



FINAL REPORT 2015

For Public Release

Part 1 - Summary Details

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

CRDC Project Number: US1301

Project Title: The physiology of cotton crop nutrition, shade & waterlogging

Project Commencement Date: 2012 **Project Completion Date:** 2015

CRDC Research Program: 2 Industry

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Part 3 – Final Report

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

Background

1. Outline the background to the project.

Australia contributes approximately 12% of the world's total cotton production, and is the third largest exporter of cotton fibre. Most Australian cotton is cultivated in New South Wales, (70% of the total production), with the remainder cultivated in Queensland, an area that extends from Emerald in Queensland to Hay in New South Wales (Hearn and Fitt, 1992). Australian cotton is generally furrow irrigated with only a small proportion rainfed. There has been a dramatic increase in cotton production in Australia from 45,000 tonnes in 1970s to 600,000 tonnes in 2000s, with an average increase in lint yield of 1.8% per year (Constable, 2004). Despite this enormous improvement in cotton production systems, the cotton yield in Australia remains substantially subject to various abiotic stress factors including drought, heat, waterlogging and cloudy conditions.

Waterlogging is an important factor that adversely affects cotton yield. Australian cotton is cultivated on heavy clay soils with inherently low drainage and a summer dominant rainfall pattern poses significant risk of intermittent waterlogging. In addition, the reproductive phase of cotton, which starts by late December through January, often coincides with heavy summer rains in cotton producing regions. As the reproductive phase of cotton growth is most sensitive to stress-induced damage, exposure to waterlogging at this phase can significantly reduce yield. A degree of damage to cotton is expected if heavy rainfall occurs just after an irrigation event. Heavy lint yield losses have been recorded in Australian cotton under persistent rainfall and cloudy weather during the 2009-2010 and 2010-11 cotton seasons (CRC, 2010-11).

Waterlogging-induced growth and yield reduction are the result of a complex syndrome caused by O₂ deficiency in the soil. Soil hypoxia impairs root growth and subsequent water and nutrient uptake. An inhibited supply of nutrients and water influences leaf development, light interception and photosynthetic efficiency leading to growth reduction. In addition, soil waterlogging alters the level of phytohormones in root tissues; specifically it accelerates biosynthesis of 1-aminocyclopropane 1-carboxylic acid (ACC). This ACC is converted into ethylene in the presence of O₂ and ACC oxidase in aboveground plant parts (Bradford and Yang, 1980). Elevated ethylene accumulation in cotton tissues can stimulate leaf senescence and fruit abortion (Lipe and Morgan, 1973).

Tolerance to waterlogging in plants is a complex phenomenon that depends on tolerance to by-products of anaerobiosis and elemental/molecular toxicities. Plants exhibit a variety of modifications to survive in O₂-deficient environments. Development of aerenchyma is one of the most common responses in many plant species at the anatomical level. Aerenchyma facilitates oxygen diffusion into root tissues (Jackson et al., 2008). Other morphological changes include increased root porosity via development of adventitious root and hypertrophied lenticels, and rapid shoot elongation in some waterlogging-tolerant species. Modifications of water relations, stomatal changes, decreased transpiration and photosynthesis are the physiological adaptive responses in plants. Metabolic adaptations, including energy production via fermentation, metabolic adjustments and anaerobic protein synthesis are also crucial for survival of plants exposed to low O₂ concentration.

Absence of any apparent changes in cotton roots in terms of aerenchyma formation (Conaty et al., 2008), as well as the slow rate of energy production through anaerobic respiration, make

cotton relatively sensitive to waterlogging. Cotton roots rapidly respond to soil O₂ deficiency, showing symptoms of growth inhibition under mildly hypoxic conditions (O₂ < 10%) within a short time (Huck, 1970). Inhibited root growth restricts nutrient uptake and interferes with various physiological process, causing overall yield reduction. Yield loss in cotton is directly associated with the duration for which root roots remain under O₂ deficient environments. For example, an inundation period of 4 to 16 h (when soil O₂ < 10 %) caused a 8% reduction in cotton lint yield, while prolonging inundation time to 32 h increased yield losses to 18% (Hodgson, 1982). Similarly, 27 – 30% yield reduction was recorded in response to 4 to 9 d of waterlogging, respectively (Wu et al., 2012). Despite significant improvements in cotton production systems, limited effort has been made in improving tolerance to waterlogging. Waterlogging tolerance in cotton is a complex trait, which depends on several environmental and physiological factors. Screening and breeding for waterlogging tolerance alone may not be adequate, as the waterlogging-tolerant cultivars identified in one experiment may appear intolerant in other trials. Therefore, understanding the impact of environmental factors and plant adaptation to waterlogging is critical for developing efficient waterlogging tolerance strategies. Physiological and biochemical modifications can provide clues to understanding plant tolerance mechanisms to waterlogging and assist in devising techniques for reducing yield losses under stressful conditions.

Objectives

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

The broad objective of this project was to develop an understanding of the key physiological processes regulating yield losses in cotton under waterlogged environments. Three independent glasshouse experiments and five field experiments were conducted. The specific objectives of these experiments were to:

1. investigate the effect of overcast/low-light conditions on cotton growth and yield under waterlogged environments (Field Experiment 1 conducted over two seasons);
2. understand the mechanisms of waterlogging damage in cotton by studying physiological responses of various layers of the plant canopy (Field Experiment 2 conducted in the second season);
3. optimise the application rate and time of an anti-ethylene agent, aminoethoxyvinylglycine (AVG) for waterlogged cotton (Glasshouse experiment 1 & Field experiment 3 conducted over two seasons);
4. study the relationship between ethylene release and waterlogging sensitivity in cotton (Glasshouse Experiment 2), using contrasting cultivars and
5. identify key factors regulating fruit losses in waterlogged cotton using an ethylene-mutant (Glasshouse Experiment 3).

Methods

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

Field experiments

Site and climate

Field experiments were conducted at the Australian Cotton Research Institute (ACRI), Narrabri (30.318°S, 149.788°E), NSW, Australia during two consecutive years (2012-13 and 2013-14). The Narrabri town is situated in the north-west of NSW. It is a major cotton producing region of Australia, situated in the Namoi Valley of north-west plains of NSW, Australia. The climate of the region is arid to semi-arid with hot summers (daily maximum 35.3°C, minimum 19.4°C) and mild winters (daily maximum 17.0°C, minimum 3.4°C). The region experiences a summer-dominant rainfall pattern, with an average annual rainfall of 642 mm, and average maximum and minimum temperature 26.7°C and 11.6°C, respectively (Australian Bureau of Meteorology, 2009). The soil of this area is classified as endocalcareous, medium grey Vertosol (Isbell, 1996) with 60 – 65% clay fraction, 8.0 – 8.8 pH, and low in organic matter and N contents.

Cultivar

A commercial cotton cultivar Sicot 71BRF (*Gossypium hirsutum* L. [Bollgard II® Roundup Ready Flex®], CSIRO Australia) (Stiller, 2008) was used for all the field experiments. This compact growth habit cultivar is well suited to all Australian production systems (CSD, 2008), and since 2009 it has been widely cultivated in Australian cotton growing areas (CSD, 2012). The plant contains Cry 1 Ac and Cry 2 Ab genes encoded for resistance to lepidopteron and CP4- two copies of EPSPS for tolerance to glyphosate application (Monsanto, St. Louis, MO).

Soil waterlogging

Seeds were sown on 23rd October 2012 and 30th October 2013 for the first and second year of field experiments, respectively, using a commercial planter with ridges at 1 m apart on a laser-levelled field. High input management and insect control were practiced throughout the cropping seasons (Hearn and Fitt, 1992). The field experiment had three treatment areas, waterlogged at early (WL_{early}) and late (WL_{late}) reproductive phase and a non-waterlogged control with four replicates of each treatment randomly assigned within each block. The crop was allowed to grow till the peak squaring stage (83 and 77 DAP in 2012-13 and 2013-14, respectively), and was then exposed to WL_{early}. Irrigation duration was extended up to 96 h and 120 h by keeping the water flowing in central four rows of each WL plot in 2012-13 and 2013-14, respectively. A 2nd waterlogging (WL_{late}) treatment was also imposed to a separate set of plots at peak reproductive phase (104 and 101 DAP in 2012-13 and 2013-14, respectively) for 120 h during each year. These waterlogging treatments have been induced using a similar method employed by Bange et al. (2004), who successfully imposed waterlogging and root-zone hypoxia in similar soils. The NWL blocks received standard 8 h irrigation at the same time of the WL_{early} and WL_{late} treatments.

Some variations in the layout and treatment plan were introduced during the field experiments as an attempt to generate treatment effects. In order to induce soil waterlogging, in 2012-13 the ridge height of WL plots was kept lower (5 cm) than NWL blocks (15 cm high). Observing a significant impact of ridge height on the growth of the WL crop in 2012-13, the ridge height of WL and NWL blocks were kept the same (15 cm) in 2013-14.



Figure (A) Field-grown crop exposed to soil waterlogging and shade, (B) changes in leaf colour after 9 d of continuous shade

Soil water

A calibrated neutron moisture meter (503DR Hydroprobe[®], CPN International, Martinez, CA) was used to measure volumetric soil water (mm) throughout the soil profile from 20 cm to a depth of 120 cm. Probe tubes were located in the central row of each treatment plot. A soil moisture sensor (Gopher[®]) was also used to determine the soil water content by measuring the dielectric constant of soil and water. Increase in soil water content changes the dielectric constant values, and a calibrated sensor estimates the volumetric soil water (mm).

Yield and yield components

In field fruit retention

In order to calculate fruit retention (FR) during the crop development, five randomly selected plants from the central row of each treatment plot were tagged. Total number of fruits and fruiting sites were recorded from all fruiting branches of each plant one day before waterlogging (pre-WL), one day (post-WL) and seven d (post-recovery) after termination waterlogging and at crop maturity (final). The average values were used to calculate fruit retention (FR) of each treatment plot from the ratio of retained fruits to total fruiting sites..

Maturity, quality and yield

Starting at first open boll, lint from open bolls was collected from one square metre of the central row of each plot every week to estimate crop maturity (60%) time. Collected lint was used for calculating final seed cotton yield (m^{-2}), number of bolls (m^{-2}) and weight of individual boll. The seed cotton was ginned using a Continental Eagle 20-saw gin to obtain lint yield and ginning turnout. A subsample of lint was taken for determining lint quality such as fibre length, fibre strength and micronaire using a high volume instrument (HVI). In addition, at 60% maturity, five plants from the central row of each plot were harvested below the cotyledons to measure the final fruit number per plant, plant height and total number of nodes per plant.

Glasshouse experiments

Growth conditions

The first glasshouse experiment was conducted at the Darlington glasshouse, the University of Sydney, Australia, while the 2nd and 3rd glasshouse experiments were conducted at the Department of Biological Sciences, Macquarie University Australia. Seeds of cotton cultivars (specific for each experiment) were surface cleaned with distilled water and planted into plastic pots after overnight imbibition. The seeds were allowed to germinate in (30 × 24 cm; height × diameter) plastic pots each containing 9 kg finely mixed red silt loam Ferrosol soil from Robertson, NSW Australia. Fertiliser viz. (MgNO₃)₂, KNO₃, (NH₄)₂SO₄ and NH₄NO₃ and CaCO₃, was added to achieve the final nutrient composition as N 0.68, P 0.17, K 1.4, Ca 2.8, S 1.1 and Mg 0.41 g per kg of dry soil. Plants were grown under glasshouse conditions at 28/20°C day/night temperature, and 14/10 light/dark photoperiod under natural light. The light intensity during the day cycle was maintained to a minimum of 400 μ mol m⁻² s⁻¹ using supplemented light (Philips Contempa High Pressure Sodium lamps). Three plants per pot were germinated and thinned to one plant per pot after two weeks of germination.

Cultivars

Different genotypes of cotton were used for each glasshouse experiment. Similar to field experiments, Sicot 71BRF was used for the first glasshouse experiment. In the second glasshouse experiment a relatively waterlogging sensitive cotton cultivar, LA 887 was used along with Sicot 71BRF. LA 887 is a Fusarium wilt disease-resistant cotton cultivar bred for Louisiana and mainly cultivated in USA cotton production systems (Jones et al., 1991). Two cotton genotypes with variation in lint production and potentially ethylene sensitivity were used in the third glasshouse experiment. A lintless line 5B (lintless), which was originally separated from a fully linted cotton cultivar B1278 as a spontaneous mutant (Dr. Alistair Low unpublished, CSIRO Irrigation Research, Griffith, NSW). Lintless produces very little or no lint on the seeds. The control line was Empire, a fully linted cotton cultivar that is closely related to B1278. Empire is also a moderately Fusarium wilt disease-resistant cotton cultivar developed at Georgia USA (Smith and Cothren, 1999).

Waterlogging

Plants were exposed to waterlogging by immersing the pots into water-filled plastic tubs, whereas NWL pots were watered regularly to field capacity. Water level in the tubs was kept approximately 3 cm above the soil surface (Fig. 3.1 A).

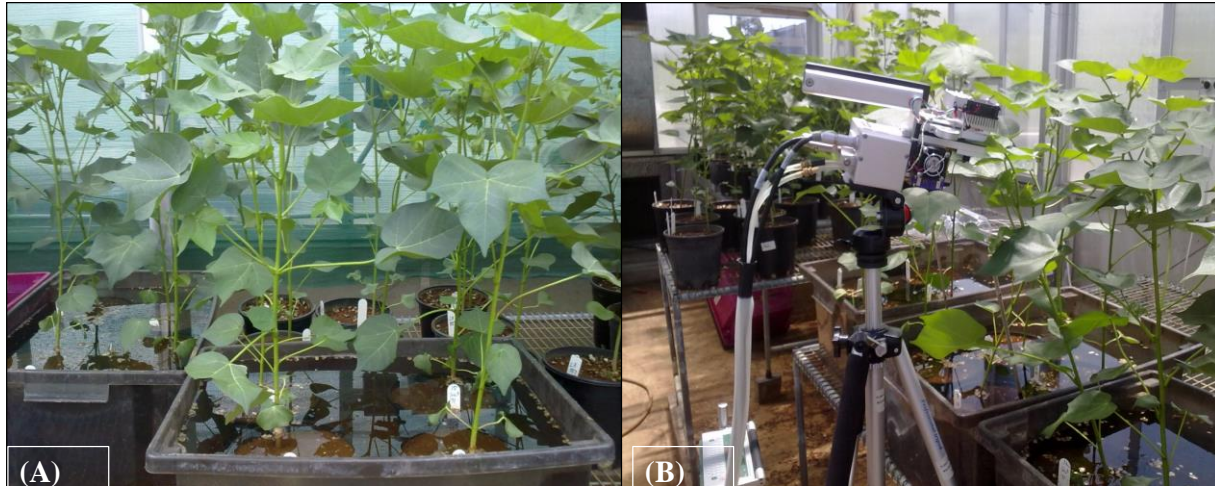


Figure 1 (A) Waterlogging treatment to glasshouse-grown plants and (B) measuring leaf CO₂ exchange rates using Li-6400

Physiological parameters

Gas exchange and fluorescence

Data on leaf CO₂ exchange and chlorophyll fluorescence parameters such as rate of photosynthesis (P_n), stomatal conductance (g_s), intercellular CO₂ concentrations (C_i), electron transport rate (ETR), maximal efficiency of PSII photochemistry (F_v'/F_m') and transpiration rate (T_r) were collected by a Licor-6400 portable photosynthesis system (Li-Cor Ltd, Lincoln, NE, USA). A pulse amplitude modulated (PAM) leaf chamber fluorometer sensor head was used for field experiments. Variables in the sensor head were adjusted ambient external conditions to provide an effective comparison between samples. The reference CO₂ concentration was set at 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ using a CO₂ mixer. Relative humidity followed ambient conditions. The system flow rate was adjusted to maintain a vapour pressure deficit between 1.5 and 2.5 kPa. Light intensity of the Licor-6400 leaf chamber was fixed at 2000 and 1800 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, for field and glasshouse experiments, respectively. In addition, the PAR value in the Licor sensor head was adjusted according to the field conditions e.g. the PAR value was fixed at 1800 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ for cloudy days, compared with 2000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ for sunny days. The temperature was set at an optimal day temperature range for photosynthesis i.e. 30°C for field and 28°C for glasshouse experiments. Measurements were taken from four leaves per plant of five different plants of each treatment plot during 1000 and 1230 h (Eastern Summer Time – Australia).

Total soluble sugar

The dried ground or fresh leaf samples were used for analysing total soluble sugar contents. Sugars from leaf tissues were extracted by 3 aliquots (2.5 mL) of 80% ethanol, and the supernatants were combined to make final volume to 10 mL using distilled water. Total soluble sugar contents were determined by anthrone assay (Yemm and Willis, 1954). Reaction mixture (100 μL of the supernatant + 3 mL anthrone reagent) was placed in boiling water bath for 10 min and then immediately cooled on ice. The absorbance was measured at 630 nm, and sugar

contents in leaf tissues were estimated from the standard glucose curve. Data on total soluble sugar were presented on leaf weight basis.

Leaf nitrogen

The dried leaf samples were ground using a sample mill (Foss Tecator Cyclotec 1093), fitted with 1.0 mm screen. Part of the sample (100 mg) was used for analysing leaf N concentrations using a CHN analyser (Model CHN 900, LECO, St. Joseph, MI). The leaf N concentrations were expressed on leaf N concentration (N %), and leaf area basis (specific leaf N, mg cm⁻²).

Ethylene measurements

Ethylene accumulation was measured from plant tissues at the end of waterlogging. To measure ethylene production, the tissues samples were transferred into 25 mL glass vials and the vials were immediately sealed with rubber septa. Gas samples (1 mL) were withdrawn from the vials after 20 – 30 min (Jackson and Campbell, 1976). Ethylene concentrations were determined by injecting gas samples into PYE series 104 gas chromatograph fitted with a flame ionisation detector (FID) and equipped with activated aluminium coated glass column. The oven, detector and injector temperatures were set at 150°C, 120°C and 120°C, respectively, and ethylene was detected after 50 sec. The fresh biomass of the leaf tissues was determined after ethylene detection, and ethylene synthesis rates were calculated as nmol g⁻¹ FW h⁻¹.

Data analysis

Data for different growth parameters were statistically analysed using JMP v. 9 (SAS Institute, Cary, NC, USA) statistical program. Linear mixed model REML (Residual Maximum Likelihood) was used to analyse the individual and interactive effects of each treatment i.e. waterlogging and shade. Respective means were compared using the Tukey's HSD test. Data on growth and yield were separately analysed for each waterlogging event (early and late).

Results

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

Objective 1: Interactive effects of waterlogging and shade

The effects of individual or combined waterlogging and shade stresses were studied on different growth phases of the cotton crop. These experiments indicated that individual and interactive effects of waterlogging shade were associated with cotton growth phase and intensity of the individual stress. Despite variable effects of shade and waterlogging on different growth components such as leaf growth, total soluble sugars and N (%) concentration, shade did not alleviate waterlogging yield losses in cotton. Instead the reductions in cotton lint yield under combined stresses were always greater than those under the individual stresses, although the interactive effect of waterlogging and shade was significant only when yield losses under waterlogging alone were relatively small. This information can assist crop managers to understand the impact of multiple stress events, which may become more frequent with global climate change.

Objective 2: Differential response of cotton canopy layers to soil waterlogging

Due to an indeterminate growth habit, cotton bolls at different developmental stages throughout the canopy layers would potentially vary in their response to waterlogging. As the developing bolls generally rely on carbohydrate supply from subtending leaves (Constable and Rawson, 1980), leaf-level physiological modifications can directly influence cotton yield. Most of the earlier experiments focused on estimating waterlogging impact in cotton by studying changes in the physiology of leaves at top of the canopy and ignored possible nutrient re-mobilisations within canopy layers. Nutrient remobilisation to the top of the canopy may mask the full effects of waterlogging on the crop. This experiment confirmed that newly pollinated flowers are relatively more sensitive to waterlogging-induced abscission than the developed bolls, implying a potential role of ethylene. It showed that physiological processes of leaves at the lower canopy level were more adversely impaired by waterlogging. On the other hand, upper canopy leaves restored physiological processes after termination of waterlogging, possibly through re-mobilisation of N from lower canopy leaves. Despite a degree of growth recovery, the cotton plants were unable to compensate yield losses in the upper canopy layers, indicating that waterlogging remediation techniques should also focus on preventing the loss of early fruits (potentially through ethylene management) rather than increasing nutrient supply alone.

Objective 3: Ethylene management

A series of glasshouse and field experiments were conducted to optimise application timing and rate of an anti-ethylene agent (AVG) for waterlogged cotton. Consistent with earlier studies, AVG significantly increased fruit production and lint yield of cotton both under WL (Bange et al., 2010) and NWL (Brito et al., 2013) conditions. My data suggested that 125 [a.i.] ha⁻¹ of AVG applied 24 h prior to waterlogging as the optimum application rate for ameliorating yield losses in WL cotton. No further yield gains were recorded by increasing AVG beyond this rate. Similar trends were suggested by Bange et al. (2010), who observed no significant effect on yield of WL cotton by increasing AVG application rate to 250 [a.i.] ha⁻¹. Possible mechanisms of AVG-induced growth and yield promotion could be through increased nutrient uptake (Leblanc et al., 2008) and fruit retention (Chen et al., 2014), respectively. This could be achieved potentially by the application of AVG and a foliar fertiliser.

Objective 4: Waterlogging sensitivity in relation to ethylene

The relationship between ethylene concentrations in cotton tissues and waterlogging sensitivity was studied in a glasshouse experiment. This study indicated that photosynthetic inhibition occurred after three d of waterlogging in LA 887 but Sicot 71BRF sustained photosynthesis rates for up to six days of waterlogging. Similar data under field conditions have been reported by Conaty et al. (2008). In addition, LA 887 accumulated significantly more ethylene than Sicot 71BRF in reproductive tissues (young squares) under waterlogging, although ethylene production in leaf tissues did not differ. This study provided clear evidence that inhibited photosynthesis and elevated ethylene production are major reasons for yield reduction in WL cotton. AVG effectively suppressed ethylene production and subsequent fruit abscission in both cotton cultivars. However, it had a limited effect on photosynthesis and shoot growth of severely

WL cotton, indicating that blocking ethylene biosynthesis alone may not be adequate to mitigate waterlogging damage and an integrated approach of fertiliser and ethylene management should be adopted.

Objective 5: Manipulation of ethylene and carbon metabolism

A glasshouse experiment explored the potential to improve performance of cotton under WL by manipulating the two major limitations i.e. elevated ethylene production and impaired photosynthesis. In addition to the chemical control of ethylene production (AVG), this study exploited genetic variability in modulating ethylene metabolism. Ethylene is required for fibre developmental process in cotton and its production in cotton tissues increases with reproductive growth (Heilman et al., 1971). Lintless cotton mutants with impaired ethylene metabolism offer an excellent option to confirm the negative effects of ethylene on cotton (Shi et al., 2006). In this experiment, AVG and a *lintless* (5B) cotton line were used to modulate ethylene metabolism, whereas CO₂ enrichment was used to manipulate the supply of photo-assimilates to sinks (Rogers et al., 1996).

No significant fruit loss in the *lintless* and AVG-treated *Empire* cultivar under WL conditions, as well as increased fruit production under elevated CO₂ supply, confirmed that waterlogging damage in cotton can be minimised by modulating ethylene and carbon metabolism. However, despite a variable response to waterlogging, ethylene production in both genotypes increased under waterlogging indicating an impaired perception rather than production of ethylene in the *lintless*. Further genetic/molecular studies are required to confirm this hypothesis. This study also indicated that cotton plants might be unable to fully benefit from an increased photo- supply of assimilates due to accelerated ethylene production and fruit loss under elevated CO₂, especially under stressed conditions. Thus, sensitivity to elevated ethylene would be a major concern for cotton adaptability to future climatic conditions when put under stress.

Outcomes

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

Table Project objectives and key findings

Objectives	Key findings	Outcome	Future directions
Relationship between waterlogging and incident light	<ol style="list-style-type: none"> 1. Waterlogging accelerates fruits shedding, while shade increases yield losses by restricting carbon metabolism. 2. Waterlogging and shade interaction becomes significant only when the effect of one of the stresses is relatively small. 	Increased understanding of mechanisms through which waterlogging and shade damage cotton crop.	Shade has a limited effect on severely WL crop, thus waterlogging-induced damage should be studied in more detail.
Growth and yield dynamics within different canopy layers of cotton in response to soil waterlogging	<ol style="list-style-type: none"> 1. Yield losses in WL cotton are associated with the accelerated abscission of young fruits 2. Waterlogging causes higher damage to leaves and fruits on lower canopy layers. 3. Better recovery of the top canopy layer of cotton plant is the result of re-mobilisation of nutrients from lower canopy layers. 	Physiological processes, regulating cotton responses to soil waterlogging, have been elucidated.	Can waterlogging damage be minimised by protecting the abscission of young cotton fruits?
Optimising AVG application rates for WL cotton	<ol style="list-style-type: none"> 1. Anti-ethylene agent, AVG has a potential to increase yield of cotton crops by limiting the loss of early fruits. 2. 125 g [a.i] ha⁻¹ of AVG applied 24 h before waterlogging has the best effects on cotton yield. 	Protocols for improving waterlogging tolerance in cotton have been optimised.	Is waterlogging sensitivity in cotton associated with ethylene concentrations?
Investigating the basis of waterlogging sensitivity in cotton	<ol style="list-style-type: none"> 1. Waterlogging sensitivity in cotton is associated with photosynthetic inhibition. 2. Accelerated ethylene release from cotton tissues impairs photosynthesis and fruit development process. 	Major causes of waterlogging damage (accelerated ethylene production and inhibited photosynthesis) in cotton have been identified	Can tolerance to soil waterlogging be induced by regulating ethylene and carbon metabolisms?
Improving waterlogging tolerance in cotton through ethylene management and CO ₂ enrichment	<ol style="list-style-type: none"> 1. Regulating ethylene through genetic or chemical application techniques induces waterlogging tolerance in cotton. 2. Elevated CO₂ supply increases fruit production, and the plants with a lower ethylene sensitivity are more responsive to CO₂ fertilisation. 	The relative role of ethylene production and carbon limitation in cotton under WL environments has been established.	Can genetic manipulation techniques offer a solution to waterlogging sensitivity in cotton?

6. Please describe any:-

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

NA

Conclusion

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

Major hypotheses of the project and conclusions

The first hypothesis of the project was, “low incident radiation exacerbate waterlogging damage to cotton” and field experiments suggested that both waterlogging and shade negatively affected nutrient uptake, photosynthesis and lint yield. This hypothesis was supported by the fact that shade can cause substantial damage to moderately WL cotton crop. However, limited additional damage to severely WL crop under shade indicated that interactive effect of shade and waterlogging depends on intensity of individual stress factors. Considering the indeterminate growth habit and higher waterlogging sensitivity to specific growth phase of cotton, it was hypothesised that waterlogging variably influences growth and yield attributes across various canopy layers. The field experimental data supported this hypothesis, as the growth recovery process was delayed in the lower part of plant canopy. WL cotton plants restored leaf N and photosynthesis in the top layer as the waterlogging was terminated. However instead of initiating the development of new fruits the plants invested these additional photosynthates in maintaining growth of the retained fruits in the top canopy layers.

As yield losses in cotton was primarily attributed to waterlogging-induced abscission of young fruits, it was hypothesised that waterlogging damage can be minimised by controlling ethylene production. Various concentrations and application timings of an anti-ethylene agent (AVG) were tested under glasshouse and field conditions. The data confirmed the hypothesis that AVG has a potential to increase waterlogging tolerance in cotton.

The role of ethylene in relation to waterlogging damage was further explored in a series of glasshouse experiments, hypothesising that waterlogging sensitivity in cotton is associated with ethylene concentrations. A limited support of this hypothesis was observed, as two tested cultivars (waterlogging sensitive and tolerant) released a similar amount of ethylene from their leaves in response to waterlogging. It suggested that there might be a threshold level of ethylene, which induces early damage in the sensitive cotton cultivar. The study indicated that photosynthesis inhibition and higher ethylene release were primary causes of yield losses in WL cotton.

Based upon the evidence that waterlogging-induced damage is associated with inhibited photosynthesis and accelerated ethylene production, it was hypothesised that yield loss in WL cotton can be minimised by regulating carbon and ethylene metabolism. Increased tolerance to waterlogging damage in an ethylene-insensitive mutant and through elevated carbon supply supported this hypothesis. Elevated CO₂ increased production of new reproductive structures but also promoted ethylene synthesis, suggesting that stressed cotton plants might be unable to benefit from CO₂ enrichment if ethylene synthesis remains un-regulated.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:

- (a) to further develop or to exploit the project technology.
- (b) for the future presentation and dissemination of the project outcomes.
- (c) for future research.

Suggested future research

This study provided insights into the physiological mechanisms of regulating waterlogging damage in cotton, and suggested techniques for improving cotton performance under this stress. It also indicated that increased sensitivity to higher ethylene concentrations in cotton tissues was a major reason for the poor performance of cotton to waterlogged environments. Thus future research should focus on this issue.

- The glasshouse and field experiments have provided supporting evidence for the positive effect of the anti-ethylene agent AVG for ameliorating waterlogging yield losses in cotton. Farm scale studies are needed to verify its economic benefits.
- Fruit abscission in cotton could be a result of a crosstalk between ethylene and developmental regulatory pathways. It would be interesting to identify the ethylene and developmental transduction components involved in abscission of cotton fruits. As a major signalling molecule, ethylene modulates cotton responses to a variety of stresses; thus the role of ethylene and AVG should be investigated for cotton crops exposed to other abiotic stresses such as drought, heat, and salinity.
- Due to the essential role of ethylene in fibre development in cotton, completely blocking the production or perception of this hormone in plants may not be useful; hence tissue-specific ethylene production in cotton should be investigated and targeted. For example, if ACC is primarily transported from hypoxic roots alone, it might be possible to block ethylene biosynthesis in root tissues through transgenic techniques without affecting its production in the developing ovules.
- Detailed studies are required to uncover the expression pattern of genes regulating fibre development and fruit abscission in cotton, specifically the candidate genes regulating ACC biosynthesis in the peduncle (point of fruit abscission) and developing ovules.

9. A. List the publications arising from the research project and/or a publication plan.

(NB: Where possible, please provide a copy of any publication/s)

Journal publications

U. Najeeb, D.K.Y. Tan, M.P. Bange, B.J. Atwell (2015) Consequences of waterlogging in cotton and scope opportunities for amelioration of mitigation of yield losses. *AoB Plants*. (*In press*) DOI:10.1093/aobpla/plv080.

U. Najeeb, D.K.Y. Tan, M.P. Bange (2015) Inducing waterlogging tolerance in cotton through an anti-ethylene agent aminoethoxyvinylglycine (AVG) application. *Arch. Agron. Soil Sci.* (*In press*). DOI:10.1080/03650340.2015.1113403

U. Najeeb, B.J. Atwell, M.P. Bange, D.K.Y. Tan (2015) Aminoethoxyvinylglycine (AVG) ameliorates waterlogging-induced damage in cotton by inhibiting ethylene synthesis and sustaining photosynthetic capacity. *Plant Growth Regul.* 76:83-98.

U. Najeeb, M.P. Bange, D.K.Y. Tan (2015) Low incident light increases yield losses in moderately waterlogged cotton by restricting photosynthesis. *J. Agro. Crop Sci.*

Submitted Papers:

U. Najeeb, M.P. Bange, D.K.Y. Tan (2015) Cotton growth and yield dynamics across canopy layers in response to soil waterlogging. *J Cotton Sci.*

U. Najeeb, D.K.Y. Tan, M.P. Bange, B.J. Atwell (2015) Manipulation of ethylene and carbon metabolism affects fruit production when cotton plants are waterlogged. *Ann. Bot.*

Conference Publication

U. Najeeb, M.P. Bange, B.J. Atwell, D.K.Y. Tan (2015) Understanding the interactive effect of waterlogging and shade on cotton (*Gossypium hirsutum* L.) growth and yield. Abstract is published in *Procedia Environ. Sci.* 29:85-86.

U. Najeeb, D.K.Y. Tan, J.S. Amthor, M. P. Bange (2013) Pre-waterlogging application of aminoethoxyvinylglycine (AVG) improves fruit retention of waterlogged cotton. SWUP-MED conference proceedings Agadir, Morocco.

U. Najeeb, D.K.Y. Tan, M.P. Bange (2013) Aminoethoxyvinylglycine (AVG)-induced improved growth and fruit retention mitigate yield losses in waterlogged cotton. Australian Cotton Research Conference, Narrabri, Australia.

Poster presentation

U. Najeeb, M.P. Bange, B.J. Atwell, D.K.Y. Tan (2015) Understanding the interactive effect of waterlogging and shade on cotton (*Gossypium hirsutum* L.) growth and yield at “Agriculture and Climate Change conference” on Adopting Crops to Increased Uncertainty. Amsterdam, the Netherlands (15-17 Feb. 2015).

U. Najeeb, M.P. Bange, D.K.Y. Tan (2014) Optimising aminoethoxyvinylglycine application rate for waterlogged cotton. E-summary of the research work presented at the 17th Australian Cotton Conference “Our Fibre, Our Focus, Our Future” Gold Coast, Queensland, Australia (5-7 August, 2014).

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

NA

Part 4 – Final Report Executive Summary

Climatic variability, typified by erratic heavy-rainfall events can cause soil waterlogging and yield losses in irrigated cotton. This project investigated the physiological mechanisms of waterlogging damage to cotton crops and suggested strategies for increasing waterlogging tolerance. Field and glasshouse studies were conducted to study the interactive effects of waterlogging and shade on growth and yield of cotton crops. The data indicated that both waterlogging and shade can significantly damage cotton growth and yield. In addition, low incident light can exacerbate yield losses in moderately WL cotton crops but had limited effect on a severely WL crop. Cotton plants maintained growth and photosynthesis of the upper canopy leaves by re-mobilising nutrients from lower canopy leaves. Thus, yield reductions in WL crops were associated with the loss of young fruits from the lowest sympodial fruiting branches. To overcome this waterlogging-induced fruit abscission, an anti-ethylene agent aminoethoxyvinylglycine (AVG) was tested under glasshouse and field conditions. The data suggested that 125 g [ai] ha⁻¹ of AVG applied at the early reproductive phase of cotton can significantly increase lint yield of WL and NWL cotton crops. The role of ethylene and AVG in regulating cotton yield was further explored in a series of glasshouse studies. These experiments indicated that accelerated ethylene production and photosynthetic inhibition as the major reasons for yield losses in WL cotton and the damage can be minimised by regulating ethylene and carbon metabolisms.

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