C$_E$ on leaf senescence which, once again, was likely the factor that reduced $A$ (Olesinski et al. 1989). Nevertheless, we accepted part of our hypothesis that T$_E$ increased $A$ under C$_E$ but not under C$_A$ conditions in control and FR plants.

We were unable to conclude that the change in $A$ was due to plants allocating resources to other parts as our results showed that there was no relationship between $A$ and vegetative biomass, reproductive biomass or total biomass. Additionally, there was no effect of fruit removal on $A$ in control and FR plants, suggesting that the rate of $A$ is not associated with higher production of source or sink strength. In other words, generation of vegetative biomass and reproductive biomass appeared to be independent of carbon assimilation. For example, the FR plants with the highest vegetative growth rate had the second-lowest photosynthetic rate. The lack of difference of $A$ between fruit treatments may also be due to the insignificant changes in canopy architecture as there was no change in leaf positions and only fruits were removed. Sadras (1996) suggested that one of the changes in $A$ could be due to changes in branch positioning, which can influence radiation interception per unit leaf area. We did not observe an interactive effect of climate factors and fruit removal on $A$. 

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We found that \( g_s \) mainly responded to the interactive effect of climate factors and DAP but not the fruit removal treatment. Warmer growth temperature significantly increased \( g_s \) at increasing DAP, indicating that leaf age may have induced stomatal resistance at leaf surface (Olesinski et al. 1989). Without the DAP effect, our result was in line with Olesinski et al. (1989) who demonstrated that \( g_s \) in control plants initially increased then decreased when the temperature reached 32°C. We also observed that there was an interactive effect between \( C_E \) and DAP on \( g_s \) and, as expected, \( C_E \) decreased \( g_s \) at increasing DAP. Without the DAP effect, this finding was consistent with Duursma et al. (2013) who also found that \( C_E \) at 640 ppm decreased \( g_s \). These results, therefore, supported our hypothesis that \( T_E \) increased \( g_s \) and \( C_E \) reduced \( g_s \) in control and FR plants. Furthermore, our results showed that the sink strength reduction did not affect \( g_s \), which suggested that \( g_s \) in cotton was not affected by the decrease or restriction in sink demand (Bustan et al. 2016; Li et al. 2007). Gas exchange can function on fruit surface; however, the lack of difference in \( g_s \) between fruit treatments indicated that gas exchange activity on cotton boll surface is very low compared to the large cotton leaf surface area. Finally, there were no interactive effects of climate factors and fruit removal on \( g_s \).

**Future study**

Future study should investigate the seed cotton yield and boll size, which can be useful in understanding cotton productivity based on the response of the source-sink relationship in cotton under increased [CO\(_2\)] and warmer air temperature. Future study may also investigate the interactive effect between vapour pressure deficit, warmer temperature and fruit removal treatment on the response of cotton. The interactive effect between these factors should enable us to understand the cotton source-sink relationship under drought conditions, which is an urgent issue for the Australian agricultural industry. For data collection, shed fruit and removed fruit data should be collected separately so that we can track how plants balance resources, as Marcelis et al. (2004) found that decreased source strength led to reduced sink strength (increased shed fruits). This method would provide a better understanding of whether cotton promotes more reproductive growth or sheds existing productive components such as squares, green bolls and open bolls. Future research should investigate the sequence of biomass partitioning, which may be done by removing different fruit loads, e.g. 25%, 50%, 75% and 100% of fruit removed from plants. This method would allow us to determine whether plants limit source strength or stimulate reproductive growth to balance sink demand.
Finally, further study may investigate the rate of growth in both vegetative and reproductive components to determine shifts in direction in biomass partitioning.

Conclusions

We found that fruit removal did not change the total plant biomass across the treatments, which suggested that there was a shift in biomass partitioning towards vegetative growth and reproductive growth (i.e. new growth and existing components). For instance, the square biomass increased significantly as a result of fruit removal. Our results showed that $C_E$ increased $A$ but, at the same time, decreased $A$ at the fastest rate at increasing DAP. $g_s$ increased under $T_E$ and decreased under $C_E$ as DAP progressed. $g_s$ was not affected by fruit removal, indicating that cotton was not affected by the reduction in sink strength and that gas exchange activity responded mainly to climate change factors at the leaf surface. Our results indicated that $T_E$ reduced reproductive biomass and total plant biomass but not vegetative biomass, more significantly under $C_A$ than $C_E$ conditions in all plants, whereas the reduction in sink strength via fruit removal significantly induced vegetative growth. Moreover, the development of green bolls into open bolls was faster under $T_E$ conditions and, consequently, $T_E$ increased open boll retention on the plants by an average of one fruit per plant. We demonstrated that plants with higher $A$ did not particularly generate more vegetative biomass, reproductive biomass or total plant biomass, suggesting no association between carbon assimilation and plant biomass production. These results explore the source-sink relationship, finding that elevated climate factors can change the strength of sources or sinks and gas exchange activity in cotton, and these findings should enable us to understand the response of this high-value crop to future climate.

Acknowledgement

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Amendments made to the original thesis:

1. Amended the citation for Li et al. (in press) as Li et al. (2019) and Li, X., Smith, R., Choat, B. & Tissue, D. 2019, ‘Drought resistance of cotton (Gossypium hirsutum L.) is promoted by early stomatal closure and leaf shedding’, Functional Plant Biology, vol. 47, no. 2, pp. 91-98.
2. Corrected Figure 2.4 (left).
3. Corrected leaf water potential symbol (Ψleaf) throughout the document.