

**PROMOTING RESILIENCE IN RAIN GROWN COTTON SYSTEMS WITH PLANT  
GROWTH REGULATORS**

By

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### **Declaration of Originality**

This thesis reports the original work of the author, except as otherwise stated.

The material of this thesis has not been submitted previously for a degree at this or any other university.

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## **Abstract**

Australian rain grown cotton production is characterised by significant climate variability, notably extremes of temperature and rainfall. Consequentially, water limitation is a focal constraint; with other abiotic stressors directly impacting and/or compounding impacts, on both production quantity and lint quality outcomes. Such elevated system exposure to climate risk results in increased variability of annualised lint production, quality and profit outcomes, as well as annualised and industry-proportionate production areas.

Management of climate risk across geographically diverse Australian rain grown cotton production areas is currently centred around the tactical manipulation of multiple agronomic management and genetic solutions simultaneously, so as to moderate existing system limitations and challenges and reach the water limited yield potential of the system. These existing strategies deliver capacity to introduce management diversity to the farming system, but lack application agility, have increased costs associated with implementation and critically, the rainfed-nature of the system often precludes opportunities for implementation. However, leveraging principles underpinning these existing strategies can inform development of novel adaptive management approaches utilising plant growth regulators (PGRs) that augment management diversity, to enable in-season responsive (agile) management of system challenges and limitations under variable climatic conditions; offsetting climatic variability and improving system resilience to climate extremes. Currently, a scarcity of detailed information exists regarding cotton crop and plant responses to exogenous Gibberellin, Cytokinin and Gibberellin biosynthesis inhibiting PGR treatment scenarios, notably in early growth and under water-limited Australian rain grown production contexts. Understanding such responses can enable future development of criteria frameworks for strategic, predictive applications within Australian rain grown cotton systems, that successfully manage climate risk and promote system resilience.

This study evaluated the utility of exogenously applied PGRs to tactically modulate cotton growth and development under rain grown production contexts. Utilising a top-down approach with a plant, crop and systems level focus; promotion of system resilience to climatic variability, through the provision of increased crop water accessibility, was examined via three physiological response strategies. Where applicable, the effectiveness of these approaches was evaluated to establish resource use efficiency benefits and / or economic values of the treatment propositions, to Australian rain grown production systems. Obtaining consistency of cotton plant response to

PGR treatment scenarios is customarily problematic and dependent upon a multiplicity of temporal and spatial appraisals, to facilitate accurate characterisation. By drawing on preliminary glasshouse and comprehensive field experiments over three production seasons (2016-19) at three locations in north-west New South Wales, provision of a broad-scale indication of specific treatment efficacy is presented. Using a prophylactic approach, treatments were positioned around achieving vegetative stage growth responses; thereby also negating endogenous hormonal influences associated with reproductive growth. Moreover, to progress research resourcefully, initial selection of specific PGR chemistry utilised published data; from horticulture and tree crops, irrigated cotton and international rain grown cotton production contexts. Concordantly, three significant bodies of investigative work are presented by physiological response strategy.

Adenine-type cytokinin, 6-benzyladenine (6BA), was evaluated for the capacity to promote cotton root biomass accumulation, to improve crop access to skip row soil water reserves. Under water-limited rain grown production settings, optimising root system architecture to increase the effective exploration of the cotton plant root growth area (root space), provides a buffer to the occurrence and impact of water deficit stress, notably in drier seasons. Moreover, when seasonal conditions permit, augmented access to soil water reserves facilitates extended crop growth for potential yield and lint quality benefits. Treatments were applied as seed priming and seed coatings in preliminary glasshouse evaluations, and as singular and triplicate (at 10 day intervals) foliar applications from four leaf stage, in both preliminary glasshouse and field experiments over the 2016/7, 2017/18 and 2018/19 production seasons at the Narrabri and Bellata experimental sites.

No 6-Benzyladenine treatments induced augmentation of root biomass accumulation or associated plant utilisation of soil water reserves were demonstrated. Initial glasshouse experiments facilitated rapid screening of multiple treatment modalities and verified no anticipated treatment impacts on short term root length, root dry weight and proportionate root biomass accumulation responses. Across field experiments, depth-stratified core-face sampling under the plant line and adjacent skip row demonstrated no foliar treatment differences in the presence of live cotton roots at mid flowering or maturity. No treatment differences in associated plant utilisation of shallow and deep soil water reserves were verified by either corresponding analysis of gravimetric soil moisture or non-destructive electromagnetic conductance surveys during both early and mid-flowering.

No treatment impacts on yield components, lint yield or quality were evidenced. No intra and inter-seasonal treatment impacts on total biomass accumulation and LAI were evidenced across field experiments. Early 6-Benzyladenine treatments induced square shedding in higher concentration triplicate foliar application treatments, resulted in early and mid-flowering fruiting pattern alterations and maturity impacts. Of the crops that were grown to maturity, principally no treatment differences in yield components, ginning turnouts, lint yields and lint quality parameters were evidenced.

The 6-Benzyladenine treatments evaluated were not effective in increasing root biomass accumulation, subsequent access to soil water reserves or follow-on lint yield and quality outcomes in these experiments. Treatment component and application regimes failed to induce the sought physiological changes under Australian rain grown cotton production system genetic, environmental and management contexts. Assumption is made that geographical, climatic, genetic and procedural differences may have been contributing aspects to the favourable outcomes demonstrated internationally.

Two bioactive gibberellins were evaluated for capacity to promote early plant canopy and structural biomass development; to intercept radiation and drive (above and below ground) growth and resultantly improve access to skip row soil water resources. Developing optimal early canopy biomass and resultant plant root / shoot structure in Australian rain grown cotton, may afford water deficit adaptation opportunities during critical early flowering to physiological cut-out stage. Increased access to soil water reserves via augmenting canopy capacity (Pn) to drive growth, may result in benefits to reproductive (number of sites produced and associated retention) capacities during squaring and early flowering. Gibberellins A<sub>3</sub> and A<sub>4,7</sub> were foliar applied from 4 leaf stage, both as single and triplicate applications at 10-day intervals. Preliminary screening and concept evaluation assessments were completed through early glasshouse and field experiments over the 2016/7, 2017/18 and 2018/19 production seasons at the Narrabri and Terry Hie Hie experimental sites.

Synopsis of findings for both total dry weight accumulation and corresponding differences in leaf area index (LAI) claim no consistent contributory impact of the specific GA<sub>3</sub> and GA<sub>4,7</sub> treatment scenarios evaluated. Early controlled environment experiments showed increased plant heights but no change in total dry weight accumulation, in response to both foliar and seed priming GA<sub>3</sub> treatments. Across the five field experiments, no GA<sub>3</sub> or GA<sub>4,7</sub> foliar treatment differences in

total dry weight accumulation were evidenced at early squaring, early or mid-flowering. In the field, no GA<sub>3</sub> treatment impacts on canopy capacity as leaf area index (LAI), were verified at early squaring or early flowering, with one of the two experiments showing an increased LAI at mid flowering. Correspondingly, GA<sub>4,7</sub> treatments evidenced no effects in LAI accumulation at early squaring, early- and mid-flowering.

Moreover, no positive treatment impacts of either GA<sub>3</sub> or GA<sub>4,7</sub> treatment applications on root biomass accumulation or associated plant utilisation of soil water reserves, across multiple treatment scenarios were demonstrated. Preliminary glasshouse screening of foliar and seed priming GA<sub>3</sub> treatments verified no treatment impacts on root length and root biomass. Seed priming treatments caused an undesirable inversely proportionate decrease in root biomass to treatment concentration, with a consequent decrease in proportionate root biomass accumulation. In the field, mid flowering core-face sampling of GA<sub>4,7</sub> treatments confirmed no treatment differences in the depth-stratified presence of live cotton roots to 1m, both under the plant line and adjacent skip row. Both GA<sub>3</sub> and GA<sub>4,7</sub> treatments demonstrated no differences in plant utilisation of shallow and deep soil water reserves.

The evidenced lack of root growth and canopy capacity differences as well as associated increases in height and the production of fruiting sites, had flow on implications for early fruit retention and subsequent maturity and yield components. GA<sub>3</sub> and GA<sub>4,7</sub> impacts on reproductive growth across all field experiments, showed increased development of total fruiting sites at early squaring, with a contrasting decrease in fruit retention and subsequent total square counts. No changes in average square weights were evident for either the GA<sub>3</sub> or GA<sub>4,7</sub> treatments. No flow-on GA<sub>3</sub> or GA<sub>4,7</sub> treatment differences in proportionate reproductive biomass, square number and size, green boll number and size were evident at early flowering. Delayed maturity was evidenced as smaller open boll sizes, however no corresponding differences in lint yield were noted.

Bioactive Gibberellin A3 and A4,7 treatment scenarios evaluated resulted in no changes to early canopy or structural (above and below ground) biomass accumulation, or the subsequent accession of additional soil water reserves. Concurrent increases in the development of early fruiting sites evidenced an increased rate of sympodial node development. However, under resource-constrained Australian rain grown production settings; the associated decreased retention (increased shedding) of early squares, suggests impact of the plants lack of accession of additional soil water reserves commensurate with the increased early production of total sites.

Moreover, inadvertent delays in maturity were achieved as a result of decreased retention (only) and not indicative of a true stasis via a delay in node development; suggesting water use efficiency consequences requiring further quantification.

Six anti-gibberellin PGRs were evaluated for the capacity to slow vegetative growth or induce vegetative physiological stasis, to manipulate the phase-transition to reproductive growth and shift subsequent crop peak resource demand to alternate (favourable) climatic conditions. The rain dependant nature of the production system delineates a priority to plant at the earliest opportunity, often earlier than the optimum window. Ensuing crop development results in the critical early flowering to physiological cut-out period coinciding with peak seasonal evaporative demand and the probable occurrence of extreme heat events. Temporally delaying crop maximum water requirements into a later climatic period of lower mean temperatures and vapor pressure deficits can improve or stabilise annualised yield outcomes and improve the downside risk associated with crop establishment outside the optimum window. Widely utilised Mepiquat chloride, in addition to cotton-atypical PGRs Chlormequat chloride, Paclobutrazol, Uniconazole, Trinexapac-ethyl and Prohexadione-calcium, were soil-drench (glasshouse only) and foliar applied from 4 leaf stage, both as single and triplicate applications at 7-day intervals. Preliminary screening and concept evaluation assessments were completed through early glasshouse and subsequent detailed field experiments during the 2016/17, 2017/18 and 2018/19 production seasons at the Narrabri and Terry Hie Hie sites.

Preliminary glasshouse-based evaluations of single treatment applications, both foliar and soil applications of Paclobutrazol and the foliar Uniconazole application produced 16-, 15- and 11-day delays in progression to 50% population first square, respectively. Early square shedding events caused by cold shock and the impact of sucking insect pests meant no substantiating differences in progression to population 50% first flower were demonstrated in the two subsequent preliminary field trials.

Preliminary glasshouse and field evaluations of foliar Chlormequat- and Mepiquat chloride, Paclobutrazol and Uniconazole soil and foliar applications, as well as Prohexadione-calcium foliar treatments; evidenced flow-on decreases in total dry mass accumulation at early flowering. However, no consistent treatment differences in canopy development, mid-flowering fruit load and subsequent lint yield, turnout and yield components were demonstrated in field assessments. Preliminary glasshouse and field evaluations of Trinexapac-ethyl demonstrated no LAI

differences at early- or mid flowering. Both total and proportionate reproductive biomass, as well as green boll numbers and size, were decreased in early flowering with no consistent impacts evident at mid-flowering. Early open bolls were comparatively smaller at mid-flowering, being commensurate with delayed development. Treatment induced delays in progression to physiological cut out and maturity were evidenced in both preliminary field experiments. No treatment impacts were observed on yield components at maturity, on ginning turnout or lint yield in both preliminary field experiments.

Foliar Paclobutrazol and Trinexapac-ethyl treatments were further evaluated during subsequent field experiments during the 2017/18 and 2018/19 production seasons, at both the Narrabri and Terry Hie Hie experimental sites. Detailed biomass assessments were utilised both as a proxy for reproductive phase change measurements and to characterise delays in reproductive growth achieved. Where apparent, induced delays in reproductive development were consistently produced by primarily a decreased accumulation of total fruiting sites; being evidenced as a decreased comparative square load during early squaring and augmented by a decreased retention of squares on those sites. Average square size was not impacted by reduced retention. Time of sowing impacted Paclobutrazol treatment responses; earlier sowings (only) evidencing comparatively reduced square counts, total number of sites grown and retention at early squaring. Trinexapac-ethyl treatments across all experiments had comparatively fewer squares, fewer total sites and a reduced retention of squares to those sites at early squaring.

Delays in reproductive growth induced by Trinexapac-ethyl treatments coincided with decreased total dry weights and LAI at early squaring across 3 out of 4 experiments, the effect being still evident at early flowering. Trinexapac-ethyl treatments induced average delays in progression to physiological cut-out and maturity of 15 and 12 days respectively. Yield and yield component responses varied across experiments, owing to hot and dry late season conditions experienced; decreased boll numbers and resultant lint yield comparative to the control in a proportion of experiments confuses the notion of a similar boll load, just moved temporally. Moreover, lint quality changes in micronaire and strength indicate possible influences of increased assimilate provision to the boll load; whether this as a result of a smaller comparative boll load or compensatory growth, and / or increased retention of later fruit, resulting in changed climatic conditions (from the control) through boll filling, is uncertain and requires confirmation.

This thesis begins to inform the discourse regarding system and crop level adaptive management opportunities to offset climate variability and promote system resilience, under resource-constrained Australian rain grown cotton production systems. Under capricious climatic conditions, in-season responsive (agile) management of system challenges and limitations introduces management diversity, reduces management implementation expenses and increases opportunity for management implementation. The resultant outcome being increased farmgate profits, through the provision of increased crop resource use efficiencies and improved yield and fibre quality outcomes, with less annualised variability. Unique Australian rain grown cotton production system-level adaptive management opportunities are identified and the utility of specific PGRs to accomplish, through the strategic and reliable manipulation of cotton plant growth and development, is explained. The data contributes to the understanding of cotton plant phenological responses to specific exogenous PGR treatment scenarios, under resource-constrained rain grown production settings and where applicable provides insight as to the systems-value of such PGR-driven adaptive management strategies.

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## Abbreviations

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<b>Abbreviation</b>	<b>Term Represented</b>
6BA	6-Benzylaminopurine
ABA	Abscisic Acid
ABARES	Australian Bureau of Agricultural and Resource Economics
ABS	Australian Bureau of Statistics
ACRI	Australian Cotton Research Institute
ACSA	Australian Cotton Shippers Association
ANOVA	Analysis of variance
APVMA	Australian Pesticide and Veterinary Medicine Authority
Aux	Auxin
AuxTI	Auxin Transport Inhibitor
BOLL	Open bolls per m <sup>2</sup> (OzCOT output parameter)
BOM	Bureau of Meteorology, Australian
CA	Cotton Australia Inc.
CAS	Chemical abstract service
CASRN	Chemical abstract service registry number
CC	Chlormequat chloride
Ck	Cytokinin
Ch.	Chapter
CRDC	Cotton Research and Development Corporation
CSD	Cotton Seed Distributors Ltd
CSIRO	Commonwealth Scientific and Industrial Research Organisation
Cut out	Physiological cut out
DAP	Days after planting
EC <sub>a</sub>	Apparent bulk soil electrical conductivity
EC <sub>t</sub>	Weighted total EC <sub>a</sub>
ET <sub>c</sub>	Crop Evapotranspiration
Eth	Ethylene
EthAI	Ethylene action inhibitors
EthBI	Ethylene biosynthesis inhibitor
ETS	Emissions Trading Scheme
FF	50% plant population first flower
FR:TOT	Proportionate reproductive biomass (dry matter)
Fsqu	50% plant population first square

GA <sub>3</sub>	Gibberellin A <sub>3</sub>
GA <sub>4,7</sub>	Gibberellin A <sub>4,7</sub>
GaBI	Gibberellin biosynthesis inhibitor
Gibb	Gibberellin
HSD	Honestly significant difference (refer: Tukey's post hoc analysis)
HVI	High volume instrument for cotton quality measurement
IPCC	Intergovernmental Panel on Climate Change
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
LA	Leaf area
LAI	Leaf area index
LSD	Least squares difference
MC	Mepiquat chloride
MDBC	Murray Darling Basin Commission
Monsanto	Monsanto Australia Ltd
NWS:NOAA	National Weather Service: National Oceanic and Atmospheric Administration
NAWF	Nodes above white flower
NFF	National Farmers Federation
NFFB	Node of first fruiting branch
No.	Number
NSW	New South Wales
OB	Open bolls
OBMB	On-board module builder
OGTR	Office of the Gene Technology Regulator
Paclo	Paclobutrazol
PGR	Plant growth regulator
PhxCa	Prohexadione calcium
P <sub>n</sub>	Net canopy photosynthetic capacity
Rain grown	Raingrown, Rain-fed, Dryland
RG	Rain grown
RMP	Resistance management plan
RRF	Roundup Ready Flex ® cotton cultivar
RSA	Root system architecture
Rt:Sh	Root:Shoot
SCBO	Seed cotton grams per boll (OzCOT output parameter)
SPAR	Soil plant atmosphere research (unit)

t	Tonne (metric)
TEP	Trinexapac ethyl
TTF	Time to 50% plant population first flower
TTFsqu	Time to 50% plant population first square
Unicon	Uniconazole
VPD	Vapour pressure deficit
VSN	VSN International
WUE	Water use efficiency

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## **Publications by the Candidate Relevant to the Thesis**

### **Conference publications**

**Welsh, C.J.** (2018). Gaming the system: using PGRs to develop resilient future cotton systems. In *Proceedings of the 2018 Australian Cotton Conference “Pushing Boundaries”*, Gold Coast, Australia: Cotton Australia and the Australian Cotton Shippers Association. Retrieved from <http://australiancottonconference.com.au/program/presentation-papers/>

### **Poster presentations**

**Welsh, C.J.**, Bange, M., and Tan, D.K.Y. (2017). Utilising plant growth regulators to develop resilient future cotton systems. In *Proceedings of the 2017 Australian Cotton Research Conference “SciCott2017: Cotton Science Delivering Impact”*, Canberra, Australia: Association of Australian Cotton Scientists. Retrieved from <http://cottonresearch.org>

Dodge, W., Young, A., **Welsh, C.**, Bange, M., Mahan, J., and Payton, P. (2018). Blue Pill / Red Pill: Yield Phenotyping in a Rain Grown Matrix. In *Proceedings of the 2018 Beltwide Cotton Conferences*, San Antonio, United States of America: National Cotton Council of America. Retrieved from <http://www.cotton.org/beltwide/proceedings/2005-2018/index/htm>

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“ond gyda Duw pob peth sydd bosible.” (Mt. 19:26, NKJV Cymraeg).

## **Chapter 1 Introduction**

### **1.1 Significance of the Australian cotton industry**

Modern Australian cotton (*Gossypium hirsutum L.*) production is characterised by high yielding, high quality, rain grown broadacre and semi-intensive irrigated row crop systems; requiring relatively high outlays of water, fertiliser and crop protection inputs (Braunack, 2013; Liu, Constable, Reid, Stiller, & Cullis, 2013; Luo, Bange, Johnston, & Braunack, 2015; Roth, Harris, Gillies, Montgomery, & Wigginton, 2013). Cotton is an economically significant crop to the Australian economy and is a global leader by yield, quality parameters and water use efficiency (CA, 2018a).

Australian irrigated lint yields exceed annual means of major cotton producing countries, consistently yielding 2.5 times the global mean (CA, 2018b). Industry irrigated mean yields of 2.2 t.ha<sup>-1</sup> (2009-14) (Roth, 2014) have been achieved due to breeding and improvements in crop, pest and post-harvest management (Hedayati, Brock, Nachimuthu, & Schwenke, 2019; Liu et al., 2013). Yields are achieved concurrently with increasing industry water use productivity; Australian irrigated production being three times more water efficient than the global mean (NFF, 2017) and demonstrating a ten-year, 40% increase in water use productivity (Roth, 2014).

Australian cotton is of consistently high quality; dry autumns in eastern Australian production regions facilitate optimal lint colour and grade quality (Strict Middling and Middling) of 1-5/32" to 1-1/4" (37-40; 29 mm – 32 mm) staple length, across the entire crop (ACSA, 2020; CA, 2018a; Roth, 2014). Australian cotton is in demand globally for production of high-quality fine to medium count yarns for use in the woven and knitted apparel sectors (van der Sluijs, 2017). Resultantly, Australian cotton commands a premium price; the long term mean price of Australian cotton (21 years, 1991-2012) being A\$460 per bale (Hedayati et al., 2019; Roth, 2014).

Cotton is one of Australia's largest rural export earners with over 99% of production being exported into a highly globalised market; the gross value of Australian cotton export revenue exceeds A\$2.0 billion annually (CA, 2015; Luo, Behrendt, & Bange, 2017; A. Williams et al., 2014). The Australian cotton industry is the third largest cotton exporter globally, producing 3%

(avg.) of global cotton stocks (CA, 2015, 2018a). Currently over 70% of Australian production is sold to China to complement the governmental release of lower quality stocks (ABARES, 2018).

Australian cotton production is highly profitable and a significant contributor of agriculture based income in the current eastern Australian production areas. Irrigated cotton gross margins exceed grain and sugarcane production per ha, whilst single skip rain grown cotton gross margins customarily exceed sorghum/chickpea double crop production on a return per mm of soil moisture basis (CSD, 2017; Hedayati et al., 2019). Cotton represents between 30 and 60% of the gross value of agricultural production in regions where it is grown (CA, 2018a; Roth, 2014) and typically makes up the largest proportion of income (approximately 66 %) in terms of gross value of production for enterprises based around cotton production (A. Williams et al., 2014). Australian cotton production helps underpin over 150 regional communities and 1200 farm enterprises across NSW, Qld and Victoria (CA, 2018a); in an average year, the industry employs up to 14,000 people in these regional and remote rural economies (CA, 2018a; CRDC, 2013).

## **1.2 A research focus on rain grown cotton production**

Study terms of reference are centred around rain grown cotton production, due to both; greater opportunities to promote system resilience (relative to irrigated production), as well as Australian cotton-industry-identified potential for significant sector growth. Elevated rain grown system exposure to climate risk results in increased variability of annualized lint production, quality and profit outcomes, as well as annualized and industry-proportionate production areas (CA, 2018b; Conaty et al., 2018; Powell & Welsh, 2019). Such relative increased magnitude of notably yield variation, affords greater opportunities to promote management amendments of economic benefit (McBratney, Whelan, Taylor, & Pringle, 2000).

Additionally, the Australian cotton industry has documented strategic imperatives for short to medium term (<25 years) research and commercial outcomes around improved system profitability and sustainability; citing an emphasis on significant expansion in production areas, an increased yield potential (Barnett, 2014; CRDC, 2013), doubling of input use efficiencies (CRDC, Cotton Australia 2013, 2018), halving yield and quality volatility (CA, 2018a; CRDC, 2013) and the rapid implementation of novel technology and improved management practices to position the industry as a leader in sustainable agriculture (CA, 2018a; CRDC, 2013). Industry

commissioned spatial analysis to determine the national-scale scope of both established and potential cotton growing regions, documented the expansion of rain grown cotton production into existing summer coarse grain crop production areas, as the optimal opportunity for sustainable and achievable overall industry growth; taking precedence from other identified location-specific, irrigated opportunities within southern and northern growing zones (Breckwoldt & Walton, 2014). Simultaneously, the widespread commercial uptake of transgenic Bollgard®3 technology promoting crop resilience to *Helicoverpa spp.* and a concomitant less-restrictive resistance management plan offering a wider planting window, reduced refuge and pupae busting requirements; offers greater flexibility in the production of cotton within the rain grown farming system and is viewed by industry as having potential to further drive expansion into such areas (Grundy & Klepper, 2016; Lowien, 2016).

### **1.3 Utilising adaptive management to contend with climate risk and promote agroecological production system resilience**

Agroecological system resilience refers to the capacity of the production system to absorb disturbance, reorganise and adapt, in order to maintain the same purpose-based identity (Lin, 2011; Peterson, Eviner, & Gaudin, 2018). Management of climate risk across geographically diverse Australian rain grown cotton production areas is currently centred around the tactical manipulation of multiple agronomic management and genetic solutions simultaneously, so as to moderate existing system limitations and challenges and reach the water limited yield potential of the system. These existing strategies (e.g. timing of planting, cultivar choice, row configuration) deliver capacity to introduce management diversity to the farming system, but lack flexibility in application, have increased costs associated with implementation (e.g. split nitrogen applications) and critically, the rainfed-nature of the system often precludes opportunities for implementation. However, leveraging principles underpinning existing strategies can inform development of novel adaptive management approaches utilizing plant growth regulators (PGRs) that augment management diversity, to enable in-season responsive (agile) management of system challenges and limitations under variable climatic conditions; offsetting climatic variability and improving system resilience to climate extremes.

Cotton growth and development is regulated in-part by plant phytohormones and can be manipulated via the application of synthetic PGRs to influence endogenous hormone

concentrations, transport and / or associated receptor sites; plant responses being dependent on cultivar (genetic), management and environmental contexts. In-crop PGR applications can facilitate active regulation of developmental processes through “fine tuning according to need”; both by affecting the optimal growth or development stage via treatment timing, and / or the intensity of manipulation via treatment dosage. Currently, a scarcity of detailed published information exists regarding cotton crop and plant responses to exogenous Gibberellin, Cytokinin and Gibberellin biosynthesis inhibiting PGR treatment scenarios, notably in early growth and under water-limited rain grown production contexts. Understanding such responses can enable future development of framework criteria for strategic, predictive applications within rain grown cotton systems, that successfully manage climate risk and promote system resilience.

#### **1.4 Central research question**

Is it possible to tactically modulate cotton growth and development using plant growth regulators, to create novel adaptive management strategies that promote rain grown production system resilience, offering economic lint yield benefits and / or resource (water) use efficiencies?

#### **1.5 Objectives**

This study evaluated the utility of exogenously applied PGRs to tactically modulate cotton growth and development under rain grown production contexts. Promotion of system resilience to climatic variability through the provision of increased crop water accessibility, was examined via understanding agronomic responses within the wider farming system context. Research to assess benefits of specific PGR opportunities utilised a top-down approach from a systems, crop and ensuing plant level focus; investigations being pursued only to the extent required to understand and validate outcomes.

Obtaining consistency of cotton plant response to PGR treatment scenarios is customarily problematic and dependent upon a multiplicity of temporal and spatial appraisals, to facilitate accurate characterization. By drawing on preliminary glasshouse and comprehensive field experiments over three production seasons (2016-2019) at three locations in north-west New South Wales (NSW) (Australia), an indication of specific treatment efficacy is presented, relative to:

- the direct impacts of PGR chemistry; selection, dosage, application methodology, frequency and timing of application scenarios (“treatment scenario”), on specific plant and crop phenology, in addition to,
- the cascade of crop and plant level responses to treatment impacts; as explained by reproductive development, retention, maturity, yield and lint quality outcomes.

Nomenclature of active ingredients in PGR products, as with those of other crop protectants or pharmaceuticals, are typically copious and indistinct. Within this study, the common name as assigned by the International Organisation for Standardisation (ISO) is primarily referred to; being short, distinctive, non-proprietary and providing distinct compound identification of which different forms (salts, isomers) may exist. Systematic chemical names based on International Union of Pure and Applied Chemistry (IUPAC) nomenclature, Chemical Abstracts Service (CAS) and “other” names, as well as CAS Registry Number (RN), structure, formula and molecular mass and commercial source of relevant PGRs are detailed in Appendix 1; acronyms being described in the Abbreviations section. Using a proactive approach, PGR treatments were positioned around achieving vegetative stage growth responses; thereby also negating endogenous hormonal influences associated with reproductive growth. Moreover, to progress research resources efficiently, initial selection of specific PGR chemistry exploited published data; from horticulture and tree crops, irrigated cotton and international rain grown cotton production contexts. Specific references are cited against treatments (where relevant) in the Materials and Methods (Treatments) section of Chapters 4, 5 and 6. Initial foliar treatment applications under glasshouse and early field studies were singular applications; being compared directly with seed soaking and seed coating treatments. Later field studies evaluated the effectiveness of triplicate foliar applications in maintaining efficacy for extended time periods, to achieve the desired phenological manipulations.

Concordantly, three significant bodies of investigative work are presented by physiological response strategy (Table 1.1). (1) Adenine-type Cytokinin, 6-benzylaminopurine (6BA), was evaluated for the capacity to promote cotton root biomass accumulation, to improve crop access to skip row soil reserves (Chapter 4). (2) Two bioactive gibberellin PGRs were evaluated for capacity to promote early plant canopy and structural biomass development; to intercept radiation and improve (above and below ground) growth, improving access to skip row soil resources

(Chapter 5). (3) Six anti-gibberellin PGRs were evaluated for the capacity to slow vegetative growth or induce vegetative physiological stasis, to manipulate the phase-change transition to reproductive growth and shift subsequent crop peak resource demand to alternate (more favourable) climatic conditions (Chapter 6). Furthermore, where applicable, the effectiveness of these approaches was evaluated, to establish resource use efficiency benefits and / or economic values of the treatment propositions, to rain grown production systems (Table 1.1, Chapter 7).

**Table 1.1 Multi-scale analysis of cotton growth and development responses to exogenous PGR treatment scenarios under glasshouse and rain grown field conditions.**

<b>Body of work</b>	<b>PGR group</b>	<b>Plant level</b>	<b>Physiological response strategy</b>	<b>Measurements</b>
Chapter 4	Adenine-type Cytokinin	Crop / plant	<p><b>“Exploiting the skip”</b></p> <p>Promotion of root biomass accumulation and consequent plant root growth area (root space) to increase access to skip row soil water reserves</p>	<p><b>Direct impact:</b> root growth, soil water</p> <p><b>Secondary response:</b> biomass and canopy development, fruit development and retention, lint yield and components, lint quality</p>
Chapter 5	Gibberellins	Crop / plant	<p><b>“Fitness for environment”</b></p> <p>Promotion of early canopy and structural biomass development; augmenting canopy capacity to drive (above and below ground) growth and increase access to soil water reserves</p>	<p><b>Direct impact:</b> root growth, soil water, biomass and canopy development</p> <p><b>Secondary response:</b> biomass and canopy development, fruit development and retention, lint yield and components, lint quality</p>
Chapter 6	Gibberellin Biosynthesis Inhibitors	Crop / plant	<p><b>“Dynamic fruiting”</b></p> <p>Slowing vegetative growth / inducing physiological stasis, to manipulate crop level reproductive phase-transition and shift crop peak resource demand to alternate (favourable) climatic conditions</p>	<p><b>Direct impact:</b> early fruit development and retention</p> <p><b>Secondary response:</b> biomass and canopy development, fruit development and retention, lint yield and components, lint quality</p>
Chapter 7	As relevant	System / crop	<p><b>“Value of the opportunity”</b></p> <p>Quantifying the system yield, input efficiency and economic values of the PGR responses</p>	<p>Long-term modelled lint yield and water use efficiency outcomes.</p> <p>Economic gross-margin analysis.</p>

## **Chapter 2 Review of Literature**

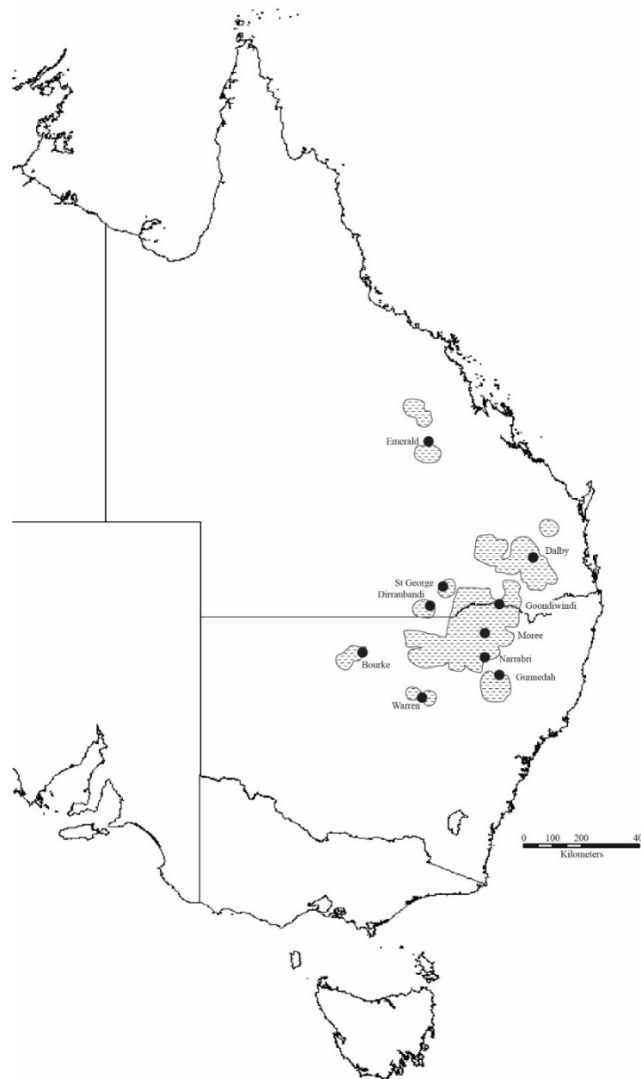
### **2.1 Introduction**

The purpose of this review is to examine current literature regarding adaptive management strategies to promote agroecosystem resilience of rain grown cotton production systems. In brief, the review characterizes Australian rain grown systems and outlines how specific abiotic (water) stressors contribute to existing and projected future systems challenges. Current agronomic adaptation strategies to manage climate risk and promote system resilience are described, drawing out their underpinning agronomic and physiological mechanisms. Ultimately by leveraging identified strategies, this review highlights the potential for the application of plant growth regulators to create novel agronomic adaptation solutions to strengthen system resilience, leading to improved production efficiencies and economic yield and lint quality benefits.

### **2.2 Characteristics of Australian rain grown cotton production systems**

Rain grown cotton is produced over a large, 115 175 km<sup>2</sup> area of eastern Australia, exhibiting significant variation within temperature, rainfall and soil characteristics across the growing region (Breckwoldt & Walton, 2014). Yield is reliant on, and responsive to, the volume of stored soil moisture accumulated prior to planting, in-crop rainfall received and in-season temperature profiles and humidity, affecting evaporative demand, notably during reproductive stages of flowering and boll filling (Anwar & Darbyshire, 2017; Eveleigh & Stiller, 2003; Tesdell, Othman, & Alkhoury, 2018).

Production is mostly situated in long-season, warm temperate summer rainfall environments in eastern Australia (Eveleigh & Stiller, 2003; OGTR, 2002), where soils have high water holding capacity and in-season rainfall is more reliable (Anwar & Darbyshire, 2017). Established areas for rain grown production include; the Liverpool Plains (Quirindi), Upper Namoi (Gunnedah), Gwydir (Moree) and arid western regions in the Lower Namoi (Wee Waa, Walgett) of NSW, as well as the Macintyre Valley (Goondiwindi), Darling Downs (Dalby) and Central Highlands (Claremont ,Biloela) in Queensland (Figure 2.1) (Alexandra, 2012; Bange, Carberry, Marshall, & Milroy, 2005; Liu et al., 2013).



**Figure 2.1 Current rain grown cotton production area (shaded polygons) totalling approx. 115 175 km<sup>2</sup>; not all extrapolated extent area being under production simultaneously, map orientated north and based on Breckwoldt and Walton (2014).**

Soils are typified by brown, grey or black alluvial soils with Vertic properties of basaltic origin; with sodic subsoil constraints common (Dang et al., 2008; Dang et al., 2006). Production also occurs on adjoining transitional soil types differing in structure, depth and sodicity (Breckwoldt & Walton, 2014). Soil plant available water holding capacity (PAWC) determines land-use suitability for rain grown cotton. Archetypal cracking clay soils of production regions can store between 150-220 mm of plant available water in a 130 cm profile (up to 300 mm in a 1.5 m

profile), particularly in a long fallow lead-in scenario following a wet winter; with 150 mm of storage capacity being perceived as the minimum for production suitability (Eveleigh & Stiller, 2003; Roth et al., 2013).

Australian cotton is grown commercially as an annual summer-grown crop, predominately in the 400-800 mm summer rainfall zone (CA, 2018a, 2018b; Roth et al., 2013). Rain grown cotton crops can receive significant proportions of their water requirements from in-season rainfall events, however seasonal total crop water demand generally exceeds rainfall supply, necessitating reliance on stored soil moisture to fulfill the deficit (Roth et al., 2013). Opportunity production also occurs in arid regions (~100 mm rainfall) leveraging large stored soil moisture reserves, however the most favourable areas have summer-dominant annual rainfall means of at least 600 mm (Conaty et al., 2018; CRDC, 2002). Cotton's perennial and indeterminate growth habit enables a lengthy production season of up to 180 calendar days (Constable & Bange, 2015). In-crop mean monthly rainfall (October to March) associated with long-season production areas ranges from 353 to 491 mm and is highly variable (standard deviation >125 mm) (Table 2.1) (Bange et al., 2005; Constable & Bange, 2015; Roth et al., 2013).

**Table 2.1 Mean monthly in-crop rainfall totals (mm) and distribution (+/-1 standard deviation), for the Australian cotton growing season (October to March) in rain grown cotton production regions across New South Wales (NSW) and Queensland (QLD) (Bange et al., 2005).**

Region	Oct	Nov	Dec	Jan	Feb	Mar	Total <sup>(1)</sup>
Breeza, NSW	53 +/-37	59 +/-44	65 +/-41	72 +/-62	61 +/-56	47 +/-48	358 +/-136
Wee Waa, NSW	48 +/-39	54 +/-46	55 +/-45	79 +/-69	64 +/-64	52 +/-57	353 +/-148
Bellata, NSW	49 +/-37	60 +/-49	62 +/-49	83 +/-67	66 +/-55	47 +/-44	368 +/-140
Moree, NSW	48 +/-32	56 +/-44	58 +/-47	71 +/-54	68 +/-61	58 +/-62	361 +/-136
Croppa Creek, NSW	46 +/-36	58 +/-47	65 +/-54	76 +/-58	70 +/-68	52 +/-52	368 +/-127
Goondiwindi, QLD	49 +/-33	60 +/-47	71 +/-47	80 +/-64	71 +/-58	59 +/-66	389 +/-129
Dalby, QLD	58 +/-39	74 +/-51	79 +/-62	84 +/-56	79 +/-64	64 +/-51	453 +/-125
Biloela, QLD	52 +/-38	74 +/-45	98 +/-60	104 +/-68	98 +/-77	66 +/-53	491 +/-144
Emerald, QLD	40 +/-39	59 +/-46	88 +/-67	100 +/-85	97 +/-83	68 +/-68	453 +/-190

<sup>(1)</sup> Total in-crop rainfall, October to March

Sustained (30-year) lint yield improvements in the Australian cotton industry have been attributed to availability of new locally-bred cultivars (45%) and to such cultivars responding to improved system management (rotation, fertiliser, water management, disease, pest and weed management)

(55%) (Braunack, 2013; Liu et al., 2013). When effective summer rainfall totals are accounted for, rain grown cotton has similar yield (0.1 bales/ha/year) and water (rainfall) use efficiency (0.00042 bales/mm/year) increases to those obtained under irrigation (Constable, Llewellyn, Wilson, & Stiller, 2011). Full season cultivars with long fibre characteristics are best suited to Australian rain grown conditions; enabling the crop to better capitalise (via an indeterminate growth pattern) on irregular rainfall events and counteracting impacts of associated water deficit stress on lint quality (Eveleigh & Stiller, 2003). Varieties suited to rain grown production being able to extract up to 40 mm more soil water than less suited varieties, because of a longer growing period (Constable et al., 2011).

The commercial use of transgenic varieties is widespread, with 99% of overall industry production (by area) utilising biotechnology; with a 94% uptake of Bt (Bollgard®) traits and 99% uptake of herbicide tolerant (RR Flex® and Liberty Link®) traits (all traits Bayer CropScience Pty Ltd) (CA, 2018a). Bollgard® cultivars contain the Cry1Ac, Cry2Ab and Vip3a Bt (*Bacillus thuringiensis*) proteins enabling effective control of major Lepidopteran pests, including *Helicoverpa* spp.; significantly reducing the amount of pesticides applied (Luo, Bange, Braunack, & Johnston, 2016; Wilson et al., 2013). Current cultivars also include Roundup Ready Flex® traits, containing a gene from the soil bacterium *Agrobacterium tumefaciens* that confers ability to control both broadleaf and grass weeds with over the top (OTT, in crop) glyphosate applications. This practice is both threatened by, and a contributor to, glyphosate resistance in farming system weed populations; however, in-crop weed control is necessary as resource competition with cotton can substantially reduce lint yield (Walker et al., 2005).

Australian rain grown cotton production is co-dependant on integration with other grain, oilseed and pulse crops and is often recognised as underpinning farm profitability, or as a lesser frequent “opportunity rotation” that adds profitability and affords key system benefits, such as;

- a disease break for cereal disease organism lifecycles and a non-host for nematodes and fungi,
- the ability to use glyphosate and target problem weeds with group A herbicides, as an alternative to sorghum group C and K in-crop herbicide options, and because of cotton’s deep taproot,

- access soil moisture and leached N at depth and within sodic subsoil layers during summer, on account of greater salt tolerance (than sorghum, for instance) and deep taproot (Grundy & Klepper, 2016).

Rain grown cotton is customarily planted into warm soil conditions (min 14°C and rising) with a full soil moisture profile between October and December; depending on the timing of rainfall events that facilitate planting and subsequent germination, which will vary between seasons and locations (Anwar & Darbyshire, 2017; Luo et al., 2016) . Seed is normally placed between 2.5 and 5 cm depth depending on seedbed conditions and depth of surface soil moisture, with a planting density of 7-8 plants.m<sup>-2</sup> (OGTR, 2002). Row spacing and configuration is dependent on yield expectations, soil water holding capacity and available starting soil moisture, machinery configuration, seasonal climatic outlook and grower risk aptitude (CSD, 2017). Crop nitrogen is utilised from preceding crop soil resources, applied pre-plant or increasingly as a post-planting side dressing application as urea or anhydrous ammonia, allowing for adjustment of rates to best suit the prevailing in season conditions (Braunack, 2013). Cotton is chemically defoliated to remove leaf prior to being mechanically harvested between March and May (Constable & Bange, 2015).

### **2.2.1 Changing contexts of production.**

Australian rain grown cotton production systems operate within globally-sourced and rapidly evolving environmental, social-cultural, economic and political-legislative environments; that through complex nature–culture negotiations, substantially shape both the production system scope and outcomes (Alexandra, 2012; Bange et al., 2016a; Tendall et al., 2015). Resultantly, dynamic production drivers such as:

- existing climate variability;
  - policy based industry regulation;
  - changing societal perceptions of production;
  - increased competition for environmental resources;
  - the emerging impacts of climate change;
  - as well as the influence of shifting terms of trade and technology advancements;
- all impact rain grown cotton system resilience, through adding provision demand and altering

regional competitiveness, and conceivably social and economic stability (Alexandra, 2012; Ge et al., 2016; Steffen et al., 2001; Tendall et al., 2015). Significantly, the location and time-specific interaction of these production drivers create distinctive abiotic-stress mediated challenges and limitations related to the sequencing of cotton in Australian rain grown farming systems. Resultantly, these key production drivers require consideration when understanding opportunities to beneficially manage or manipulate crop growth and development.

### ***Limitations on annualised production and profit due to existing climate variability***

Australian rain grown cotton production is characterised by significant climate variability, notably extremes of temperature and rainfall, influencing both the industry wide rain grown lint yield as well as the annualised and industry proportionate production areas (Ge et al., 2016; Luo et al., 2017; Pereira, 2016). Published mean annual commercial lint yields for Australian rain grown cotton systems range between 794-930.7 kg.ha<sup>-1</sup> depending on the source and time period analysed and demonstrate wide year to year yield variation (ABARES, 2019; CA, 2018b; Conaty et al., 2018; Roth, 2014). Australian rain grown field and simulation studies also document high year to year yield variability (113.5-2179 kg.ha<sup>-1</sup>) (Bange et al., 2005; CRDC, 2002); agronomic determinants of yield being predominately associated with stored soil moisture at planting, as well as rainfall and temperature both during reproduction and across the entire growing season as a whole (Bange, Constable, McRae, & Roth, 2010; Turner, 1986). Anwar and Darbyshire (2017) modelled the effect of climate (rainfall, mean temperature and extreme heat days) on lint yield across four rain grown cotton regions in Qld and NSW and determined inter-seasonal lint yield to be highly variable (CV: 33-49%). Conaty et al. (2018) also documented the historical incidence (1 in 5) of very low yields (<550 kg.ha<sup>-1</sup>) associated with the impact of seasonal conditions on CSIRO rainfed germplasm evaluations.

Seasonal climate variability also affects crop profitability and resultantly annual production areas. Increased crop gross margin profitability, as well as comparative profit advantage with determinate summer grain and pulse options, is associated with seasons with above mean in-crop rainfall (Conaty et al., 2018; Grundy & Klepper, 2016; Powell & Welsh, 2019). Consequently, rainfed cotton crops are not planted every season; planting decisions are driven by the substantial availability of stored soil moisture, as well as favourable prevailing commodity prices and to a lesser extent, long-range rainfall predictions (Ford & Forrester, 2002; Redfern & Welsh, 2014).

Resultantly the annual proportion of Australian cotton plantings that rain grown cotton represents is dynamic between 5 and 25% of the total green area planted (Conaty et al., 2018; Roth, 2014). Annualised production areas also vary; between 20 and 200000 green ha, averaging 63000 ha, for the period 2007-2017 (CA, 2018b; Dowling, 2019; Roth, 2014).

***Increased management frameworks associated with changing societal perceptions of agricultural production***

Changes in wider community values, associated shifts in consumption patterns, perceptions regarding the social acceptability of agriculture and the maintenance of connected natural resources, in addition to a growing consumer awareness and concern regarding sustainable and ethical fibre production, require; increased industry accountability, transparency and communication of production practices in order to maintain a “social licence” to farm (Falguera, Aliguer, & Falguera, 2012; Teagle, 2010; Ticehurst & Curtis, 2015). Current social discourse associated with the sustainability of modern (Australian) rain grown cotton production, includes impacts of the production system on:

- wider riverine catchments and aquatic systems (groundwater and rivers), through on-farm modification of field runoff/ drainage patterns;
- farm biodiversity enhancement;
- maintaining soil quality (tillage, erosion, salinity);
- increasing water use efficiency;
- chemical use for pest and disease management;
- the use of transgenic technology;
- dust and odour emissions;
- the greenhouse gas footprint of cotton production, associated carbon sequestration and energy use (Roth, 2011; Teagle, 2010; Tendall et al., 2015).

Participation within formalised structures and programs to facilitate such accountability and transparency, often requires rigidity in production practices, thereby limiting potential management options, and impacting directly on profitability through increased labor requirements

associated with reporting and documentation obligations; with improved end-market accessibility and/or increased lint price outcomes not always evident.

### ***Production changes resulting from government policy and industry regulation***

Overarching government policy and political standpoints, as well as industry regulation; shape current production practices and may influence future industry scale and/or profitability (Bange & Constable, 2008b; Braunack, 2013; Ticehurst & Curtis, 2015). Introduction of commercial novel biotechnology Bollgard®3 (BG3) varieties resulted in modifications to the existing Bollgard®II Resistance Management Plan (RMP). The current BG3 RMP aims to; minimise exposure of *Helicoverpa* spp. to the *Bacillus thuringiensis* (Bt) proteins Cry1Ac, Cry2Ab and Vip3A, dilute potential population resistance by ensuring provision of populations of susceptible individuals and removing resistant individuals at the end of the cotton season (Monsanto, 2016b). The BG3 RMP achieves these aims through legislative jurisdiction over commercial production practices regarding; planting timing restrictions, the compulsory planting of a designated proportional area of refuge crops, the control of volunteers and ratoon cotton, end of season pupae destruction (via tillage) and pesticide application limitations (Monsanto, 2016b). Such changes to defined production practices have the potential to impact the scope and productivity of rain grown cotton, for e.g., enabling exploitation of both early/late season planting opportunities that had previously been excluded by preceding RMP planting window restrictions, or the increased appeal of cotton as a rotation option to traditional zero tillage broad acre (grain, oilseed) producers, on account of reductions in tillage requirements associated with end of season pupae destruction (Grundy & Klepper, 2016).

### ***Increased competition for environmental resources***

Water governance and policy reform is an emerging global focus resulting from population growth driving an increased global food demand, and associated reductions in land and fresh water availability due to competing environmental applications (Bange et al., 2016a; Lautze, de Silva, Giordano, & Sanford, 2011; Ticehurst & Curtis, 2015; Y. Wang, 2003). In an Australian context, the Murray–Darling Basin (MDB) accounts for approximately 91% Australia’s total cotton production area (ABS, 2016a), with cotton contributing an mean of 18% of the total gross value of irrigated agricultural production in the Basin (2006–07 to 2014–15) (ABS, 2016b).

Increasing conflict for fresh water resources in the MDB is being addressed via the Murray Darling Basin Plan (MDBA, 2016), with a focus on improving on-property water use efficiency (WUE) of irrigated agriculture and by reallocating water access from agriculture to the environment (Ticehurst & Curtis, 2015). Such competition for water between urban, industrial and agricultural use will compound drought effects (Nachimuthu & Webb, 2017) and drive an increased relative significance (by yield output) of rain grown cotton production (A. Williams et al., 2014); necessitating intensified agronomic management interventions to stabilise annualised yield variation associated with a reduced reliance on irrigation.

### *The emerging impacts of climate change*

Projected future climate change is a multi-faceted, complex challenge for global cotton industries and has the potential to profoundly affect the sustainability of Australian rain grown production via both legislative and direct ecological impacts; at farm-enterprise, ecosystem and wider associated community levels (Bange et al., 2016a; Garnaut, 2008, 2011; IPCC, 2014). Agriculture is a significant contributor (16%) of Australian greenhouse gas emissions (Keogh & Thompson, 2008). Resultantly, future-imposed government regulations aimed at climate change mitigation through emissions reductions (Bange et al., 2016a; Braunack, 2013; Teagle, 2010) may also result in changed operating settings for rain grown production, as an indirect consequence of climate change. Potential system impacts of proposed Emissions Trading Scheme (ETS) being associated with; the limited system opportunities for sustained carbon offsets, increased fuel and energy costs, and reduced competitiveness with other OS markets that have no ETS (Braunack, 2013).

The emerging direct ecological effects of climate change are envisaged to increase occurrences and compound the impacts of abiotic stressors on plant growth and yield, via elevated CO<sub>2</sub> levels, reduced future system water availability, increased inter-annual and seasonal variability, increased frequency of extreme climatic events, as well as modified weed, pest and pathogen pressures (Altieri, Nicholls, Henao, & Lana, 2015; Chapman, Chakraborty, Dreccer, & Howden, 2012; Tompkins & Adger, 2004; A. Williams et al., 2014). System water deficits being driven by altered precipitation patterns, increases in mean temperature and resultant modified transpiration regimes (due to lower humidity and net evaporation increases) (Bange & Constable, 2008b; Pereira, 2016; A. Williams et al., 2014).

Comparatively little understanding exists regarding the resultant interactive system impacts on Australian cotton productivity (Bange et al., 2016a), which are suggested to be diverse, significant, and location-specific (Altieri et al., 2015; Tompkins & Adger, 2004); modelling by A. Williams et al. (2014) suggesting a possible decrease in cotton yields without the effect of CO<sub>2</sub> fertilisation. Resultantly, climate change induced modification on cotton growth and yield could significantly impact cotton production practices and may conceivably change the historic location of production (Bange et al., 2016a), with only a proportion of system modifications likely to be compensated for via market systems (Chapman et al., 2012).

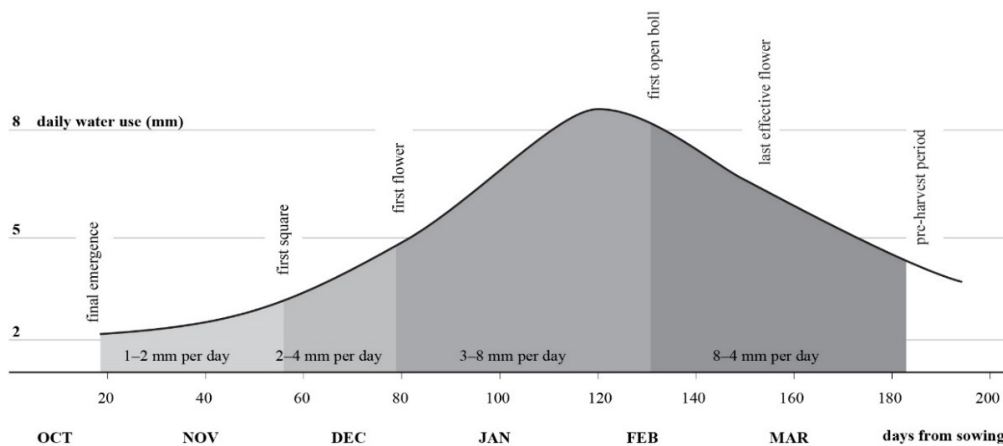
### **2.3 Abiotic stress mediated production challenges and system sequencing limitations**

Australian cotton production is predominately driven by water availability. Other abiotic stressors, such as; excessive variances in temperature (heat and cold-chill) (Zahid et al., 2016), macro / micro nutrient deficiencies (Pereira, 2016), gaseous pollutants (Ozone, CO<sub>2</sub>) and soil constraints such as mechanical compaction (Antille, Bennett, & Jensen, 2016) and sodicity (Dodd, Guppy, Lockwood, & Rochester, 2013), can also all directly impact and / or compound the effect of water deficit stress on yield and quality in rain-grown systems. Combinations of these stressors can cause unique, location-specific effects that cannot be predicted from individual stressors (Chapman et al., 2012; Nachimuthu & Webb, 2017). Consequently, a range of physiological iterations of combined abiotic stress impacts are evidenced (Pereira, 2016), through unique, water-driven production challenges and system sequencing limitations, as well as the resulting annualised yield gap between potential and achieved yields (Bange et al., 2016a; Constable & Bange, 2015). Consequently, such location- and / or system-unique production challenges and sequencing limitations, require consideration when understanding opportunities to profitably manage or manipulate crop growth and development.

#### **2.3.1 System, crop and plant level implications of water deficit stress**

Moisture determinants of production in Australian rain grown cotton systems include the timing and volume of seasonal rainfall, as well as prevailing atmospheric vapour pressure deficits affecting evaporative demands (A. Williams et al., 2014). Depending on seasonal rainfall patterns, Australian rain grown yield potential can range from less than 300 to over 2000 kg lint.ha<sup>-1</sup>; averaging 800 kg lint.ha<sup>-1</sup> (Conaty et al., 2018; Ford & Forrester, 2002).

Total rain grown cotton crop water requirements are an accumulation of the daily crop evapotranspiration (ET<sub>c</sub>) over the whole season and under Australian conditions range from 650-770 mm, with a maximum daily water use between 7 and 12 mm.day<sup>-1</sup> during peak flowering and early boll development (CRDC, 2012) (Figure 2.2). In most Australian rain grown cotton production regions, seasonal crop water demands of up to 729 mm ET<sub>c</sub>, habitually exceed the highly variable 400-800 mm production region mean rainfall supply (Roth et al., 2013). Consequently, a critical reliance exists on stored soil water to offset the deficit between in-crop rainfall and crop ET<sub>c</sub> requirements and to buffer against the associated high probability of occurrence of in-season moderate to severe moisture stress (Eveleigh & Stiller, 2003; Lascano & Baumhardt, 1996; Rich et al., 2016).



**Figure 2.2 Nominal seasonal daily water use (mm.day<sup>-1</sup>) for cotton in Australian production environments (CRDC, 2010).**

Water deficit stress causes changes in cotton plant physiological characteristics such as decreased net photosynthesis rates and stomatal conductance. Such changes result in diminished plant growth (principally leaf area and rate of leaf expansion), sink limitations (fewer vegetative and sympodial nodes / fruiting sites), premature senescence and a decrease in yield and quality parameters such as fibre length (Bange et al., 2017; Loka & Oosterhuis, 2014; Singh, Pandey, Naskar, & Shirke, 2015). Cotton crop response to water deficit stress depends on; the duration, intensity and recurrent frequency of limited water conditions, the varietal-specific endurance capacity, relative maturity of the crop and the occurrence of other compounding biotic and abiotic stressors (Singh et al., 2015). Under commercial production conditions, water deficit stress will reduce lint yield by ~19 kg.ha<sup>-1</sup> for each day of stress (viz. leaf water potential less than -2.4 MPa)

during early flowering, with cumulative interactions of stress events from successive phenological growth stages resulting in significant (48%) yield losses (Hearn & Constable, 1984b; Yamada & Grimes, 1982).

### **2.3.2 Water availability and system sequencing limitations**

Sequence rotation with grain and legume crops is essential to the sustainable production of rain grown cotton; continuous (back to back) rain grown production results in decreased water use efficiency and run-off related soil losses (Hulugalle, Entwistle, Weaver, Scott, & Finlay, 2002). However, cotton is a complex crop to include in rain grown production sequences; inclusion and frequency of, being based on but not limited to available soil moisture (Grundy & Klepper, 2016). Principally, system sequencing challenges are centred around the capacity to efficiently harvest, store and utilise limited water resources for profitable yield outcomes.

#### ***Crop lead-out system rotation legacies and yield impacts***

Cotton has a greater ability to scavenge and make use of water and nutrients deep in the profile compared to other summer crop options, which can have legacy impacts that last over 12 months and result in lower subsequent grain crop yields compared to after other non-cotton summer crops (Baird, Lonergan, & Bell, 2018). Cotton rotation lead out effects include; an extended time post-cotton to sufficiently recharge soil profile to enable a successful following cover / grain crop, and minimal soil cover to prevent erosion and improve water infiltration due to BG3 resistance management plan (RMP) tillage requirements, for *Helicoverpa sp.* pupae control or the sparse quality of standing cotton stubble if defoliated prior to the 31<sup>st</sup> of March. The cumulative outcome on both wider-system profitability and potential in-crop management opportunities, being; a greatly reduced potential to double crop back to grains or winter chickpeas following cotton (Baird et al., 2018; Lowien, 2016).

#### ***Sequencing lead-in fallow requirements***

Comparatively high levels of stored soil water are essential for reliable rain grown cotton production; to offset the deficit between in-crop rainfall and crop ET<sub>c</sub> requirements (CRDC, 2012), as well as high probability of occurrence of in-season moderate to severe moisture stress (Eveleigh & Stiller, 2003). A long fallow rotation lead in, with substantial standing-stubble

ground cover, being best-practice requisite for rain grown cotton crop establishment (CA, 2020), but presenting both associated opportunity costs and system / crop management complexities (Baird et al., 2018).

Cotton requires relatively longer fallow lead in periods than other grain crops on account of minimum soil moisture requirements for production; being greater than 50% and ideally 100% PAWC and equating to at least 140 mm of stored soil moisture or 60-90 cm profile depth of wet soil (Grundy & Klepper, 2016). Generalised crop sequences from the Darling downs, Goondiwindi, eastern Gwydir and upper Namoi regions, where cotton was a planned inclusion within rotation, detail regional uniformity in winter cereal production followed by a long fallow (12 months) into cotton (Grundy & Klepper, 2016). Such crop choices are primarily driven by the need to capture and take best advantage of available rainfall; placing emphasis on the maintenance of a standing cereal stubble cover that affords protection to the soil during the lengthy lead in period to cotton and slows down water movement on the soil surface, resulting in improved infiltration and consequently soil moisture accumulation (Grundy & Klepper, 2016). Additionally, remaining cereal stubble cover post cotton planting (i.e. in-crop), provides cotton seedling protection against sand blasting and lowers soil water loss from skipped inter-row areas (Henggeler, Shaw, & McLellan, 2000; Kirkegaard et al., 2014).

The principal overhead associated with long fallow lead-in to cotton is comparatively decreased fallow and subsequent water-use efficiencies; systems with less time in fallow (e.g. Wheat-Canola-Wheat) demonstrate an increased system water utilisation (Bell, Whish, & Zull, 2019). However, the significantly higher returns for crops (such as cotton) planted on higher plant available water, compensates (on an annualised profit basis) for the lower efficiencies of fallow water accumulation and use (Bell et al., 2019). This scenario is also further influenced by the cost structure and risk appetite of the farming enterprise as well as the availability of labour; higher intensity systems will increase inputs of labour and machinery and increase the risk of crop failures (Bell et al., 2019). The ramifications for crops following long-fallows (e.g. cotton production) being that it is more critical to optimise management and maximise input (water) use efficiencies, in order to convert the extra water available efficiently into increased yields.

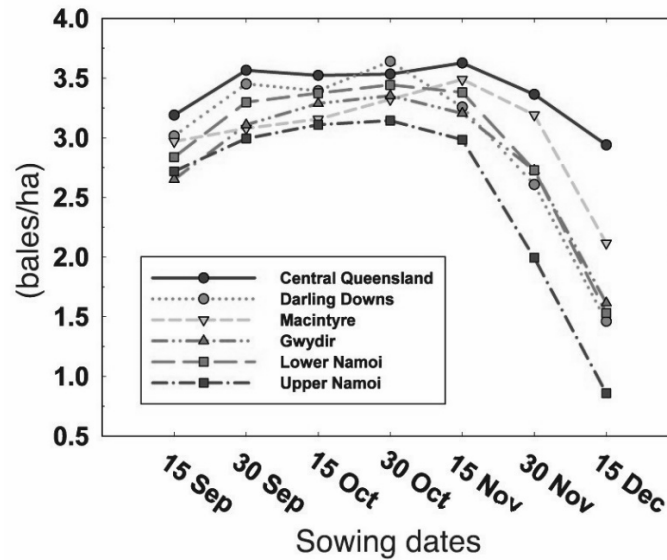
### **2.3.3 Water availability and opportunity challenges impacting the timing of planting**

Rain grown cotton planting operations are climate dependant and consequently, timing varies across production regions and between seasons (OGTR, 2002). Best practice cotton planting operations occur in spring when soil temperatures reach 14° C at 10 cm soil depth, in early morning for three consecutive days, and forecast mean daytime temperatures for the week following are on a rising trend (CA, 2020; OGTR, 2002). Planting window restrictions apply to the use of transgenic varieties; all Bollgard®3 crops and refuges must be planted into moisture between August 1 and December 31 each year (Monsanto, 2016b). Under rain grown conditions, planting opportunities are also defined in terms of the accumulation of adequate accumulated soil moisture reserves (>50% PAWC or 150 mm stored soil moisture) coinciding with subsequent planting rainfall events (30 mm rain over three days); the risk of failing to economically break-even being reduced with greater soil moisture resources (Baird et al., 2018; Bange, 2019; CRDC, 2002).

Total season length varies between regions, with longer season areas affording greater planting date flexibility; Breeza (NSW), a shorter season, cooler production area having a temperature-driven planting window of only approximately 40 days (CRDC, 2002; Eveleigh & Stiller, 2003). Simulation assessments using OzCot (Hearn, 1994) to determine the occurrence and distribution of summer crop planting opportunities across Australian rain grown cotton production regions, defined November as having the greatest probability of adequate planting conditions (Baird et al., 2018; Bange, 2019; CRDC, 2002). Based on the risk of failing to obtain a defined planting opportunity or probability for ideal planting conditions; across Breeza, Gunnedah, Dalby, and Moree production areas, growers may miss out on planting in three out of 10 years, with other production areas (Wee Waa, Bellata, Croppa Ck, Emerald, Biloela, Goondiwindi) having less favourable probabilities (Baird et al., 2018; Bange, 2019). Consequently, a priority exists to plant at the earliest possible (legislation, temperature and moisture defined) opportunity; with any delay in planting increasing the reliance on the probability of additional rainfall to provide subsequent planting opportunities (CSD, 2017; Grundy & Klepper, 2016).

Physiological simulations of mean crop yield as influenced by time of planting across current rain grown production areas, delineates an optimal planting window across production regions between the 15<sup>th</sup> of October and 15<sup>th</sup> of November (Bange, 2019) (Figure 2.3). Planting times

away from the optimum window reduce the mean yield and increase yield variability; planting prior to 30 September and after 30 November (15 November for Darling Downs) incurring significant yield penalties (Bange, 2019).



**Figure 2.3** Change in expected mean crop yield with planting date; yields being predicted using a single skip configuration and a plant available water holding capacity of 200 mm (Bange, 2019).

Planting later than the optimum date range can reduce lint yield potential due to a shortened production season; additional impacts on lint quality are also possible due to a delayed crop maturity coinciding with unfavourable late autumn climatic conditions at harvest (Bange, 2019). In contrast, early planting enables a longer production season on the proviso of adequate soil water resources and in crop rainfall events; increases in total crop water use of up to 10% being possible (compared to crops planted at end of the window) (CSD, 2018a). Early planting production risks are associated with the occurrence of flowering during December and January, as opposed to February and early March; meaning maximum crop water requirements coincide with peak evaporative demand and the probable occurrence of extreme heat events (CSD, 2018a; Grundy & Klepper, 2016).

Specific crop physiological development stages respond differently to temperature and water availability (Abdulmumin & Misari, 1990; Roth et al., 2013); end of season yield outcomes being more dependent on in-crop rainfall occurring during flowering than soil moisture at planting (Bange et al., 2005). Resultantly, it is critical that both adequate soil moisture and rainfall is available during this reproductive period for maintaining both yield and fibre length (CRDC,

2002). Additionally, extreme temperatures (i.e. daily maximum temperature in excess of 35° C) during peak flowering can also influence cotton yield via; an inhibition of pollen tube growth causing reduction in pollen viability at flowering, a reduction in photosynthesis and increase in respiration collectively reducing assimilate availability, a resultant reduction in boll size and an increase in square and boll shedding (Anwar & Darbyshire, 2017; McDowell, Bange, & Tan, 2007; Song et al., 2015). Australian rain grown cotton yield reductions associated with extreme heat events at flowering (maximum temperatures > 35° C) being between 115-375 kg.ha<sup>-1</sup> (Anwar & Darbyshire, 2017).

#### **2.3.4 Water availability and crop establishment challenges**

Poor, uneven plant stand establishment subverts the effectiveness of in crop management interventions to both increase yield and use efficiency outcomes, and promote system resilience. Target plant populations for rain grown production are between 6 and 8 plants.m<sup>-1</sup>. However, due to the rain grown nature of production, variable stands resulting from poor crop establishment have limited capacity for compensatory growth (as opposed to irrigated crops), with reduced yield outcomes probable, irrespective of the emerged plant population (Bange, 2019).

Poor or failed crop establishment occurs when one or more biophysical, system or environmental factors coincide to prevent the effective placement, germination and/or emergence of cotton seed, during or after the planting operation, resulting in a non-uniform or highly variable plant stand with two or more gaps greater than 50 cm in length every 5 m of row (Milroy, Bange, & Hearn, 2004; Reddy, Brand, Wijewardana, & Gao, 2017). The cumulative agronomic outcomes being a reduction in stand viability and / or poor ensuing early-season canopy and root growth, with lasting effects on light interception, water access and crop resilience against seedling diseases and insect infestations; resulting in variations in crop maturity, with ongoing yield and lint quality impacts (Constable & Shaw, 1988; Oosterhuis & Jernstedt, 1999; Reddy et al., 2017).

Circumstances contributing to establishment failure vary spatially (within and between fields and regions) and temporally (within and across seasons) (Reddy et al., 2017). The inability of cotton to emerge from depth compared with other crops, impairs crop establishment under marginal moisture conditions (CSD, 2018a). Successful germination depends on placing the cotton seed in adequate surface contact with moist soil to enable water imbibition. Once germination has

commenced, emergence is then dependant on both radicle and hypocotyl penetrating the under- and-overlying soil environs, respectively. Deep placement of cotton seed into adequate soil moisture below 5 cm profile depth challenges the hypocotyl's ability to push cotyledons through to the soil surface, due to both the greater depth of overlying soil requiring penetration and decreased seed energy reserves associated with the development of varieties with higher lint turnouts (and smaller seed size / density) (Grundy & Klepper, 2016). Moreover, many Australian production regions have very shallow structurally stable topsoils (Hulme, 2015); resultantly sub soil constraints can further compound emergence issues associated with moisture-seeking planting practices under marginal conditions.

### **2.3.5 Soil physical degradation and chemical constraints impacting plant access to soil water reserves**

Optimum crop management within northern (NSW, Qld) rain grown farming systems depends on maximising the infiltration and storage of rainfall in the soil, and the subsequent extraction of this moisture for crop growth (Passioura & Angus, 2010). Soil characteristics that limit these processes may be physical (compaction, layers of high bulk density and gravel), chemical (sodicity, salinity, high or low pH, nutrient deficiencies and the presence of toxic ions such as chloride, carbonate or bicarbonate) or biological (diseases and pathogens) (Page et al., 2018). The biggest challenges for rain grown cotton producers in the region being understanding how to effectively manage soils where multiple constraints are present to maximise access to soil water reserves (Dang et al., 2010).

#### ***Sodicity associated with Vertosol (Australian) cotton production soils***

In Australia, the surface and subsurface soils of the majority of cotton growing regions (Vertosols) are considered sodic; ranging in Exchangeable Sodium Potential (ESP) from 2 to 25% at 15 cm profile depth (Dang et al., 2006; Ghosh et al., 2011; Ghosh et al., 2010). Recent simulations to quantify the spatial extent of soil constraints identified 69% of 113 420 km<sup>2</sup> northern (NSW, Qld) rain grown cropping area as having soils with sodic characteristics (Orton et al., 2018). Cotton yields decrease under sodic conditions due primarily to inhibition of above- and below-ground growth (root area exploration) and an associated decrease in potassium and phosphorus uptake by the reduced root biomass (Hulugalle, Broughton, & Tan, 2015; Nachimuthu & Webb, 2017).

Sodic soils are characterized by elevated concentrations of sodium (Na) on soil exchange sites (often >6%), that can weaken the bonds between soil particles when wet, leading to poor structural stability in water and susceptibility to dispersion and a collapse soil of structure (Ghosh et al., 2010; Rengasamy & Marchuk, 2011). At the surface, structural degradation can result in the formation of surface seals (wet) and crusts (dry), which reduce water entry into the soil profile and impede seedling emergence (Ghosh et al., 2010). In subsoils, it can lead to the formation of dense soil layers with reduced water holding capacity, poor aeration and increased soil strength; impacting plant root penetration and subsequent access to water resources (Rengasamy & Olsson, 1993). Structural degradation associated with soil sodicity can also impact planting operations and cause increased runoff and erosion (Oster & Jayawardane, 1998).

### ***Traffic-induced soil compaction***

Compaction is a significant cause of soil degradation in Australian cotton cropping systems, (Braunack & Johnston, 2014; Daniells, 1989; D. McGarry, 1990). Traffic-induced soil compaction associated with the recent uptake of on-board-module-building (OBMB) pickers and strippers is viewed as a rapidly emerging issue facing northern (NSW, Qld) rain grown cropping systems, as well as being a significant impediment to the integration of cotton as a crop rotation within the broader farming system, particularly where it only features once every 3-5 years (Grundy & Klepper, 2016; Nachimuthu & Webb, 2017).

Cotton root elongation is affected by soil strength (Antille et al., 2016); traffic induced compaction results in a shallow and sparse cotton root systems and reduced spatial exploration of the soil space by the root system, with subsequent reduced access to soil water reserves (D. McGarry, 1990). Impacts of traffic-induced soil compaction include; an increased yield gap between mean and potential (cotton) yields and reduced water use efficiency due to root growth inhibition (Antille et al., 2016). Due to influences on water infiltration and hydraulic conductivity, traffic-induced compaction is also linked to increased erosion and runoff; having wider catchment soil degradation impacts (Antille et al., 2016). Harvest-traffic (compaction) associated yield reductions in central Qld wheat, sorghum and maize crops have been quantified as 15–30% of comparative non-trafficked areas, representing financial losses to farmers of ~AU\$160–350 ha<sup>-1</sup> (Neale, 2011); with similar conclusions being drawn by Bennett, Woodhouse, Keller, Jensen, and Antille (2015) for cotton. The total cost of traffic-induced compaction (as lost

productivity) to Australian rain grown systems being estimated at \$850M p.a. (Woodhouse, Bennett, & Jensen, 2013).

### **2.3.6 System production challenges associated with projected climate change impacts**

Recent IPCC (2014) reporting reaffirms that climate change and variability will impact global food and fibre production due to direct (abiotic stress) impacts on plant growth and development; via elevated CO<sub>2</sub> levels, reduced future system water availability, increased frequency of extreme climatic events, as well as modified weed, pest and pathogen pressure (Altieri et al., 2015; Pereira, 2016; A. Williams et al., 2014) . System water deficits being driven by altered precipitation patterns, increases in temperature and resultant modified transpiration regimes (due to lower humidity and net evaporation increases) (Bange & Constable, 2008a; Pereira, 2016; A. Williams et al., 2014). Projected increases in atmospheric [CO<sub>2</sub>] may alter water use and water use efficiencies in cotton plants, within the probable context of reduced water availability. However, controlled environment (SPAR), glasshouse and field experiments investigating cotton plant water use under high [CO<sub>2</sub>] have reported contrasting outcomes of lower (due to lower stomatal conductance and resulting transpiration rates), higher (due to increased leaf area and rapid depletion of soil water resources) and no relative difference in crop water use (viz. no difference in transpiration), compared to ambient [CO<sub>2</sub>] (Ephrath, Timlin, Reddy, & Baker, 2011; Hunsakera et al., 1994; Samarakoon & Gifford, 1995). Such impacts may result in reduced system resilience to disturbance, being evidenced as decreased yields, decreased input use efficiencies and increased annualised yield variability; consequently, presenting a future need for flexible management strategies to adjust the production system.

## **2.4 Agronomic adaption strategies to promote agroecosystem resilience**

### **2.4.1 The imperative for agroecosystem resilience.**

Agricultural specialization, resulting in low-diversity, monoculture production systems, grown under a narrow scope of optimized conditions, and the economies of scale these systems enable; has driven productivity and labour-use efficiency gains in modern commercial agroecosystems such as the Australian cotton industry (Ge et al., 2016; Peterson et al., 2018)(Ge et al., 2016; Peterson, Eviner, and Gaudin, 2018). However, the impact of multiple, capricious system drivers of change, evidenced through abiotic stress mediated system limitations and challenges; significantly influence the long-term sustainability of such highly specialized, controlled systems and concentrated agricultural landscapes (Ge et al., 2016; Peterson et al., 2018; Tendall et al., 2015). Resultantly, to maintain profitability and ensure the ongoing viability of production, an imperative exists for sustained increases in; yield and lint quality (with reduced year to year variability), crop resource use efficiencies, and farm-gate lint and seed values, whilst reducing input costs and maintaining biodiversity (Bange et al., 2016a; Liu et al., 2013; Tendall et al., 2015). The concept of system resilience is a rationale being progressively applied within sustainable management responses to system limitations and challenges (Ge et al., 2016; Tendall et al., 2015; Tompkins & Adger, 2004). System resilience refers to the capacity of an agroecological production system to absorb disturbance, reorganise and adapt, to maintain the same (purpose-based) identity; being defined by the objectives and limitations of the specific production system (Ge et al., 2016; Lin, 2011; Peterson et al., 2018). Traditional (existing) farming constructs are repositories of principles and measures that can be leveraged by modern agricultural systems, to improve efficiencies with little added risk and improve resilience to climatic extremes (Altieri et al., 2015; Bange et al., 2016b; P.S. Carberry, Bruce, Walcott, & Keating, 2011)(Carberry et al, 2011; Bange et al., 2016; Altieri, Nicholls, Henao, and Lana, 2015).

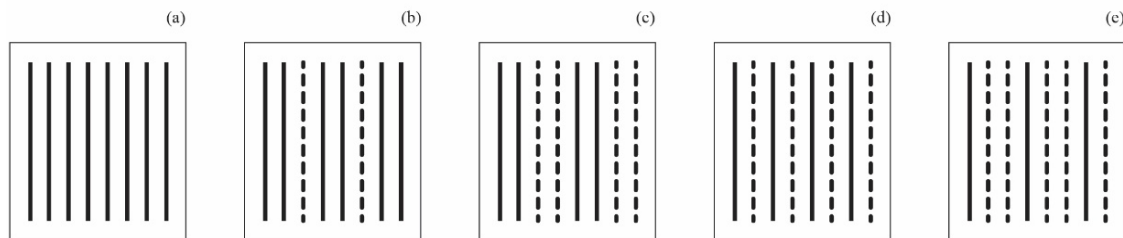
### **2.4.2 Existing agronomic adaption strategies to promote agroecosystem resilience**

Agronomic adaptation strategies to manage production (climate) risk and promote system resilience have been centred around the tactical adjustment of practices and technologies (Rickards & Howden, 2012), but can also occur as systems modifications or at a transformative (industry) level (Barlow et al., 2013); central being the key role of the farmer as decision maker

(Hayman, Rickards, Eckard, & Lemerle, 2012). Greater production (climate) risks are associated with rain grown cotton in comparison to alternative grain or pulse crops, because production occurs over an extended growing season and is agronomically highly specialised, requiring an increased intensity of both management and capital expenditure (Antille et al., 2016). Management of such risk across the geographically diverse rain grown cotton production areas is centred around the manipulation of multiple agronomic management and genetic solutions simultaneously, so as to moderate existing abiotic stress mediated system limitations and challenges, and reach the water-limited yield potential of the system (Bell et al., 2019; Chapman et al., 2012; Kirkegaard, 2017). Consequently, manipulating row configuration to increase access to soil moisture, varying planting date to optimise soil moisture availability during peak crop resource demand, cultivar changes and manipulating crop maturity for timeliness are currently utilised in rain grown cotton systems to manage climate risk.

***Manipulation of row configuration to promote plant access to soil moisture reserves***

Specialised skip row planting configurations are used as an agronomic management strategy to mitigate climate risk and potential associated financial loss (Bange et al., 2005; Payero, Robinson, Harris, & Singh, 2012). Skip row planting configurations are deviations from conventional 1m spaced solid plantings with entire rows omitted (Bange et al., 2005; Luo et al., 2016) (Figure 2.4).



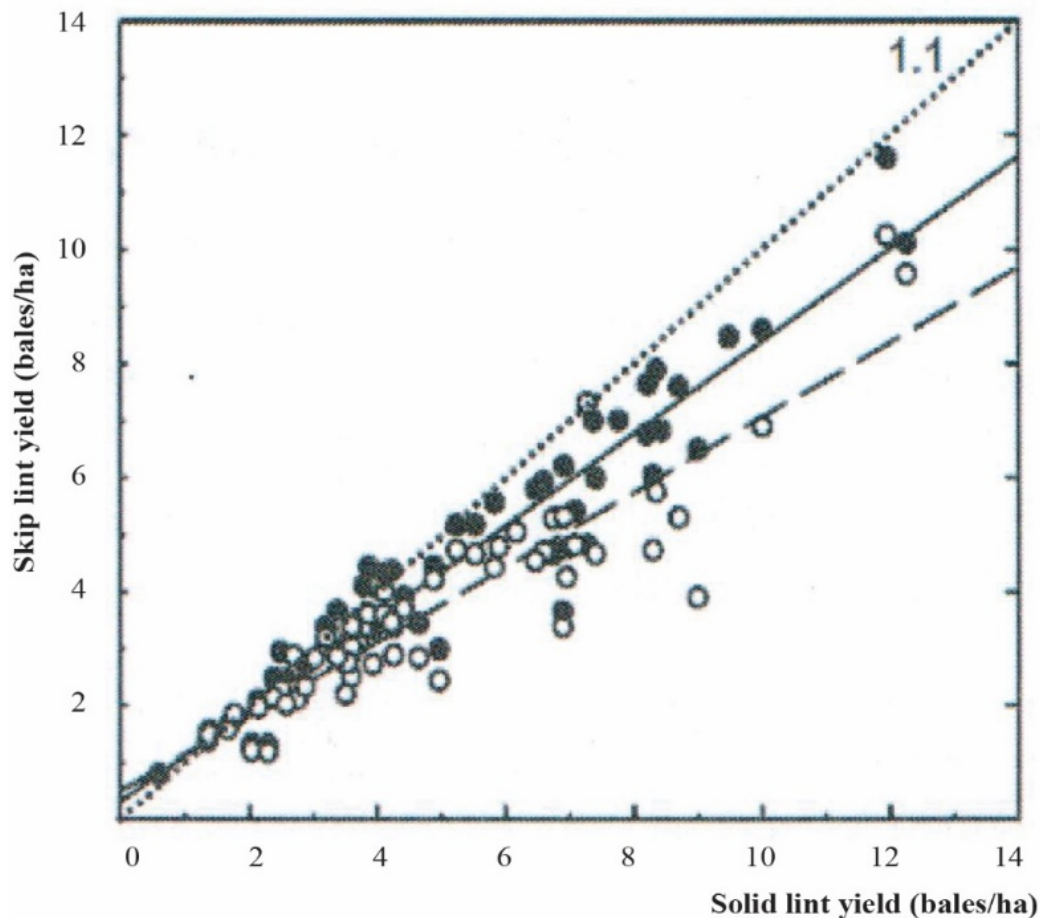
**Figure 2.4 Solid and skip row planting configurations utilised in Australian rain grown cotton production systems, solid lines (—) representing planted rows, dashed lines (- - - -) representing skipped rows on conventional 1 m row spacings; (a) solid plant (100% planted), (b) single skip (67% planted), (c) double skip (50% planted), (d) alternate (1-in1-out) (50% planted), (e) super single (33% planted). Other less frequently utilised configurations include wide row configurations of 1.5 m row spacings (67% planted area), being essentially solid row configurations with considerably wider row spacings (CRDC, 2002).**

Skip row configurations increase plant access to soil moisture reserves adjacent to the plant line by increasing the area accessible by cotton plant roots (the root space) and consequently the amount of profile-stored soil water available to the plant; providing a buffer to the occurrence and

impact of water deficit stress (notably in drier seasons) and when seasonal conditions permit, enabling extended crop growth for potential yield and lint quality benefits (Bange et al., 2005; Payero et al., 2012). The underlying premise being a reduction in the length of time where the crops are in water-deficit stressed conditions during the flowering and boll filling period (Bange et al., 2016a).

Selection of appropriate configurations for specific environment and economic circumstances involves multiple, often complex considerations (Bange et al., 2005). Selection of row configuration that best suits individual rain grown situations varies with; existing machinery configuration and equipment availability (Bange et al., 2012), current farming system rotations (including the row spacing of prior crops) (Grundy & Klepper, 2016), rainfall patterns and seasonal forecasts, ground cover requirements for reducing soil evaporation as well as the risk of runoff and soil erosion (Howden, Gifford, & Meinke, 2010).

Another primary consideration for row configuration selection is the enterprise (management) capacity to carry economic risk associated with such impacts on economic returns and associated input costs. A trade off exists between the higher potential lint yields of solid planted crops v. the lower lint yield variability, lower lint quality variability and reduced variable costs of skip configurations (CSD, 2018a). When solid plant yields are high (i.e. seasons when moisture is less limiting), an associated yield penalty for skip configurations exists (Figure 2.5); single skip when solid yields exceed 1.6 bales/ha, and double skip / alternate, when solid yields exceed 1.5 bales/ha (Bange et al., 2005).



**Figure 2.5 Skip row configuration yield potential in relation to solid plant cotton; relationship between single and double skip row and solid row lint yields derived from measured field data of Goyne (2000), Pyke (1991), Marshall et al. (1994) and Hearn (unpublished) as published by Bange et al. (2005), 1:1 lines (.....) are denoted, single skip (—), double skip (- - - -),**

Profitability is influenced not only by yield, but lint quality and relative input costs. Skip row configurations markedly reduce the risk of price discounts due to short fibre or low micronaire (Bange et al., 2005); financial gains from skip row cotton production compared to solid plant being derived predominantly from production cost savings and the reduced incidence of lint quality discounts (Grundy & Klepper, 2016). The gross margin for skip row cotton only becomes less than that of solid plant once the potential yield of solid exceeds  $1400 \text{ kg}\cdot\text{ha}^{-1}$  for single skip and  $1090 \text{ kg}\cdot\text{ha}^{-1}$  for double skip configurations; when potential staple length discounts are accounted for substantial changes take place for solid compared to skip configurations; gross margins of solid plant stay below skip row configurations (Bange et al., 2012; Grundy & Klepper, 2016).

Furthermore, row configuration can influence rain grown cotton crop season length per given planting date; early October plantings can be as large as 14 days difference between solid and super single planted crops, with other row configurations falling within this range relative to the width of the skip (Bange & Constable, 2008b). Resultantly, the optimum time of planting rain grown crops differs between solid and skip row configurations; wider row configurations are of benefit in earlier planting opportunities to extend the soil moisture available to the crop, notably around flowering (Bange et al., 2005; Bange & Constable, 2008b). Conversely, late season plantings can have trouble reaching maturity prior to inclement late autumn weather hindering harvest; an earlier maturity being encouraged by tighter row configurations (Bange et al., 2005; Bange & Constable, 2008b).

Finally, soil PAWC has significant impact on the choice of row configuration; solid row configurations being most suitable for high PAWC soils, whereas skip row configurations facilitate profitable production on lower PAWC soils, notably in marginal rainfall areas (Bange et al., 2005). However, consideration is required toward underlying soil physical properties that may affect lateral and vertical root distribution and water infiltration (e.g. compaction and sodicity) (Bange & Constable, 2008b; CSD, 2017). The degree to which the soil profile was wetted at planting did not increase the advantage of skip row relative to solid configurations; cotton utilises minimal amounts of soil water prior to first flower (6–9 weeks after planting) and due to prevailing summer dominant rainfall distributions in production areas, a high probability exists of rainfall between planting and flowering (Bange et al., 2005)(Bange, Carberry, Marshall and Milroy, 2005).

Resultantly, use of skip row configurations offer; significant water deficit risk mitigation in years where in-crop rainfall and/or soil water at planting is limited (Montgomery & O'Halloran, 2008), reductions in year to year production variability and reduced input costs (e.g. Bollgard®3 licence fees). However, yield potential may be compromised by skipping rows if seasonal conditions (rainfall totals) are better than anticipated (Milroy et al., 2004).

#### ***Varying planting date to optimise soil moisture availability during peak crop resource demand***

Cotton crop physiological and legislative (resistance management plan) changes associated with the introduction of Bollgard®3 cotton varieties have enabled increased planting date flexibility

in Australian rain grown cotton systems. The use of Australian transgenic cotton varieties provides improved *Lepidopteran* spp. insect pest control; resulting in an increased early fruit retention, a shorter fruiting cycle and consequently for warmer production areas, enables later planting-window establishments to maintain yield and improve fibre length and micronaire (Bange & Constable, 2008b; Braunack, Bange, & Johnston, 2012). Manipulating crop planting dates provides a systems-level strategy to manage climate risk and promote system resilience by:

- Targeting optimal combinations of planting opportunities and available soil moisture (Lowien, 2016);
- Reducing the occurrence of cold shock temperature impacts on early growth and resultant incidence of seedling diseases, early in the season (Bange & Constable, 2008b); and,
- Transitioning phases of crop maximum water use into time periods of lower atmospheric VPDs (Bange et al., 2016a), thereby relieving water deficit stress impacts associated with the occurrence of peak flowering (Lowien, 2016). The overarching outcomes being to maximise the use of limited water (and other inputs such as nitrogen) whilst maintaining yield and improving lint quality (Braunack et al., 2012; Luo et al., 2016).

Optimal planting dates will vary between cotton regions and seasons, due to the need to match cotton's water requirements to local rainfall patterns and soil water availability (Braunack et al., 2012; Hearn, 1995); later window plantings giving the crop more time to capture (summer dominant) rainfall during peak crop demand (Bange, 2019). As defined earlier (Figure 2.3), crop physiological simulations demonstrates increasing mean yields until late October, with an optimum planting time for most rain grown production regions based on mean yields from 15 October to 15 November (Bange, 2019; Eveleigh, 2018). In all regions, mean yields of single skip cotton were less when planted prior to September 30<sup>th</sup>; 15<sup>th</sup> November being latest planting date with no yield penalties, except for Darling Downs being 30<sup>th</sup> October (Bange, 2019; Bange et al., 2005). Planting times outside this window will reduce mean yields but also increase potential yield variability (Bange, 2019). However, an imperative exists to plant earlier in the season due to the risk of later season planting opportunities not eventuating. Additionally, the option of changing planting time is not feasible in short season (colder) areas, notably around the eastern Darling Downs and upper Namoi regions (Luo et al., 2016). Finally, consideration must also be given to crop maturity (and consequently harvest) timing as influenced by planting time;

being dependant on long-term mean late autumn rainfall distributions so to avoid the negative lint quality impacts of a wet harvest (Bange et al., 2005; CRDC, 2012).

***Cultivar change to incorporate key adaptive traits specific to limited water environs***

A longer-term yet high-efficiency adaptation strategy is the breeding and application of novel cultivars that incorporate key adaptive traits specific for environment, to maintain yields and lint quality and improve sustainability (when compared with existing varieties) under inter and intra-seasonal climate variability (Challinor et al., 2014; Chapman et al., 2012; Kaloki, Luo, Trethowan, & Tan, 2019; Ramirez-Villegas & Thornton, 2015).

At a whole-farm level, a key premise of the strategy being to select cultivars that have differing adaptive traits in order to buffer climate (variability) risk and accommodate associated changes in management (Bange et al., 2016a). In regions where there are significant risks of abiotic stress impacts, the application of cultivars that demonstrate increased resilience to heatwaves (Bibi, Oosterhuis, & Gonias, 2008), waterlogging (Conaty, Tan, Constable, Sutton, & Field, 2008) or water deficit (Stiller, Read, Constable, & Reid, 2005) and / or that demonstrate relative efficiencies in resource use (water and nutrition), will be applicable as per disease tolerance or increased staple length traits currently (Bange et al., 2016a). Across rain grown production regions, growing season length as determined by either thermal dependence, terminal drought or economic-based selection for “earliness” (being timeliness), will inform cultivar season-length trait applications (Bange et al., 2016a). Indeterminate and late-maturing cultivars being suited to rain grown production areas with long seasons with (generally) adequate resources, whilst shorter season (cooler) regions or when terminal drought may occur, should preference more determinate, early maturing cultivars (e.g. shorter phenological stages, higher fruit growth rates) (Bange & Milroy, 2004). Cultivar selection with specific fibre properties can mitigate climatic and / or management challenges; e.g. longer fibre cultivars reducing short fibre discounts in water-stressed environments, or high micronaire cultivars minimizing low micronaire discounts as a result of cool or stress environments during boll maturation (Bange et al., 2016a).

### ***Manipulating crop maturity for timeliness or “economic earliness”***

Reducing the time to maturity (“timeliness” or “economic earliness”) and managing a crop to achieve a targeted economic yield threshold is often utilised as a strategy for increasing yield stability under overseas, comparatively shorter rain grown production conditions (Bange & Milroy, 2004; Raper & Gwathmey, 2015); where season length can be determined by an end of season “terminal” drought (Stiller & Constable, 1998) and cooler late-autumn temperatures can impact the efficacy of harvest preparation and subsequent harvest efficiency (Raper & Gwathmey, 2015). The premise is not currently associated with Australian rain grown production under ideal planting dates, but may have application (via use of earlier maturing, more determinate varieties) for later planting dates where season length is substantially shorter (Raper & Gwathmey, 2015; Stiller & Constable, 1998) or under water-limited conditions where establishment occurs within the optimal planting window and the crop is grown predominately on stored soil moisture (i.e. drought), managed to reduce the time to maturity (shortening the fruiting cycle) and achieve a targeted, lesser economic yield threshold (Bange et al., 2016a).

Crop management for earliness involves minimising the time in which it takes to set and mature a profitable boll load, via leveraging favourable early season conditions combined with cultivar choice, growth regulator applications, insect management and/or nutrition; to quickly produce adequate vegetative growth to support a profitable boll load without forcing the plant to reach physiological cut out too early (Bange & Constable, 2008b; Raper & Gwathmey, 2015). Over aggressive attempts to maximise early maturity resulting in significant yield penalties (Raper & Gwathmey, 2015). Under Australian rain grown conditions, early crop maturity may avoid lint quality down grades and avoid late season insect protection (Bange & Constable, 2008b). However, these outcomes being balanced against reduced lint yield due to shorter periods of reproductive growth and maturity (Bange & Milroy, 2004). An improved application efficiency of defoliant and boll openers, a reduction of weathering effects on open bolls, as well as a reduction in compaction associated with running heavy harvesting equipment on wet ground during late autumn / winter harvests are benefits associated with favourable (warmer) conditions at harvest (Raper & Gwathmey, 2015). Resultantly, the maximum profit potential of the crop within a wider rain grown system context can be captured, due to the capacity to manipulate crop growth and development to best suit the prevailing environment.

### **2.4.3 Adaptive management to promote agroecosystem resilience**

Due to the complex relationships and non-linear feedbacks between evolving social, ecological, and climatic production system drivers, the administration of productive crop systems through “predict and control” management processes has been succeeded by adaptive management strategies, that demonstrate greater efficacies in promoting system resilience and sustainability (Alexandra, 2012; Tompkins & Adger, 2004). Adaptive management strategies address production risk by incorporating stakeholder perspectives, prior management knowledge and iterative (i.e. in-season) system learning, to gain critical knowledge and understand potential outcome trade-offs; management actions are bespoke and proceed irrespective of system knowledge shortfalls (Alexandra, 2012; Johnson, 1999; Tompkins & Adger, 2004). Adaptive management strategies also leverage system and stakeholder capacities that promote and maintain ecological resilience and facilitate flexibility in stakeholder (institutions / farmers) positions; enabling in-season responsive (agile) management to system challenges and limitations under variable climatic conditions (Gunderson, 1999). Therefore, set against management for a single optimal state of resource (as traditional “predict and control” strategies), adaptive management strategies administer within a range of acceptable outcomes whilst avoiding system (yield, input utilisation) failures and irreversible negative effects (Johnson, 1999). Operationally, adaptive management strategies are centred around; productivity, stability of production, resistance to declines in yield components in the face of disturbance (ecological resilience), and/or rapid recovery to baseline functionality when conditions improve (engineering resilience) (Peterson et al., 2018).

### **2.4.4 Matching inter- and intra-seasonal climatic variability with management diversity**

The interaction of genetics, environment and management ( $G \times E \times M$ ) provides an approach to realizing the yield potential of cropping systems (Hatfield & Walthall, 2015). In water-limited Australian rain grown cotton production systems, differences in management, climate and the interaction between, have a greater influence on annualised yield outcomes than the underlying genetics of the cultivar (Braunack, 2013; Liu et al., 2013). However, in comparison to other field crops, identifying the sensitivity of particular aspects of cotton production to different climate

variables is relatively complex because of the cotton plants indeterminate growth habit (A. Williams et al., 2014).

Many current agronomic management strategies deliver capacity to introduce diversity in management practices to guard against climate variability and promote system resilience. However such practices can lack agility (requiring commitment to a strategy at the beginning of a season e.g. split planting dates), have increased costs (an irreversible negative effect) associated with implementation (e.g. multiple plantings and / or nutrient applications) and the rain fed nature of production system removes opportunities for management diversity (system failure) (e.g. may not get second planting opportunity). However, the key principles underpinning such strategies, can be leveraged to inform the development of novel adaptive management approaches to promote system resilience and realise yield potential (Table 2.2). Consequently, specific novel adaptive strategies that increase both the volume and accessibility of stored soil water and its optimised use thereafter, as well as the promotion of rapid and dynamic beneficial crop responses to specific environmental contexts; are potentially key foundations to the design of future novel adaptive management strategies for rain grown cotton production systems.

**Table 2.2 Key physiological rationales underpinning existing agronomic adaptation strategies to manage production risk and promote agroecosystem resilience.**

Strategy	Level of impact <sup>(1)</sup>	Mechanism(s) of impact	Outcome	Rationale basis (physiological, agronomic)
Row configuration	Crop	Designed omittance of planted rows.	Increases cotton plant root growth area (root space).	<b>Increase access to stored soil water adjacent to the plant line.</b>
Planting date	System	Planting date variation across enterprise for optimal combinations of planting opportunities and available soil moisture.	Transition crop max. water use to periods of lower temp. and VPD, minimising water deficit stress impacts.	<b>Optimise the use of limited crop water inputs (starting available soil water and rainfall).</b> <b>Improve seasonal growing conditions.</b>
Nitrogen management	Crop	Total crop nitrogen requirement applied in split applications tailored to prevailing climatic conditions; 1 <sup>st</sup> in prior cereal crop, 2 <sup>nd</sup> at planting and 3 <sup>rd</sup> in crop as side dressing	Increased mineralisation and migration of early N applications to deep in profile, reducing denitrification risk. Reduced compaction via incorporation with prior crop and planting.	<b>Change in crop response to environment (manipulation of vegetative growth to maintain WUE and avoid delayed maturity).</b>
Soil management practices	System	Alternate crop rotation and use of cover crops.  Increase length of lead-in fallow.  Implementation of no-till, controlled-traffic farming practices and stubble retention.	Minimises soil compaction and improved soil structure.  Decreased runoff, increased soil water infiltration and decreased evaporation to retain soil moisture.	<b>Increased access to stored soil water adjacent to the plant line</b> <b>Increased plant available water content.</b>
Cultivar selection	System	Application of novel cultivars with adaptive traits specific to environ; e.g. long staple cultivar to address short fibre discounts in limited water environs.	Maximise yield and quality outcomes under variable climatic conditions in specific production contexts.	<b>Change in crop response to environment.</b> <b>Increased crop resilience to abiotic stress.</b>
Crop maturity management	Crop	Reducing time to crop maturity and managing to achieve yield threshold; via manipulation of cultivar choice, PGR applications, nutrition and insect management.	Increased yield stability in (overseas) short season areas.	<b>Rapid crop response to water-driven production opportunities.</b> <b>Reduce resource (water) use; allowing for other crops in system.</b>

<sup>(1)</sup>Crop level impacts refer to tactical adjustments of practices and technologies vs systems level modifications.

## **2.5 The role for plant growth regulators in facilitating novel adaptive management solutions for rain grown production**

Plant growth regulators (PGRs), otherwise known as growth retardants, plant bioregulators, synthetic plant hormones, or plant growth substances are widely utilised in modern agriculture (including cotton), horticulture and viticulture as agronomic management tools and systems adaptation solutions, to promote efficient propagation, production and optimal yield outcomes (Oosterhuis & Robertson, 2000; Rademacher, 2015). “True” plant growth regulators are organic compounds of innate plant hormones, their synthetic analogues or compounds relating to their biosynthesis, translocation (including hormone receptor blockers) and metabolic regulation (Cothren & Oosterhuis, 2010; Koprna, De Diego, Dundalkova, & Spichal, 2016; Oosterhuis & Robertson, 2000; Rademacher, 2015). In contrast and not referred to within this study, “atypical” plant growth regulators are characteristically herbicides that induce a local and/or transient phytotoxic effect, similarly “plant bio-stimulants” comprise complex, indeterminate mixtures of ingredients (often based on extracts of seaweeds or hydrolysates of abattoir by-products) and may contain PGR-like components (Rademacher, 2015). Forthcoming reference to “plant growth regulators” (PGRs) denotes “true” type PGRs.

### **2.5.1 Realising strategic manipulation of cotton growth and development utilising PGRs**

PGRs represent diverse chemistries and modes of action, are biologically active in small concentrations, non-phytotoxic, have no nutritive value and primarily through exogenous application, provide numerous possibilities for beneficially altering crop growth and development (Cothren & Oosterhuis, 2010; Oosterhuis & Robertson, 2000; Rademacher, 2015). Classical plant hormone categories comprise Auxins, Gibberellins, Cytokinins, Abscisic Acid, and Ethylene; additionally, Brassinosteroids and Jasmonates have phytohormonal functions, whereas the classification of polyamines and salicylic acid is still contentious (Rademacher, 2015). Plant hormones are formed at specific cytological or morphological locations, notably apical and root meristems, as well as developing reproductive structures; they may act in the tissue of production or are translocated to more remote target tissues (Rademacher, 2015). PGRs directly and specifically impact this intrinsic hormone system of higher plants, or elicit a physiologically similar metabolic or developmental response; either through the inhibition of biosynthesis and /

or translocation of endogenous hormones, or the blocking of hormone receptors at the site of action (Rademacher, 2015).

Adaptive management of crop growth and development using PGRs may provide opportunity to further enhance and tailor crop genetic characteristics to unique production and climatic contexts; improving the system capacity to absorb climatic variability (i.e. resilience) via increased production efficiencies and economic yield and lint quality benefits. Many phenological targets for PGRs are also goals for plant breeding initiatives; however, PGRs enable active in-crop regulation of plant processes, through agile “fine tuning” of growth and development under variable in-crop climatic conditions, thereby adjusting a given genotype to specific environment and management contexts (Rademacher, 2000). Accordingly, multiple plant developmental processes can be actively regulated using PGRs to facilitate bespoke adaptation to abiotic stimuli, including; enhanced photosynthesis, alteration of carbon partitioning, increased root:shoot ratios and subsequent improved nutrient and water uptake, modification of crop canopy architecture, acceleration or delay of seed germination, dormancy breaking in woody perennials, stimulation or reduction of shoot elongation, induction of flowering and fruiting and corresponding reduction or increase of fruit set and acceleration or delay of senescence processes including fruit ripening and defoliation (Cothren & Oosterhuis, 2010; Koprna et al., 2016; Oosterhuis & Robertson, 2000; Rademacher, 2015).

However, achieving consistency in manipulation of plant response to exogenous PGR applications can be problematic due to specific PGR compound interactions with both the heritable characteristics of the crop and the prevailing environmental and cultural-management contexts under which the crop is produced (Cothren & Oosterhuis, 2010; Oosterhuis & Robertson, 2000). PGRs (notably auxins) can induce diametrically opposed effects on a specific plant physiological process at differing concentrations (Cothren & Oosterhuis, 2010) or when applied to differing plant growth stages (Rademacher, 2015). Moreover, in order to penetrate the plants epidermis and membranes, appropriate formulants (solvents, surfactants and or water conditioners) must also be incorporated with the active ingredient (Rademacher, 2015; Rethwisch et al., 2017).

Resultantly, an understanding of cotton crop responses to PGR treatment scenarios under specific production settings is required in order to achieve consistency in manipulation of crop growth and

development, for strategic (adaptive) management purposes. Both global commercial utilisation of PGRs in cotton, as well as elicited phenological responses documented in research, can provide insights as to such capacities.

### **2.5.2 Conventional PGR manipulation of cotton growth and development in commercial production**

PGRs are commonly used in commercial cotton production for the management of crop growth and development, essentially as vegetative growth retardants during flowering, as ripeners and crop termination and defoliation compounds (Cothren & Oosterhuis, 2010; S. Williams, Bange, & Constable, 2019); with the overarching aim of lint yield and lint quality improvement and increased production efficiencies (Cothren & Oosterhuis, 2010; Oosterhuis & Robertson, 2000). Applications occur via seed treatments / in-furrow applications at planting, or in aqueous solution as foliar applications within the cropping season (Cothren & Oosterhuis, 2010). Current Australian cotton PGR registrations include; 37 registered products containing Ethephon for the acceleration of ripening and defoliation, 22 registered products containing Gibberellin biosynthesis inhibitor Mepiquat chloride (MC) for the control of vegetative growth, 25 products containing diphenylurea-type synthetic Cytokinin Thidiazuron for defoliation, and one product containing auxin Indole-3-butyric acid (IBA) and “nutritional ingredients”, for the promotion of early root growth (AVPMA, 2020).

Australian commercial use frameworks around the application of MC for the control of vegetative growth (canopy manipulation) in flowering, reference; crop growth rates via proportionate internode elongation and change in the number of nodes (as vegetative growth rate, VGR), varietal response considerations and the prevailing climatic conditions, crop fruit retention rates, soil type and fertility (S. Williams et al., 2019). Notably under rain grown production conditions, the magnitude and timing of likely future water deficit scenarios requires consideration with regards to application rates of MC; yield penalties being associated with high application rates and concurrent / subsequent water deficit stress conditions (Eveleigh, Marshall, Kelly, Ford, & Quinn, 2010). Currently MC is infrequently utilised in Australian rain grown cotton production for the initiation of physiological cut out, but can be of utility for such in very late or uneven crop maturity scenarios (S. Williams et al., 2019).

### **2.5.3 Cotton plant responses to exogenous PGR treatment scenarios as documented in research**

A review of current global research regarding the utility of PGRs in cotton production systems was conducted to update Cothren and Oosterhuis (2010), with a synopsis tabulated and presented in Appendix 2. From this research, specific groupings of growth promoting and inhibiting compounds, being relevant to the scope of physiological rationales described in Section 2.4.4, will be regarded in this contribution; specifically, naturally occurring bioactive Gibberellins, inhibitors of gibberellin biosynthesis and Adenine-type synthetic Cytokinins.

#### ***Naturally occurring bioactive Gibberellins***

Approximately 140 known Gibberellins have been identified in higher plants and / or gibberellin producing fungi, however the majority are precursors or catabolites formed during the formation of biologically active GA<sub>1</sub>, GA<sub>3</sub> and GA<sub>4</sub> (Rademacher, 2015). Phytopathogenic fungus *Gibberella fujikuroi* (imperfect stage *Fusarium moniliforme*, later *F. fujikuroi*), that causes abnormal shoot elongation in rice (“bakane disease”), is used to produce Gibberellin A<sub>3</sub> and a mixture of Gibberellin A<sub>4</sub> and Gibberellin A<sub>7</sub>, via fermentation. Chemical synthesis of Gibberellins (GAs) is possible but is too complex and expensive for commercial production (Petracek, 2017).

All Gibberellins are comprised of the ent-gibberellane structure (Cothren & Oosterhuis, 2010); full nomenclature and structural descriptions being detailed in Appendix 1. Gibberellin A<sub>3</sub> is the most widely utilised GA, with a mixture of GA<sub>4</sub> and GA<sub>7</sub> also commercially available; GA<sub>4</sub> is less persistent than GA<sub>3</sub> and is suited where a shorter acting effect is warranted (Rademacher, 2015). Due to close chemical similarity of GA<sub>4</sub> and GA<sub>7</sub>, separation in fermentation extracts is difficult; resultingly, the content of GA<sub>7</sub> in different commercial products varies between 40% and insignificant amounts (Rademacher, 2015). On a sector proportionate basis, Gibberellin A<sub>3</sub> is one of the most widely utilised PGRs commercially with total annual production exceeding 100 t; 25% being utilised to accelerate the malting process in barley fermentation and 75% utilised in viticulture, horticulture and agricultural applications (Hornsey, 2003; Rademacher, 2015).

Gibberellins have more than one primary site of action and resultantly are involved in a high proportion of cotton plant growth and development processes (Rademacher, 2000, 2015; Taylor & Cosgrove, 1989). Research involving gibberellins in cotton is centred on foliar applications of aqueous GA<sub>3</sub> solutions, notably from early squaring; evidencing broad-scale phenological impacts (Rademacher, 2000, 2015; Taylor & Cosgrove, 1989). Gibberellin induced increases in cell wall plasticity facilitates cell elongation and longitudinal internode growth, resulting in taller plants (Ergle, 1958; Hansen, Black-Schaefer, Shafer, & Larson, 1996; Larson, Black-Schaefer, Adair, & Conley, 1997) and increased above ground biomass accumulation notably of stem and petiole tissue (Ergle, 1958; Fang et al., 2018; Fang et al., 2019; Larson et al., 1997; Walhood, 1958). Concomitantly, early vegetative stage foliar applications of Gibberellin A<sub>3</sub>, has also demonstrated utility in promoting increased LAI under irrigated international production environs (Hansen et al., 1996; Larson et al., 1997; Walhood, 1958).

Although cotton is a day-neutral plant with no verified direct impacts of exogenous Gibberellins on phase change transition into reproductive growth; increases in floral bud initiation at early squaring (Fang et al., 2018), and resultant flower and mature boll numbers (Mathur & Mittal, 1964) have been evidenced in glasshouse and irrigated field grown cotton. Recent research noting that increased boll numbers and yield were attributable to early increased sucrose accumulation in floral buds, being associated with an increased leaf photosynthesis rate (Fang et al., 2019). Exogenous GA<sub>3</sub> applications have also increased leaf photosynthetic pigment content and canopy net photosynthesis rate (due to increased LAI), under glasshouse and irrigated field settings (J. C. F. da Costa et al., 2017; Hansen et al., 1996; Larson et al., 1997). Corresponding fruit retention rates are dependent on timing of measurement; evidencing an overall initial increased development response to GA<sub>3</sub> applications with associated early shedding, resultant compensatory vegetative growth, delayed maturity and reduced boll size (Walhood, 1958). This was supported by increased end of season retention rates and boll numbers, as reported by Fang et al. (2019), Subbiah and Mariakulandia (1972) and Walhood (1958). Resultant yield increases were reported by Fang et al. (2019) and Larson et al. (1997), with increases in fibre length noted by Bhatt and Ramanujam (1971) under irrigated field conditions.

Limited references regarding the physiological role of Gibberellins in root growth exist due to earlier evidence suggesting a lack of promotion of root elongation (Tanimoto & Hirano, 2013).

However, molecular mechanisms of Gibberellin signal transduction in shoots have recently been substantiated in roots; evidence suggesting Gibberellins control root growth at very low concentrations compared to shoot regulation (Tanimoto & Hirano, 2013). In cotton, under hydroponic growth conditions (with no restricting influences of soil bulk density or resource availability), duplicate foliar applications of GA<sub>3</sub> (0.04 ppm) at 30 days apart, from 36 days post planting augmented both root total volume and root absorption area at 90 days post planting (Onanuga, Jiang, & Adl, 2012).

### ***Inhibitors of Gibberellin biosynthesis***

Gibberellin biosynthesis inhibiting PGRs reduce the bioactive Gibberellin content of treated plants by disrupting specific points along the Gibberellin biosynthesis pathway, particular to the individual chemical (Rademacher, 2000). Decreased GA concentrations reduce rates of cell division in the shoot apical meristem, resulting in decreases in cell growth (expansion) and cell wall plasticity (Cothren & Oosterhuis, 2010); being evidenced at a plant level as reduced shoot elongation and key to cotton plant development, a decreased number of main-stem nodes (V. A. da Costa & Cothren, 2012; Rademacher, 2000). Globally, inhibitors of GA biosynthesis are the most commercially significant group of PGRs in terms of commercial value and treated area; decreasing the risk of lodging in oilseed and cereal crops, as well as facilitating the management of canopy architecture in cotton, ornamental horticultural plants and tree crops, for improved resource partitioning and reduced production costs (Rademacher, 2015). Gibberellin biosynthesis inhibitor (GaBI) PGR, Mepiquat chloride, is currently widely utilised in Australian cotton production systems for the control of excessive vegetative growth during flowering and to induce physiological cut-out in high-input irrigated systems (S. Williams et al., 2019). Consequently, most of research outputs in cotton are centred around the utility of both MC and closely associated CC, with significant variation existing between GaBI PGR chemicals in cotton plant phenotypic response to treatments (Appendix 2).

Chlormequat chloride (CC) and Mepiquat chloride (MC) are quaternary ammonium compounds that inhibit cyclases involved in the early stages of GA metabolism, which lead to the formation of *ent*-kaurene (Rademacher, 2015). Cotton plant response to foliar applications of Chlormequat chloride demonstrated variation based both on timing of treatment application and crop growth environment. Earlier (55 DAP) applications lowering final boll numbers and seed cotton yield via

early impacts on vegetative growth (biomass accumulation) and LAI, with subsequent reductions in net canopy photosynthetic capacity (Dhillon, Kler, & Raj, 1980; Marani et al., 1973; Thomas, 1975). In non-resource limited crops demonstrating consistent, excessive vegetative growth; later (e.g. 75-105 DAP) foliar applications increased seed cotton yield via optimising photosynthetic partitioning and consequent increases in both boll number and boll weight (Dhillon et al., 1980; N. Rao, Prabbakara-Rao, & Amarish, 1980; S. S. Rao, Tanwar, & Regar, 2016; Sawan, 2016). Bhatt and Ramanujam (1971) noted lint quality changes proportionate to treatment concentration with higher concentrations decreasing fibre strength and yield. Chloromequat chloride treatments also caused reductions in root density and growth rates (Cappy & Cothren, 1980).

Reductions in biomass accumulation associated with Mepiquat chloride foliar and seed applications, had significantly differing flow-on physiological responses depending on both the timing of application and prevailing crop growth environment. Earlier (seed soaking and early squaring foliar) applications documenting both no impact (Yeates, Constable, & McCumstie, 2005) and increases in yield components (open boll weight and number) and lint yield (Ferrari, Furlani, Ferrari, & Luques, 2015; Sawan & Sakr, 1990; Yang et al., 2014). Seed soaking applications of MC demonstrated utility in promoting root length, area and volume, with associated reductions in aerial biomass at 20 DAP under hydroponic controlled environment conditions (Chen et al., 2018). Proportionate to treatment concentration, seed soaking applications also decreased plant height and stem diameter (Ferrari et al., 2015), as well as the number of floral buds at first square (Fang et al., 2018); resulting from reduced growth rates and node development that led to delays in progression to first flower and maturity with lint yield penalties, under non-resource limited field conditions (Yeates et al., 2005). Foliar applications of MC from first square caused reductions in plant height and LAI under non resource limited growth conditions (irrigated field, glasshouse) (Gonias, Oosterhuis, & Bibi, 2012; Heilman, 1981; Walter et al., 1980). Canopy architecture changes being evidenced as increased canopy extinction coefficient, increased radiation use efficiency due to changes in the photosynthetic capacity of leaves and light distribution through the canopy (Gonias et al., 2012).

Several compounds with a nitrogen-containing heterocycle, block cytochrome P450-dependant monooxygenases, which catalyse the oxidation of *ent*-kaurene via *ent*-kaurenoic acid into GA12-

aldehyde; triazoles Paclobutrazol (Paclo) and Uniconazole (Unicon) being of practical relevance to this review. Early flowering foliar applications of Unicon in *G. barbadense* cotton, under irrigated field conditions, resulted in reduced height at maturity with an associated increase in fruiting branches, green and open bolls, with a decreased time to first open boll (Mohamed, Hanan, & Mahasen, 2010); no published outcomes being obtainable for *G. hirsutum*. Seed priming applications of Paclo in *G. barbadense* cotton, under irrigated field conditions, inhibited early vegetative stage node development and biomass accumulation, with no ongoing treatment effects evident in early flowering (Zaghlool & Ibrahim, 2005). Application of Paclo as a soil drench is widely utilised in commercial horticultural applications (tree crops and ornamentals) on account of longer efficacy of treatment impact (Rademacher, 2015). Soil drench applications after the first irrigation in field grown cotton, decreased the node of first fruiting branch with no other impacts on yield components or lint yield evident (Cimen, Basbag, Temiz, & Sagh, 2003; Cimen, Basbag, Temiz, & Sagir, 2015), however increases in micronaire were reported (Temiz, 2009). Single foliar applications from first flower in irrigated field grown cotton reduced plant height, the number of main-stem nodes and the dry weight of stems and leaves; the earlier (FF) applications having stronger impacts on growth and subsequent node development, with no impact on flowering, lint quality or yield (Ben-Porath, Levin, & Meron, 1988).

The late stages of GA biosynthesis are catalysed by dioxygenases, requiring 2-oxyglutaric acid as a co-substrate; because of their structural relationship to 2-oxyglutaric acid, acylcyclohexanediones Trinexapac ethyl (TEP) and Prohexadione-calcium (PhxCa) inhibit these reactions (Rademacher, 2015). Of note with regards to abiotic stress resilience in cotton, both TEP and PhxCa also inhibit ethylene formation by limiting the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) into ethylene (being catalysed by ACC oxidase) (Rademacher, 2015). Limited published information exists regarding the impact of either TEP or PhxCa on cotton; a single foliar application of TEP at 12-leaf (FF) demonstrated no treatment impacts on height or biomass accumulation at 50 days post treatment in irrigated cotton, grown in pots under field conditions (Correia & Leite, 2012).

### ***Adenine-type synthetic Cytokinins***

Phytohormonal Cytokinins are N6-substituted Adenine compounds that regulate many components of plant growth and development in a localised, organ-autonomous method

(Grossmann, 1991; Werner et al., 2010); stimulating cell division in meristematic tissues to promote cell and organ differentiation, counteracting auxins in apical dominance, increasing flowering, fruit set and fruit growth, influencing nutrient translocation to attract assimilates into sinks, delaying leaf senescence and conversely inducing ethylene formation and the subsequent development of abscission layers associated with juvenile fruit abortion (Burke, 2013; Rademacher, 2015). Endogenous Cytokinins are also transported via the plant vascular system, having both paracrine and long-distance activities (Werner et al., 2010) and facilitating root:shoot communication (Werner & Schmulling, 2009). However, shoot growth regulation does not depend on root-borne endogenous Cytokinins (Werner et al., 2010); especially in young growing tissue where Cytokinins are synthesized locally (Miyawaki, Matsumoto-Kitano, & Kakimoto, 2004). Endogenous Cytokinins are negative regulators of root growth; moderating root elongation via reductions in the exit of cells from the zone of cell division into cell differentiation (Ioio et al., 2007) and reducing lateral root initiation via endogenous Cytokinins blocking the first divisions of xylem-pole pericycle cells (Laplaze et al., 2007).

In addition to natural Cytokinins, synthetic compounds of different chemical classes demonstrate Cytokinin-like activity in cotton (Appendix 2); N<sup>6</sup>- substituted Adenine derivatives such as 6-Benzylaminopurine (6BA) and the heterocyclic urea Thidiazuron are cost-efficient to manufacture and have demonstrated appliance in manipulating plant growth and development in commercial horticulture and cropping scenarios (Grossmann, 1991; Rademacher, 2015; Srivastava, 2002). These differing synthetic Cytokinin structures have diverse specificities for in planta Cytokinin receptors, with comparative receptor preferences for specific ligands (Yonekura-Sakakibara, Kojima, Yamaya, & Sakakibara, 2004); resultantly, exogenous treatment effects vary depending on plant species, tissue impacted and / or developmental stage of application (Feng, Shi, Yang, & Zuo, 2017). Clarity around mode of action of these non-plant aromatic Cytokinins is still ambiguous (Grossmann, 1991; Srivastava, 2002). Plausible diphenyurea mode of action being through the inhibition of endogenous Cytokinin oxydase, thereby having a “sparing” effect on Cytokinins present in the tissue, by inhibiting their breakdown (Srivastava, 2002). Conversely, 6BA side chains are immune to attack by Cytokinin oxidase, however the enzymes that catalyse the modifications of the purine ring, are able to use BA as a substrate; thus exogenous 6BA has been demonstrated to give rise to its N<sup>9</sup>-ribosyl derivatives, as well as N<sup>3</sup>-glucoside and conjugates (Srivastava, 2002).

Recent research reviews note the inconclusive effectiveness of exogenous applications of Cytokinins in cotton; reporting variation between and within Adenine- and duphenyurea-type Cytokinin PGRs in plant phenotypic responses, with notable inconsistencies in yield benefit outcomes (Cothren & Oosterhuis, 2010; Guinn, 1986). Early vegetative stage foliar applications of 6BA, in glasshouse and rain grown production settings on the Texas high plains (USA) (Burke, 2011, 2013), demonstrated increases in early hypocotyl diameter and tap root length, with flowering stage reductions in apical dominance and reduction in water deficit stress levels, as quantified via lower chlorophyll fluorescence levels following a dark respiration bioassay challenge. Flow on responses were evident in increased mean boll weights and consequent lint yields (Burke, 2013). These studies (Burke, 2011, 2013) in addition to early vegetative growth (5 leaf) stage investigations conducted by Grossmann (1991), also reported the occurrence of phytotoxic leaf lesions and leaf abscission due to increased ethylene levels, at higher ( $100 \mu\text{mol}\cdot\text{mol}^{-1}$ ) treatment concentrations.

Glasshouse (pot) and irrigated field experiments evaluating foliar ( $5\text{-}50 \text{ mg}\cdot\text{L}^{-1}$ ) applications of 6BA during early to mid-flowering demonstrated PGR capacity to counteract water deficit stress impacts on ribulose-1,5-bisphosphate carboxylase/oxygenase (*RuBPCO*) and PEP Carboxylase (Pandey, Goswami, Kumar, & Jain, 2000) and net photosynthesis rate, transpiration rate and stomatal conductance (Kumar, Pandey, Goswami, & Jain, 2001). Pandey, Goswami, and Kumar (2003) then associating the decreased impact of water deficit stress on enzyme activation with improved seed cotton yield outcomes in glasshouse grown cotton; achieved via increased flower retention and open boll numbers. Mid-flowering applications under irrigated field conditions also increased open boll mean weights and fibre length; being associated with increased boll sucrose content and associated sucrose synthase and sucrose phosphate activity (Y. Wang, Liu, Chen, & Zhou, 2011). Recently, Fang et al. (2018); Fang et al. (2019) has also demonstrated increased flower retention, boll numbers and resultant lint yields in irrigated field grown cotton from early squaring applications; being attributable to increased leaf photosynthesis rates (and associated increases in leaf carbohydrate content, translocation rate and resultant sucrose and starch contents in floral buds).

#### **2.5.4 Opportunities for novel manipulation of cotton growth and development utilising PGRs**

Current commercial applications and outcomes from international research in irrigated field and controlled environment settings, demonstrate capacity of PGRs to tactically promote or inhibit cotton plant growth and development. Cotton plant responses to the specific Gibberellin, Cytokinin and Gibberellin biosynthesis inhibiting PGR treatment scenarios detailed, demonstrate potential to action the physiological rationales underpinning current climate risk mitigation strategies utilised in rain grown cotton production (Table 2.3).

However, interpretation of PGR studies are complex, because crop responses to experimental inputs are influenced by differences in management and environmental contexts. Many PGR treatment scenarios are not quantified in Australian cotton production systems; notably under resource constrained rain grown constructs, on Vertosol soil and / or under skip row planting configurations. Additionally, limited information exists regarding cotton plant responses to early vegetative growth stage PGR treatment applications; where any potential impacts on early canopy and root development, or reproductive phase change could be quantified.

Therefore, it is currently difficult to attain conclusive evidence demonstrating the consistent capability of these PGRs in tactically modulating cotton growth and development, to facilitate adaptive management strategies based on the physiological rationales underpinning current rain grown cotton climate risk mitigation strategies. Resultantly, complexities also exist in determining both the occurrence and magnitude of any potential system benefits associated with:

- overcoming initial growth lag through the promotion of early canopy and structural biomass accumulation, augmenting canopy capacity to drive (above and below ground) growth and increase access to soil water reserves;
- promoting early root growth biomass accumulation and consequent plant root growth area (root space), to increase access to skip row soil water reserves; and
- slowing vegetative growth or inducing physiological stasis, to manipulate crop level reproductive phase-transition and shift crop peak resource demand to alternate (favourable) climatic conditions.

Consequently, research is required to adapt these growth regulators to both rain grown cotton farming systems and the strategic manipulation scenarios that leverage the physiological rationales underpinning existing climate risk management strategies.

**Table 2.3 Specific PGR linkages to proposed adaptive management approaches, being based on physiological rationales underpinning existing agronomic climate risk management strategies; further detail as to specific PGR function in cotton provided in Appendix 2.**

<b>Current agronomic strategy</b>		<b>Plant growth regulator</b>		<b>Classical role, function in cotton</b>	<b>Proposed adaptive management strategy</b>
<b>Strategy</b>	<b>Rationale basis</b>	<b>Active ingredient</b>	<b>PGR group</b>		
Row configuration	Increase access to stored soil water adjacent to plant line	6-Benzylaminopurine	Adenine-type Cytokinin	<p><b>Promotion of cell division in meristems</b> Reduction in apical dominance, increased root length, increased open boll weight and lint length (increased lint yield) in glasshouse and irrigated field conditions Increased lateral root proliferation, decreased water deficit stress and increased yield under rain grown field conditions (Tx. USA)</p>	<p><b>“Exploiting the skip”</b> Promotion of root biomass accumulation and consequent root growth area (root space) to increase access to skip row soil water reserves</p>
Cultivar selection	<p>Change in crop response to environment</p> <p>Increased crop resilience to abiotic stress</p>	Gibberellin A <sub>3</sub> , Gibberellin A <sub>4,7</sub>	Gibberellin	<p><b>Increased cell wall plasticity resulting in cell elongation</b> Increased abscission, increased photosynthetic pigments and rate, increased height, LA and leaf weight, increased floral bud initiation and number of flowers, increased lint length and yield in Gibberellin A<sub>3</sub> under glasshouse and irrigated field conditions No evidence of utilisation of GA<sub>4,7</sub> in cotton</p>	<p><b>“Fitness for environment”</b> Promotion of early canopy and structural biomass development; augmenting canopy capacity to drive (above and below) ground growth and increase access to soil water reserves</p>
Planting date	Optimise the use of limited crop water inputs (starting available soil water and rainfall)	Chlormequat, Mepiquat, Paclobutrazol, Prohexadione calcium, Trinexapac ethyl, Uniconazole	Gibberellin biosynthesis inhibitors	<p><b>Growth retardant</b> Decreased height, internode length and leaf area, changed canopy structure. Varying flow-on impacts on flowering, boll fill and resultant lint yields and quality; depending on timing and concentration of PGR treatment under glasshouse and irrigated field conditions</p>	<p><b>“Dynamic fruiting”</b> Slowing vegetative growth / inducing physiological stasis, to manipulate crop level reproductive phase-transition and shift crop peak resource demand to alternate (favourable) climatic conditions</p>

## **2.6 Conclusions**

This review draws on knowledge of the dynamic production contexts shaping rain grown cotton systems, as well as the current application of agronomic management strategies to mitigate abiotic-stress-mediated system limitations and challenges; so as to understand how plant growth regulators may be leveraged to create novel adaptive management strategies that promote agroecosystem resilience.

Plant growth regulators (PGRs) show potential for utilization in the development of adaptive management strategies for Australian rain grown cotton production systems, based on capacity to strategically manipulate cotton growth and development; as evidenced by both global commercial applications and elicited responses as documented in research. However, knowledge gaps exist regarding (Australian) rain grown cotton crop and system level responses to specific, early vegetative growth stage, PGR treatment scenarios; aimed at achieving phenological manipulations emulating the underlying principles of current agronomic climate risk management strategies.

Fundamental analysis is centred around quantification of the direct impacts of specific PGR choice, rate, application type, frequency and timing combinations, on cotton crop growth and development outcomes; as proportionate to each proposed adaptive management strategy. Moreover, understanding is required of the resulting flow-on morphological plant and crop level responses to PGR treatments; as explained by yield, lint quality, biomass and canopy development, as well as fruit development, retention and maturity outcomes. Such understandings can be utilised to determine system benefits and to resultantly develop frameworks for the application of specific PGR treatment scenarios; facilitating bespoke crop adaptation to prevailing climatic and management contexts, to promote system resilience through increases in yield, yield stability and / or input use efficiencies.

## Chapter 3 General Materials and Methods

### 3.1 Introduction

Between October 2015 and June 2019, 11 experiments were conducted to identify and investigate applications of specific plant growth regulators (PGRs) to improve crop use efficiencies and / or deliver economic yield and lint quality benefits to rain grown production systems. Field and glasshouse experiments were carried out at three locations in north-west New South Wales, Australia (Table 3.1).

**Table 3.1 Location and year of glasshouse and field experiments.**

Experiment number (name)	Cotton Season	Location, Farm (Field)	Referring Chapter
1. (GH Seed CkGa)	2016	ACRI, Glasshouse 8N	4, 5, 7
2. (GH Foliar CkGa)	2016	ACRI, Glasshouse 8N	4, 5, 7
3. (GH GaBI)	2016	ACRI, Glasshouse 8N	6, 7
4. (2016.17 ACRI ERCF)	2016.17	ACRI, Field D2	4, 5, 7
5. (2016.17 BTA ERF)	2016.17	Bellata, “Dobikin” (Boggy Creek)	4, 5, 7
6. (2016.17 ACRI GaBI)	2016.17	ACRI, Field D2	6, 7
7. (2016.17 THH GaBI)	2016.17	Terry Hie Hie, “Myall Plain” (Tank-1).	6, 7
8. (2017.18 ACRI Resil.1)	2017.18	ACRI, Field B2	4, 5, 6, 7
9. (2018.19 ACRI Resil.2b)	2018.19	ACRI, Field A3	4, 5, 6, 7
10. (2018.19 BTA Resil)	2018.19	Bellata, “Dobikin” (Horse West)	4,5, 7
11. (2018.19 THH Resil)	2018.19	Terry Hie Hie, “Myall Plain” (Johns).	5, 6, 7

This chapter details cotton cultivar selection, site and climate descriptions, experiment design, field plot and glasshouse pot management, treatments and measurements, crop simulations and data analysis that was common to experiments discussed in Chapters 4 to 7. Materials and methods specific to each experiment are described in the relevant chapter.

### 3.2 Genotypes

Normal leaf, medium to late maturity, transgenic cultivars of upland cotton (*Gossypium hirsutum* L.) were used for all field and glasshouse experiments; being selected to reflect commercial practice. Unless otherwise specified, all glasshouse (GH) and field experiments utilised Sicot 746B3F (Stiller, 2017a); a full season cultivar with a compact growth habit, very high yield

potential, good disease resistance, high lint quality and excellent adaption to both irrigated and rain grown management in central and hot production areas (CSD, 2018b). Due to collaborating-grower preference, 2016/17 season field experiment 7 (2016.17 THH GaBI) at the Terry Hie Hie site utilised Sicot 748B3F (Stiller, 2017b); a full season cultivar with vigorous growth habit, very high yield potential, good disease resistance, high lint quality and excellent adaption to central, hot and rain grown production areas (CSD, 2018c).

Both Sicot 748B3F and Sicot 746B3F are transgenic cultivars containing the Bollgard®3 *Bacillus thuringiensis* (*Bt*) insecticidal crystal protein stack Cry 1Ac and Cry 2Ab, as well as the *Bt* vegetative insecticidal protein gene Vip3A, for the control of lepidopteron pests (Monsanto Australia n.d.). Both varieties also contain Roundup Ready Flex ® technology for tolerance of glyphosate application throughout the growth stages in both vegetative and reproductive plant parts, through the manipulation of the CP4-EPSPS protein sequence. Both these technologies were owned and licenced by Monsanto Technologies LLC® (Monsanto, 2016a).

### **3.2.1 Commercial seed treatments**

Unless specified, all seed utilised in glasshouse and field experiments were treated with Dynasty Complete® fungicide (Fludioxonil, Metalaxyl-M, Azoxystrobin, Acibenzolar-S-methyl) and Cruiser Extreme® insecticide (Thiamethoxam); for the control of seedling diseases such as damping off (*Pythium spp.*) and *Rhizoctonia solani*, for increased resistance to *fusarium spp.* and black root rot, and to control soil-borne and sucking insect pests (thrips, aphids, wireworm) during early growth. Both products are owned and licenced by Syngenta® Australia Pty Ltd; being adhered to the seed coat with Peridiam® polymer seed coating (Bayer S.A.S, Merville, France).

### **3.3 Glasshouse experiments**

Three glasshouse experiments were conducted between June and November 2016; being used to provide controlled environment-grown plant samples, for root and above ground biomass accumulation, partitioning and architecture assessments. All experiments were conducted at Glasshouse 8 (north), Australian Cotton Research Institute (ACRI) “Myall Vale” (-30.206834 S, 149.594086 E), Narrabri NSW, Australia.

Delinted, untreated and sterilised seed of Australian cotton cultivar Sicot 746B3F (Stiller, 2018) was used for all three experiments; sterilising methodology being detailed in section 3.3.2. Longer duration experiment 3 (GH GaBI) was conducted once only; experiments 1 (GH Seed Ckga) and 2 (GH Foliar Ckga) were repeated twice due to root washing methodology adjustments, with results from the second run (only) of both experiments being utilised.

All field experiments were constructed as a randomised complete block design using CycDesign V6.0 (VSN, 2016) software; blocks were replicates, blocked down the glasshouse benches which ran in an East-West direction to avoid shading. Experiments 1 (GH Seed Ckga) and 2 (GH Foliar Ckga) contained 10 replicates, experiment 3 (GH GaBI) contained six replicates due to space limitations associated with the larger planter bags.

### **3.3.1 Growth conditions**

Cotton plants were established in a loam potting medium (turf underlay, Johnsons Concrete and Landscaping Pty Ltd, Narrabri NSW) and were grown under glasshouse conditions at 28/20°C day/night temperature, under natural light.

#### ***Experiment 1 (GH Seed Ckga)***

Experiment 1 (GH Seed Ckga) was established on the 23<sup>rd</sup> August 2016. PGR treatments were pre-applied as seed-priming applications or incorporated with a polymer sticker as a seed coat; primed and coated seed was germinated in seedling tubes (Super-forestry native tree tubes (67 mm x 160 mm), Danbar Plastics, Horsham Vic). Seedling tubes were watered daily by hand using a 5 L watering can and sprinkler head (1 L/replicate). Nutrients were applied at planting and every four days thereafter with Yates Thrive All Purpose Soluble Plant Food (Dulux Group (Australia) Pty Ltd, Clayton, Vic) added to irrigation water at 1.6 g.L<sup>-1</sup> water (Table 3.2). The experiment was terminated at 13 days after planting (DAP) on the 5<sup>th</sup> September 2016.

#### ***Experiment 2 (GH Foliar Ckga)***

Experiment 2 (GH Foliar Ckga) was established on the 23<sup>rd</sup> August 2016; two seeds per seedling tube (as per Experiment 1) were germinated and thinned to one plant at establishment (cotyledon unfurled). Seedling tubes were watered daily by hand using a 5 L watering can and sprinkler head

(1 L/replicate). Nutrients were applied at planting and every four days thereafter with Yates Thrive All Purpose Soluble Plant Food (Dulux Group (Australia) Pty Ltd, Clayton, Vic) added to irrigation water at 1.6 g.L<sup>-1</sup> water (Table 3.2). The experiment was terminated at 24 DAP on the 16<sup>th</sup> September 2016.

### **Experiment 3 (GH GaBI)**

Experiment 3 (GH GaBI) was planted on the 30<sup>th</sup> September 2016; four plants per planter bag (BB1538H 7L Planter bags (150 mm x 380 mm), Botany Horticultural, Nerang QLD) were established, thinned to one plant at 14 DAP, then sited on three glasshouse benches with two replicates per bench. Planter bags were watered at 1230h daily for four mins by drip irrigation delivering 4.5 L.h<sup>-1</sup>. Nutrients were applied at planting with 1.25 g of Granulock 12Z (Incitec Pivot Fertilisers Ltd, Southbank, Vic) (Table 3.2) and supplemented at control 50% population first square (26 DAP) with 1.81 g granular urea (Incitec Pivot Fertilisers Ltd, Southbank, Vic) (Table 3.2). The experiment was terminated at 59 DAP on the 28<sup>th</sup> November 2016, coinciding with control first flower plus 7 days.

**Table 3.2 Composition of fertiliser products used in experiments 1-3.**

Nutrient	Compound	%(w/v)
<b>Yates Thrive All Purpose Soluble Plant Food</b>		
<i>(N:P:K:S:Mg:Fe:B:Cu:Zn:Mo = 25:5:8.8:4.6:0.5:0.18:0.005:0.005:0.004:0.001)</i>		
<i>(No composition data available)</i>		
<b>Granulock 12Z (N:P:S:Zn=11:21.8:1)</b>		
Nitrogen / Phosphorus	Mono-ammonium phosphate	85-90
Nitrogen / Phosphorus	Ammonium sulphate	5-10
Zinc	Zinc sulphate monohydrate	2.9
Sulphur	Sulphur	2
<b>Granular Urea (N:40.8)</b>		
Nitrogen	Urea	99.5
Nitrification inhibitor	3,4-Dimethylpyrazole	<0.15

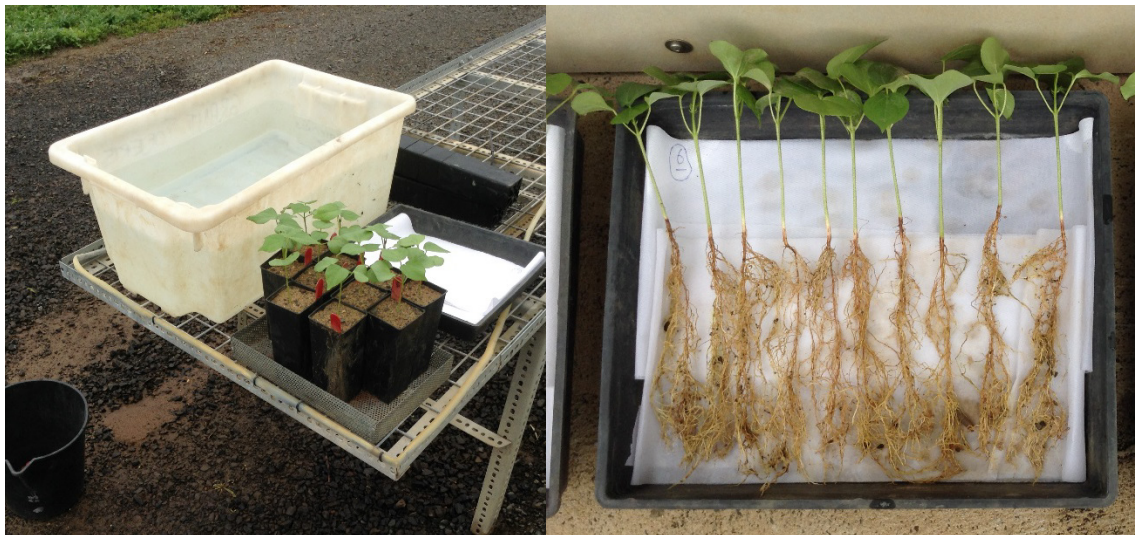
### **3.3.2 Seed sterilisation**

Untreated, delinted cotton seeds were surface sterilised to remove contamination and decrease the risk of soil-borne seedling disease, as per methodology of Salimath and Neogi (2016). Cotton

seeds were rinsed three times in deionised water (Milli-Q ultra-pure system, Merck KGaA, Castle Hill, NSW), transferred to 100 mL of 70% ethanol (200 proof, Merck KGaA, Castle Hill, NSW) plus two drops of Polysorbate 20 non-ionic surfactant (Tween 20, Merck KGaA, Castle Hill, NSW) and agitated for one minute. Seeds were drained, rinsed again twice in deionised water for two minutes each. Seeds were then sterilised by immersion in 10% sodium hypochlorite (Home brand bleach, Woolworths Ltd, Castle Hill, NSW) plus two drops of Polysorbate 20 non-ionic surfactant for two minutes, then rinse-washed three times in deionised water. Seeds were air-dried under a fume hood for 24 h at ambient room temperature in a single layer on absorbent paper towel (3-ply paper towel, Woolworths Ltd, Castle Hill, NSW).

### 3.3.3 Root washing

At termination of experiments 1 and 2, separation of the potting medium from the root system was achieved by removing the seedling tube and gently immersing the core in a 20 L tray of water to allow the potting medium to fall away without damaging the fine root structure. Individual plants (experimental units) were arranged by treatment on trays lined with greaseproof paper for further root length, stem diameter and total wet weight analysis prior to oven drying (Figure 3.1).



**Figure 3.1 Experiment 2 (2016) 24 DAP biomass harvest; root washing equipment (left) and outcome (right).**

### **3.3.4 Treatment application**

#### ***Seed coating***

Dry-process seed coating treatments were downscaled from Cotton Seed Distributors Ltd (Wee Waa, NSW) commercial seed treatment methodology (Ross, 2016); 1% of centum weight (cwt: 45.359 kg) of delinted, untreated and sterilised Sicot 746B3F cotton seed were weighed into 1L plastic takeaway food containers (Woolworths Pty Ltd, Castle Hill NSW). A treatment slurry was produced by combining 0.81 mL Peridiam® Evolution EV23003 (Blue pigment added) polymer film coating (Bayer S.A.S, Mereville, France) with 0.45 mL of the specific PGR aqueous treatment solution of known concentration (Chapter 5, Table 5.2.2.1). The slurry was added to the seed, covered and agitated for one minute or until seed was completely covered. Seed was then placed in a single layer on absorbent paper towel and air dried under a fume hood for 24 h at ambient room temperature.

#### ***Seed priming***

Delinted, untreated and sterilised Sicot 746B3F cotton seeds were fully submerged in PGR treatment solutions of known concentration for the specified time period (Chapter 4, Table 4.1; Chapter 5, Table 5.1). Seed were then drained and air dried under a fume hood for 24 h at ambient room temperature in a single layer on absorbent paper towel.

#### ***Foliar spray application***

PGR foliar treatments were applied 30 cm above the plant line with a Ryobi 3.5 L hand-held battery-operated sprayer (flow rate: 8 mL.sec<sup>-1</sup>). Simulating a field application rate of 100 L.ha<sup>-1</sup> to provide complete canopy coverage, applications were sprayed “to drip” and dried in a sheltered outdoor position; plants were moved back into the glasshouse within 2 h.

### **3.4 Field experiments**

Field based experiments were used to provide field grown plant tissue samples for; lint yield, lint quality, root and above ground biomass accumulation, partitioning and architecture, crop water extraction and electronic conductivity (EC) assessments.

### **3.4.1 Site and climate**

Three field experiment sites in the Gwydir / Namoi region were selected to enable efficient evaluation of PGR treatment responses in distinct environmental contexts that characterised wider (Australian) rain-grown cotton production settings. The region is characterised by hot summers with high daily maximum and minimum temperatures, cool winters and summer dominant rainfall with high annual variability (Table 3.3). Soil profile characteristics at all three sites were classified as grey, self-mulching, endo-calcareous grey Vertosols (Isbell, 2002), with 60-65% clay fraction, 8.0-8.8 pH, low in organic matter and N contents. Seasonal summaries of rainfall and temperature across all field experiments are provided in Appendix 3.

#### ***Terry Hie Hie (THH)***

Typifying eastern (slopes) production climates, field experiments in 2016/17 and 2018/19 were undertaken at adjacent properties “Carmona Downs” and “Myall Plain”, located between Terry Hie Hie (10 km north-west) and Moree (40 km south-east) in north-west, NSW Australia (Table 3.3, Figure 3.2).

#### ***Bellata (BTA)***

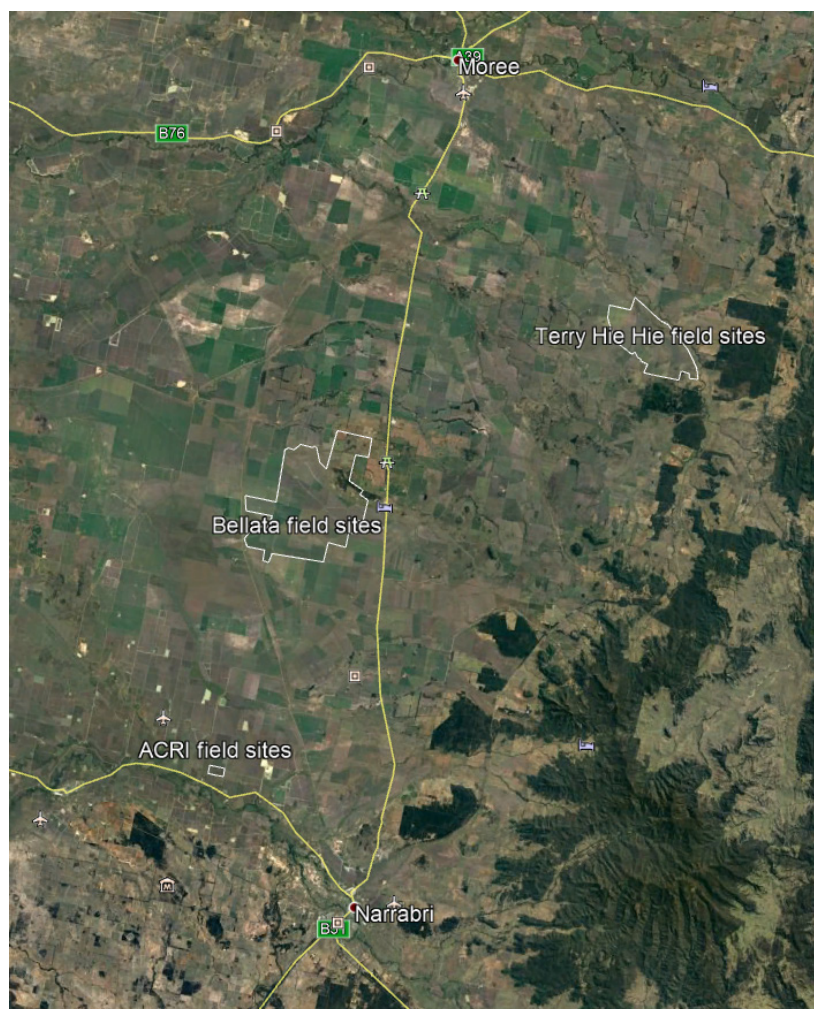
Characterising drier, hotter western (flood plain) production settings, field experiments in 2016/17 and 2018/19 were undertaken at “Dobikin”, located 10 km west of Bellata in north-west NSW, Australia (Table 3.3, Figure 3.2).

#### ***Narrabri (ACRI)***

Field experiments in 2016/17, 2017/18 and 2018/19 were undertaken at the Australian Cotton Research Institute (ACRI) “Myall Vale”, located 30 km west of Narrabri in north-west NSW, Australia; mitigating project climate risk through the option to opportunity irrigate and simulate “mean” seasonal in-crop rainfall conditions (Table 3.3, Figure 3.2).

**Table 3.3 Terry Hie Hie (THH), Bellata (BTA) and Narrabri (ACRI) field experiment site location, soil plant available water holding capacity and climate means ((BOM, 2019); <sup>(1)</sup>Terry Hie Hie (053108), <sup>(2)</sup>Bellata Post Office (053003), <sup>(3)</sup>Narrabri (Mollee) (053026), <sup>(4)</sup>Warialda (054029), <sup>(5)</sup>Moree Aero (053115), <sup>(6)</sup>Narrabri Airport AWS (054038), 2019;(CSIRO, 2019); Google Earth, 2019).**

Field Site (Lat./Long.)	Elev. (m)	Est.Soil PAWC (mm)	Mean Rainfall (mm) <i>Annual (Range)</i>	Mean Temp (°C)				
				<i>Summer</i>	<i>Summer</i>		<i>Winter</i>	
					<i>Max</i>	<i>Min</i>	<i>Max</i>	<i>Min</i>
<b>THH</b> 29°43'52.21"S, 150°05'05.77"E	290	~300	538.8 (359-743.4) <sup>1</sup>	163.1	32.93 <sup>4</sup>	15.8	18.36	0.9
<b>BTA</b> 29°56'19.73"S, 149°41'14.71"E	206	~250	602.0 (299.3-957.6) <sup>2</sup>	217.9	33.43 <sup>5</sup>	19.56	19.23	5.23
<b>ACRI</b> 30°11'50.53"S, 149°36'32.07"E	203	~200	600.5 (280.4-1002.6) <sup>3</sup>	213.6	33.9 <sup>6</sup>	19.53	18.8	4.8



**Figure 3.2 Regional map of experiment field sites located at ACRI, Bellata and Terry Hie Hie, NSW; map is oriented north, distance Moree to Narrabri is 102 km.**

### 3.4.2 Field experiment design and plot management

All field experiments were constructed as a randomised complete block design using CycDesignN V6.0 (VSN, 2016) software; blocks were replicates, blocked down the field in the direction of the planted rows. The number of replicates, row configuration, plot size and seed bed varied with experiment and are presented in Table 3.4.

**Table 3.4 Field experiment replication and row, plot and seed bed configuration.**

Experiment number (name)	Reps	Row Configuration	Plot and Bed Dimensions
4. (2016.17 ACRI ERCF)	5	Single skip (66% area)	8 row x 10 m length, 1m raised beds
5. (2016.17 BTA ERF)	5	Double skip (50% area)	8 row x 13 m length, no-till, controlled traffic, 3m centres
6. (2016.17 ACRI GaBI)	4	Alternate (50% area)	8 rows x 10 m length, 1m raised beds
7. (2016.17 THH GaBI)	4	Double skip (50% area)	8 rows x 5 m length, no-till, controlled traffic, 3m centres
8. (2017.18 ACRI Resil.1)	6	Alternate (50% area)	8 rows x 10 m length, 1m raised beds
9. (2018.19 ACRI Resil.2b)	6	Alternate (50% area)	8 rows x 10 m length, 1m raised beds
10. (2018.19 BTA Resil)	6	Double skip (50% area)	8 row x 13 m length, no-till, controlled traffic, 3m centres
11. (2018.19 THH Resil)	6	Double skip (50% area)	8 rows x 5 m length, no-till, controlled traffic, 3m centres

### *Crop management*

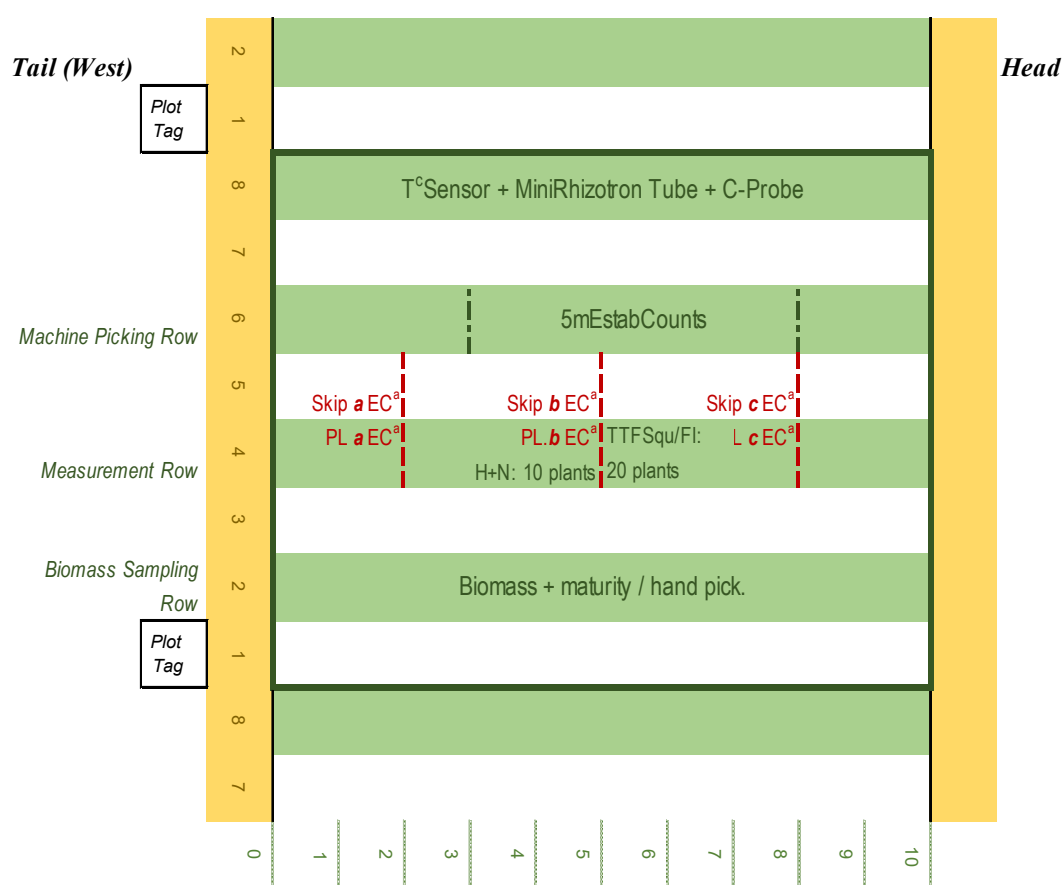
Establishment and management for all experiments followed current commercial practices (Hearn & Fitt, 1992). Planting occurred on full moisture profiles for all experiments, exceptions being experiments 10 (2018.19 BTA Resil) and 11 (2018.19 THH Resil), having 30% and 80% starting soil moisture profiles, respectively. Each experiment was managed individually with pest and weed control applied as required to maintain vigorous crop growth and reduce variation within the sampling area; per experiment, all replicates received the same management regime. Planting operations, supplementary irrigation and nutrition varied with experiment and are presented in Table 3.5.

**Table 3.5 Field experiment establishment and management protocol.**

Experiment number (name)	Planting operation		Planter	Supplementary irrigation	Nutrition
	Date	Rate (seeds.m <sup>-2</sup> )			
4. (2016.17 ACRI ERCF)	20/10/2016	13.6	Monosem NG Plus vacuum planter, twin disc openers, double rubber press wheels	Crop watered up post-plant pre-emergence, rain grown	180 kg N.ha <sup>-1</sup> , as 390 kg.ha <sup>-1</sup> prilled urea, preplant drilled into beds.
5. (2016.17 BTA ERF)	3/11/2016	10	JD MaxEmerge XP row units, cast steel press wheel, trash whippers, drag chain in loop, harrows	Rain grown	No pre plant or in crop fertiliser; residual N from prior wheat
6. (2016.17 ACRI GaBI)	21/11/2016	13.6	As per experiment 4. (2016.17 ACRI ERCF)	Crop watered up post-plant pre-emergence, rain grown	180 kg N.ha <sup>-1</sup> , as 390 kg.ha <sup>-1</sup> prilled urea, preplant drilled into beds.
7. (2016.17 THH GaBI)	21/10/2016	11	Kinze 2100 row units on tool bar, dawn row cleaners, precision planting seed firmer, double rubber press wheel, loop drag chain	Rain grown	Zn <sub>(aq)</sub> down slot at planting; residual N from prior wheat
8. (2017.18 ACRI Resil.1)	<i>Planting 1:</i> 19/10/2017 <i>Planting 2:</i> 7/12/2017	13.6	As per experiment 4. (2016.17 ACRI ERCF)	Pre-plant irrigation, 1 x supplementary irrigation at 86 DAP (13/1/18, early flowering)	100 kg N.ha <sup>-1</sup> , as 220 kg.ha <sup>-1</sup> prilled urea, preplant drilled into beds.
9. (2018.19 ACRI Resil.2b)	<i>Post hail replant:</i> 12/1/2019	13.6	As per experiment 4. (2016.17 ACRI ERCF)	Pre-plant irrigation, rain grown	100 kg N.ha <sup>-1</sup> , as 220 kg.ha <sup>-1</sup> prilled urea, preplant drilled into beds.
10. (2018.19 BTA Resil)	30/11/2018	10	As per experiment 5. (2016.17 BTA ERF)	Rain grown	No pre plant or in crop fertiliser; residual N from prior wheat
11. (2018.19 THH Resil)	13/9/2018	11	As per experiment 7. (2016.17 THH GaBI)	Rain grown	Zn <sub>(aq)</sub> down slot at planting; residual N from prior wheat

## Sampling

For all field experiments, non-destructive sampling occurred from plants growing on the inner two rows of the plot and from the inner 50% of row; destructive sampling for biomass and maturity harvests, and the placement of sensors and probes occurring on the opposite outer rows (Figure 3.3). Final machine harvests, where relevant, were also located on one of the inner two rows (Figure 3.3).



**Figure 3.3** Plan view of within plot sampling protocol for field experiments; scaled to 8 row by 10 m plot.

## Climate data sets

Complete meteorological data sets from fully serviced weather stations were measured near each field site and are presented in Table 3.6.

**Table 3.6 Weather station locations and proximal distance to specific field experiments.**

Experiment number (name)	Site, Coordinates, Operator	Proximity (km)
4. (2016.17 ACRI ERCF)	ACRI Chico (Myall Vale), 149.6 E / 30.2 S, CSIRO Agriculture and Food	1.8
5. (2016.17 BTA ERF)	Bellata Post Office (53003), 149.792 E / 29.918 S, Australian Bureau of Meteorology	10.3
6. (2016.17 ACRI GaBI)	ACRI Chico(Myall Vale), 149.6 E / 30.2 S, CSIRO Agriculture and Food	1.8
7. (2016.17 THH GaBI)	Terry Hie Hie (53039), 150.200 E / 29.8 S, Australian Bureau of Meteorology	8.9
8. (2017.18 ACRI Resil.1)	ACRI Chico(Myall Vale), 149.6 E / 30.2 S, CSIRO Agriculture and Food	1.4
9. (2018.19 ACRI Resil.2b)	ACRI Chico(Myall Vale), 149.6 E / 30.2 S, CSIRO Agriculture and Food	0.847
10. (2018.19 BTA Resil)	Dobikin Bellata, 149.7 E / 29.9 S, CSIRO Agriculture and Food	Located within experiment
11. (2018.19 THH Resil)	Terry Hie Hie (53039), 150.200 E / 29.8 S, Australian Bureau of Meteorology	9.2

### 3.4.3 Root growth measurements

#### *Core break sampling*

Timing (DAP) and plant growth stage of root biomass measurements in field experiments is detailed in Chapter 4, Table 4.2 and Chapter 5, Table 5.2. Binary presence or absence of root growth was determined at 15 cm, 30 cm, 60 cm and 90 cm depth, both under the plant line and adjacent skip row using the core-break method as detailed by Drew and Saker (1980), with all reps being sampled. Cores (0.50 m diameter) were sunk perpendicular to soil line using a tractor mounted hydraulic ram (ACRI experiments 4, 6, 8 and 9; Table 3.1) or 2-stroke petrol picket post driver (Terry Hie Hie and Bellata experiments 7, 11 and 5, 10, respectively; Table 3.1) and removed with an A-frame and block and tackle.

#### *Soil pits*

Post final defoliation in experiment 11 (2018.19 THH Resil) a tractor mounted hydraulic backhoe was utilised to dig soil pits (3 m length x 2 m depth) perpendicular to rows in the control (Trt.1) and Gibberellin<sub>4,7</sub> (Trt. 6) plots in replicate 3. Pits provided the opportunity to qualitatively evaluate root architecture (length and lateral root growth) as well as soil physical properties.

### 3.4.4 Soil moisture measurements

#### *Gravimetric*

Stratified gravimetric soil moisture ( $\text{g.g}^{-1}$ ) was quantified concurrently with core-break sampling for root growth. Following core-break partitioning for root growth, proportionate samples were weighed (wet weight, g), bagged and oven dried at  $105^\circ\text{C}$  for a minimum of 96 h. Samples were cooled for five minutes to enable handling and weighed again to obtain a corresponding dry weight (g). Gravimetric soil moisture ( $\text{g.g}^{-1}$ ) of the partitioned core was then calculated from;

$$\text{Soil moisture } (\text{g.g}^{-1}) = \frac{(\text{wet soil weight } (\text{g}) - \text{dry soil weight } (\text{g}))}{\text{dry soil weight } (\text{g})}$$

#### *Volumetric*

Cumulative volumetric soil moisture to depth (0.75 m and 1.5 m) was calculated from electromagnetic conductivity (EC) surveys, calibrated to specific field site volumetric soil moisture, as per Foley (2015) and Huth and Poulton (2007); survey timing being described in Chapter 4, Table 4.2 and Chapter 5, Table 5.2. EC surveys were conducted at six reference survey points in each plot (Figure 3.3); maintained consistently between survey dates at 2 m, 5m and 8m of row length on both the plant line of row 4 and adjacent skip (row 5) of each plot, as explained in Figure 3.3 (previous). Using an EM38 MkII (Geonics Ltd, Mississauga, ON, Canada) apparent EC ( $\text{EC}_a$ ) was recorded in both the horizontal and vertical positions on both 1.0 m and 0.5 m coils, providing four readings per survey point.

The three respective  $\text{EC}_a$  values per plot (i.e. 2 m, 5 m and 8 m) for plant line (PL) 1.0 m horizontal, 1.0 m vertical, 0.5 m horizontal and 0.5 m vertical readings were averaged to give a single “plant line” reading for each coil and dipole position (i.e. four readings in total); being replicated for the adjacent skip row. Mean EC readings were corrected for temperature as per location and survey date (Huth & Poulton, 2007) and cumulative volumetric soil moisture (mm) calculated to 1.5 m and 0.75 m depth using calibrations for the 1.0 m vertical and 0.5 m vertical EC readings, respectively. Resultant outcomes being mean plot cumulative soil moisture (mm) to 1.5 m and 0.75 m depth, for the plant line and adjacent skip row.

### 3.4.5 Treatment application

PGR foliar treatments were applied 30 cm above the plant line with a Ryobi 3.5 L hand-held battery-operated sprayer (flow rate: 8 mL.sec<sup>-1</sup>). Simulating a field application rate of 100 L.ha<sup>-1</sup>, applications were sprayed at slow walking pace (2.8 km.hr<sup>-1</sup>) to provide complete canopy coverage (viz. “to drip”). Applications were made during the early morning; facilitating a slow dry-down by avoiding hot, windy conditions and associated high evaporation rates.

## 3.5 Crop growth and development measurements

### 3.5.1 Growth

#### *Vegetative Growth Rate*

Heights and number of nodes above cotyledon of five representative non-damaged plants from each plot were measured weekly for six weeks (or as otherwise described in individual experiments) from the first PGR treatment spray application. The rate of internode increase, taken as mean treatment vegetative growth rate (cm/node), being calculated from;

$$VGR(\text{cm/node}) = \frac{\text{This week's height(cm)} - \text{Last week's height(cm)}}{\text{This week's node number} - \text{last week's node number}}$$

#### *Biomass partitioning and leaf area*

Per specified dates, plants from one linear meter of row were harvested out of all reps for each treatment, by cutting with secateurs directly below the cotyledons. For each rep, a total sample plant count and fresh biomass weight was taken, four plants sub-sampled and a subsequent four-plant wet weight obtained. Harvested four-plants were separated into pooled laminae, stems (including petioles), squares, green bolls (flowers and non-open bolls) and open bolls (two sutures on the boll dehisced); the number of each fruit type was recorded. Total Leaf Area (LA) of the four-plant sub sample was measured using a Li-Cor planimeter (LI-3100A, LI-COR Inc., Lincoln, NE, USA). Harvested samples were oven dried at 80°C for a minimum 48 h and weighed to obtain dry biomass weights. Four-plant sub sample partitioned dry weights and leaf area, were then converted to 1 m<sup>2</sup> dry weight equivalents and Leaf Area Index (LAI), respectively, on a proportionate weight basis with consideration to experiment row configuration. Mean square, green boll and open boll weights were extrapolated from fruit counts (m<sup>2</sup>) and biomass dry weights (g).

### **3.5.2 Development**

#### ***Time to First Square and first flower***

Progression of the control (Trt.1) treatments to 50% population first square and first flower was monitored weekly in all reps. Ten row-consecutive, representative, undamaged plants from each plot were tagged and the date and node location of the emergence of the first square and flower on each plant documented. Plot “first square” and “first flower” in days after planting, was denoted as the date when 50 % of plants had a recorded square or flower, respectively.

#### ***Nodes Above White Flower***

Physiological cut out, where assimilate requirements exceed supply, resulting in the cessation in development of reproductive sites, was taken as four nodes above a first position one day old (white) flower (Hearn & Constable, 1984a). Differences in PGR treatment progression to cut out was quantified via counting the number of nodes above first position white flowers to the apical shoot meristem, on five randomly selected plants per plot; sampling occurred weekly with all reps being assessed.

### **3.6 Yield and yield component measurements**

#### **3.6.1 Fruit mapping and retention**

Schematic mapping of fruit location and development occurred with each biomass harvest. Utilising the four-plant sub sample; the number of nodes, position of vegetative and sympodial fruiting branches, as well as number and positions of pinhead squares, squares, flowers, green bolls, open bolls and aborted sites, were recorded. In addition to total fruit numbers and mean weights calculated during biomass partitioning, square meter basis numbers of shed sites, total sites and retention were calculated. Early squaring retention percentages (as in Chapter 6) being calculated as the total number of squares divided by the total number of fruiting sites; fruiting site totals incorporating both shed sites and sites with squares, but not pinhead squares. Final (maturity) retention percentage calculations being determined from open boll counts relative to total fruiting sites; total sites comprising of shed / aborted sites, immature bolls and open bolls.

### **3.6.2 Maturity and lint yield**

As per individual experiment descriptions, lint yield was quantified by either hand harvesting all open bolls from a 2 m<sup>2</sup> subsection of each plot and / or machine harvesting an entire row (10 m ACRI, 15 m Bellata) with a spindle picker (modified Case International 1822), then manually weighing to obtain a seed cotton sample. Where specified, yield components and days after planting to maturity (taken as 60% open boll) were quantified through weekly hand harvests from first cracked boll (being taken as when two sutures on the boll dehisce); with open boll numbers and seed cotton weights being sequentially tallied and resultant mean open boll size extrapolated.

### **3.6.3 Ginning and quality parameters**

All seed cotton samples were weighed, ginned in a 10-saw gin (Continental Eagle Corp, Prattville, AL, USA) and resulting proportionate weights of lint, seed and trash samples obtained; total yield (g.m<sup>-2</sup> or T.ha<sup>-1</sup>) being deduced from the ginned lint weight. All lint samples were analysed for lint quality (fibre length, strength and micronaire) on a high-volume instrument (HVI) (Uster HVI 1000, Uster Technologies AG, Uster, Switzerland).

### **3.7 Crop simulation modelling**

The OZCOT crop simulation model (Hearn, 1994) was employed to validate location-based time of planting impacts on yield (Chapter 6) and to demonstrate potential system benefits from established PGR treatment responses (Chapter 7); specific parameters of such being detailed in respective chapters. OZCOT is a dynamic crop simulation model of cotton growth, development and yield; potential yield being the primary modelled output (Conaty et al., 2018). The structure and validation of the OZCOT model is detailed by Hearn (1994).

OZCOT uses a daily time-step with growth and development driven by air temperature and intercepted radiation, being modified by soil water and nitrogen status (Bange et al., 2005). Central to the model is the fruit production and survival subroutine (Hearn & da Roza, 1985); the rate of fruit production, shedding and growth being determined by carbon supply (Conaty et al., 2018). Daily carbon supply is estimated from light interception and crop level carbon assimilation rate, adjusted for respiration rates (Conaty et al., 2018). Light interception is estimated using Beer's Law (Monsi & Saeki, 2005) and leaf area is

generated using a correlation between fruiting site production and leaf area (B. S. Jackson, Arkin, & Hearn, 1988). The rate of photosynthesis, leaf expansion and fruiting is modified by ‘stress indices’ which scale the rate of these processes in relation to nitrogen supply and water availability, including the effects of waterlogging (Conaty et al., 2018). The moisture extraction routine is based on increasing supply with increasing extraction depth over time, using the Ritchie water balance approach (Ritchie, 1972).

The OZCOT model has been demonstrated to accurately simulate Australian rain grown cotton yield with respect to nitrogen availability, planting date and row configuration; accounting for 70% of measured yield variation (across 30 crops) with discrepancies being explained via impacts of factors not addressed by model (e.g. severe pest damage) (Bange et al., 2005; Hearn, 1994). Consequently, the model was utilised to develop rain grown production projections in the major Australian cotton growing regions (Hearn, 1990) and benchmark the performance of commercial crops (P.S. Carberry, Hochman, McCown, Dalgliesh, & Foale, 2002). Modification to accommodate skip row configurations also enabled production risk assessment associated with specific rain grown system queries, including row configuration selection and the impacts of climate variability (Bange & Carberry, 1998; P. S. Carberry, Hammer, Meinke, & Bange, 2000). Simulations using OZCOT were made available to industry through the ‘Whopper Cropper’ software initiative (Hammer et al., 2001) and is the cotton module of APSIM ([www.Apsim.info](http://www.Apsim.info)).

The model requires site related (weather data, soil characteristics and starting soil moisture), agronomic (planting date, row configuration, plant stand, irrigation dates and defoliation date), and genotype inputs (Hearn, 1994). The model does not account for the effects of; insect pests, diseases, weeds, management failures and soil nutrient limitations other than nitrogen (Conaty et al., 2018). Of relevance in a rain grown context, the model does not simulate the effects of climate and management on lint quality (Conaty et al., 2018).

### **3.8 Data analysis**

Data for all experiments was statistically analysed using GenStat v16.0 (VSN, 2017) software at the  $P=0.05$  level of significance. Following one-way Analysis of Variance (ANOVA) assessment in randomised blocks; respective treatment means were compared with the control using Tukey’s HSD test to account for experiment-wise type I error rate. Alternatively, where applicable, two-sided, two sample t-tests (one variate with treatment as a group factor) were utilised for direct comparison with the control (Trt.1). Pearson

product-moment correlation coefficients (Pearson's  $r$ ) were computed to assess the relationship between variables. Generalised linear regression analysis was utilised to determine treatment differences from the control in progression to physiological cut out and maturity (as 60% open boll). Detailed application of specific analysis methods being noted in each chapter.

## **Chapter 4 “Exploiting the Skip”: Adenine-type Cytokinin Plant Growth Regulator, 6-Benzylaminopurine, to Improve Access to Soil Water Reserves**

### **4.1 Introduction**

Optimising yield outcomes in rain grown cotton systems is contingent on both maximising soil water reserves via infiltration and storage of rainfall in the soil profile, as well as subsequent effective extraction for crop growth (Passioura & Angus, 2010). Correlation exists between root system size and crop resilience in limited water scenarios (Werner et al., 2010); a larger root system being advantageous in facilitating the absorption of water in deep soil profile layers (Fukai & Cooper, 1995; Yue et al., 2006). Resultantly, the rationale behind skip row planting configurations can be used to develop PGR based adaptive management strategies, i.e. optimising root system architecture to facilitate increased access to such soil water reserves. Foreseeably providing growth and subsequent yield and lint quality advantages under water-limited rain grown settings (Rogers & Benfey, 2014; Werner et al., 2010). On this basis, understanding cotton plant and crop responses to specific Adenine-type Cytokinin treatment scenarios, can potentially enable the development of a framework criteria for strategic applications within Australian rain grown cotton systems that manage climate risk by increasing plant and crop access to soil water resources.

Enhanced utilisation of stored soil water reserves, and any associated crop growth and yield benefits, are closely related to both the root space available and critically the penetration and proliferation of the plant root system within that space (Hulugalle et al., 2015; Luo et al., 2015). Specialised skip row planting configurations that omit entire rows, are currently utilised in Australian rain grown cotton production systems as an agronomic management strategy to mitigate climate risk (Bange et al., 2005; Payero et al., 2012). Skip row configurations increase plant access to soil moisture reserves adjacent to the plant line by augmenting the cotton plant root growth area (root space); providing a buffer to the occurrence and impact of water deficit stress (notably in drier seasons) and when seasonal conditions permit, enabling extended crop growth for potential yield and lint quality benefits (Bange et al., 2005; Grundy & Klepper, 2016; Payero et al., 2012).

Plant water extraction from such soil reserves is dependent on the spatial exploration of the soil by the root system; although associated plant metabolic costs can be significant (Lynch, 2015). Stored soil moisture at depth provides a more reliable, but somewhat inaccessible, source of water than in-crop rainfall (Wasson et al., 2012); being advantageously utilised

toward the end of the crop cycle for increasing carbohydrate supply to the fruiting bodies (Condon, Richards, & Farquhar, 1993). Research by Goyne (2005) indicated limitations in skip row water extraction patterns in Australian Vertosol cotton production soils. Extraction commenced in and close to the plant line, prior to the middle of the skip, where its (later season) use down the profile was rapid to a mean depth of 120 cm. Water use at depth in the skip row was last to be extracted, with a higher end of season (residual) soil water content comparative to the plant line (Goyne, 2005). Manipulating root system architecture (RSA) towards a distribution of roots in the soil that optimises water access and uptake (e.g. facilitates absorption of water in deep soil layers), can increase the total amount of water available to the plant and reduce the length of time where the crops are in water-deficit stressed conditions, notably post-anthesis (Dorlodot et al., 2007; Wasson et al., 2012).

Plant phytohormones are significant endogenous regulators of cotton root system architecture (Osmont, Sibout, & Hardtke, 2007); being influenced by abiotic factors such as soil strength and moisture status (Bengough et al., 2005), temperature and volatile organic compounds emitted from proximate plants, via modulation of hormone homeostasis or signalling (Osmont et al., 2007). Early vegetative growth stage foliar applications of N6-substituted synthetic Adenine-type Cytokinin PGR, 6-Benzylaminopurine (6BA), were shown to promote adventitious root system architecture under water limited, rain grown international production environments in the high plains of Texas (Burke, 2011, 2013). However, a scarcity of detailed information exists regarding cotton plant (and notably RSA) responses to exogenous Adenine-type Cytokinin applications, under rain grown production and notably vertic, skip row soil contexts. Consequently, an opportunity exists to leverage research from international limited water cotton systems, to evaluate function and suitability in skip row configurations on vertosol soils, under rain grown production settings.

This chapter details a series of glasshouse and field experiments, conducted to determine the capability of Adenine-type Cytokinin, 6-Benzylaminopurine, to increase root biomass in early vegetative stage cotton plants, with the objective of increasing plant access to soil water reserves. Specifically, these experiments aimed to:

- Quantify the impact of specific Adenine-type Cytokinin PGR treatment choice, rate, application type, frequency and timing combinations, on plant root biomass accumulation and access to soil water reserves; and

- Understand the resulting morphological plant and crop level responses to treatments, as explained by; yield, lint quality, biomass and leaf area accumulation and fruit development, retention and maturity outcomes.

Specifically, the hypothesis tested was;

H<sub>0</sub>: Adenine-type Cytokinin 6BA applied in early vegetative growth stage, will induce no detectable plant phenological response on plant root biomass accumulation and consequent access to soil water reserves.

## **4.2 Materials and methods**

To test aims and respond to the null hypothesis, experiments across three production seasons were structured based on rationale (Table 4.1). Preliminary screening occurred under controlled environment conditions in experiments 1 and 2. Appraisal of a recurring (as opposed to single dose) treatment application methodology, occurred during season one in field experiments 4 and 5. Dosage assessments occurred in field experiment 8 during season two. Field experiments 9 and 10 provided the final concept evaluation in season three.

### **4.2.1 Site description**

To enable hypothesis assessment of 6BA treatments on rain grown cotton plants, seven different cotton experiments were undertaken under both controlled environment (spring 2016) and rain grown field conditions across multiple locations during the 2016-17, 2017-18 and 2018-19 cotton seasons. Specific site locations, climatic conditions, experimental design, crop and plot management protocols are detailed in Chapter 3, Sections 3.4.1 and 3.4.2.

### **4.2.2 Treatments**

For the purpose of this analysis, treatments utilised per experiment are detailed in Table 4.1; with full nomenclature, structural descriptions and proprietary sources being provided in Appendix 1.

**Table 4.1 Treatments per experiment, as included in chapter 4 analysis; preliminary screening under controlled environment conditions in experiments 1 and 2, appraisal of triplicate application methodology in field experiments 4 and 5, dosage assessment in field experiment 8 and concept evaluation in field experiments 9 and 10.**

<b>Exp. No., name</b>	<b>Trt. No.</b>	<b>Compound</b>	<b>Conc. (mg.L<sup>-1</sup>)</b>	<b>Application</b>	<b>Timing</b>	<b>Application Frequency</b>
<b>Experiment 1. (GH Seed Ckga)</b>						
	1	Untreated control				
	4	6BA	0.01	Seed prime, 20 h submerge	Seed	Once
	5	6BA	850.00	Seed prime, 10 sec. submerge	Seed	Once
	6	6BA	9.97 mg a.i./cwt.	Seed coating	Seed	Once
	7	6BA	49.99 mg a.i./cwt.	Seed coating	Seed	Once
<b>Experiment 2. (GH Foliar Ckga)</b>						
	1	Untreated control				
	2	6BA	150.00	Foliar, to drip	2 leaf	Once
	3	6BA	25.00	Foliar, to drip	2 leaf	Once
	4	6BA	33.00	Foliar, to drip	2 leaf	Once
	5	6BA	67.00	Foliar, to drip	2 leaf	Once
<b>Experiment 4. (2016.17 ACRI ERCF)</b>						
	1	Untreated control, water spray only				
	2	6BA	25.00	Foliar, to drip	4 leaf	Once
	3	6BA	25.00	Foliar, to drip	4 leaf	3 x 10 d
<b>Experiment 5. (2016.17 BTA ERF)</b>						
	1	Untreated control, water spray only				
	2	6BA	25.00	Foliar, to drip	4 leaf	Once
	3	6BA	25.00	Foliar, to drip	4 leaf	3 x 10 d
<b>Experiment 8. (2017.18 ACRI Resil. 1)</b>						
	1	Untreated control, water spray only				
	3	6BA	100.00	Foliar, to drip	4 leaf	3 x 10 d
	4	6BA	25.00	Foliar, to drip	4 leaf	3 x 10 d
<b>Experiment 9. (2018.19 ACRI Resil. 2b)</b>						
	1	Untreated control, water spray only				
	2	6BA	25.00	Foliar, to drip	4 leaf	Once
	3	6BA	25.00	Foliar, to drip	4 leaf	3 x 10 d
<b>Experiment 10. (2018.19 BTA Resil)</b>						
	1	Untreated control, water spray only				
	2	6BA	25.00	Foliar, to drip	4 leaf	3 x 10 d

### 4.2.3 Measurements

Quantification of cotton plant response to PGR treatment applications varied contingent on individual experiment rationale (Table 4.1). Crop measurements per experiment are described in Table 4.2, with referenced sampling and processing methodologies being detailed in Chapter 3, Sections; 3.4.2 plot sampling protocol, 3.4.3 soil moisture (EC<sub>v</sub>, volumetric, gravimetric), 3.4.4 root growth (core break sampling), 3.5.1 growth (biomass, leaf area), 3.6.1 fruit mapping and retention, 3.6.2 maturity, 3.6.3 lint yield (hand and machine harvests), 3.6.4 ginning and 3.6.5 quality parameters.

**Table 4.2 Details of plant and crop measurements taken per experiment as included in Chapter 4 analysis; all replicates being sampled.**

Exp	Root growth		Soil moisture		Growth and development (above ground)					Yield components		Yield		Quality
	Biomass	Coreface	Soil Cores	EC <sub>a</sub>	VGR	Biomass	Leaf area	Fruit retention	Maturity	Seed cotton	OB no. and size	Lint	Ginning	HVI
1.	13 DAP	-	-	-	Early vegetative, 13 DAP									
2.	24 DAP	-	-	-	Early vegetative, 24 DAP									
4.	-	Post harvest, 237 DAP	-	-	>25 DAP 10-day intv. 6 weeks	Mid flowering, 124 DAP			-	1 m <sup>2</sup> hand harvest, 182 DAP	10 m machine harvest, 231 DAP			
5.	-	-	-	-	>25 DAP 10-day intv. 6 weeks	Mid flowering, 113 DAP			-	1 m <sup>2</sup> hand harvest, 152 DAP	15 m machine harvest, 181 DAP			
8.	-	-	-	78 DAP (FF), 110 DAP (mid flowering)	>25 DAP 10-day intv. 12 weeks	55 DAP (FSqu), 81 DAP (FF), 116 DAP (mid flowering)			Maturity 1 m <sup>2</sup> hand harvests from 134 DAP, 10-day intervals		10 m machine harvest, 210 DAP	Obtained from machine harvest		
9.	-	Mid flowering, 108 DAP <sup>(1)</sup>	-	109 DAP (mid flowering)	>25 DAP 10-day intv. 6 weeks	44 DAP (FSqu), 66 DAP (FF)								
10.	-	-	-	-	>25 DAP 10-day intv. 6 weeks	66 DAP (FF)								

<sup>(1)</sup> Earlier (first flower) attempts to draw cores were unsuccessful due to wet sub soil conditions.

#### 4.2.4 Data analysis

One-way analysis of variance (ANOVA) with Tukey's post-hoc HSD test was used to determine treatment differences in:

- Root length, root dry weight and Root:Shoot (Rt:Sht) ratio in experiments 1 and 2.
- Height, leaf dry weight, stem dry weight and total dry weight in experiments 1 and 2.
- Total dry weight, LAI and proportionate reproductive biomass (Fr:Tot); at early squaring in experiments 8 and 9, at early flowering in experiments 8, 9 and 10, at mid-flowering in experiments 4, 5 and 8.
- Total number of fruiting sites, square counts and mean weights, total fruit retention; early squaring in experiments 8 and 9.
- Square and green boll counts and mean weights; early flowering in experiment 8, 9 and 10.
- Square, green and open boll counts and mean weights; mid-flowering in experiment 4 and 5 and 8.
- Final open boll counts and mean weight; experiment 4 and 5, and 8.
- Lint yield, turnout; experiment 4, 5 and 8.
- Lint length, strength and micronaire; experiment 4, 5 and 8.
- Volumetric soil moisture, cumulative to depth; early and mid-flowering in experiment 8.
- Mean weighted total apparent bulk soil electrical conductivity (EC<sub>t</sub>) to depth, mid flowering in experiment 9.

A two-sided T-test (two sample, one variate with group factor) was utilised to determine treatment differences in stratified gravimetric soil moisture in experiments 4 and 9. Generalised linear regression was utilised to determine treatment differences from the control in experiment 8 for progression to maturity (60% open boll). Methodologies of data analysis referenced are detailed in Chapter 3, Section 8.

#### 4.3 Results

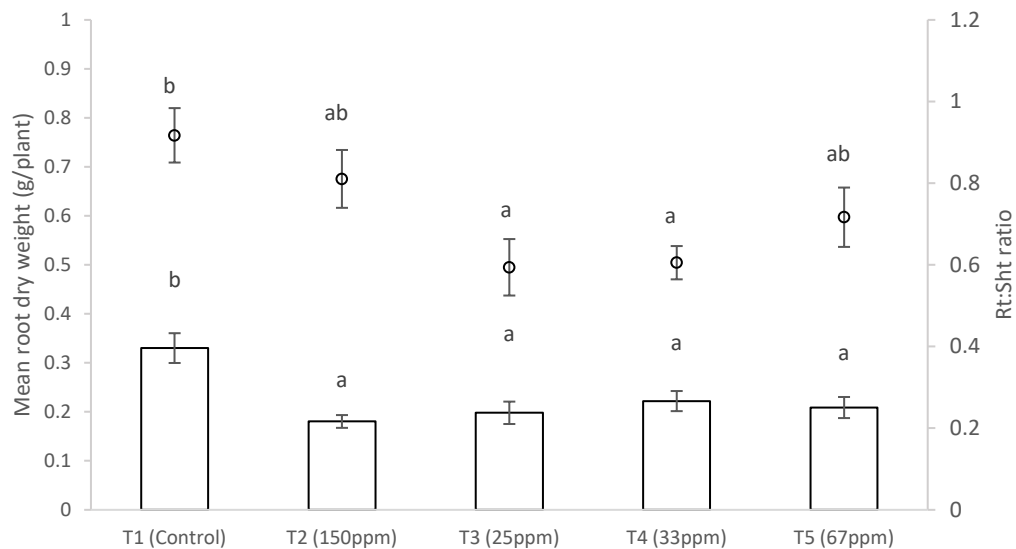
Presentation of results across all experiments have been organised as primary PGR treatment impacts on root biomass accumulation and access to soil water reserves and secondly, quantification of any consequential crop and plant growth responses.

### 4.3.1 Glasshouse preliminary screening; experiments 1 and 2

Experiments 1 and 2 were conducted under glass house conditions as short-duration preliminary screening investigations, to evaluate varying concentrations of seed coating and seed priming (experiment 1), as well as foliar (experiment 2) 6BA treatment applications.

#### *Promoting plant root biomass accumulation*

Experiment 1 (seed coating / priming) was terminated with a biomass harvest at 13 days after planting (DAP) and demonstrated no treatment differences from the control (Trt.1) in root length ( $P=0.487$ ), root dry weight ( $P=0.159$ ) or the ratio of root dry weight to shoot dry weight (Rt:Sht ratio) ( $P=0.359$ ). Experiment 2 (foliar) was terminated with a biomass harvest at 24 DAP (8 days post treatment) and showed no treatment differences from the control (Trt.1) in root length ( $P=0.947$ ). However, root dry weights ( $P<0.001$ ) of all 6BA treatments were less than the control; Rt:Sht ratios of treatments 3 and 4 were also less than the control (Trt.1) ( $P=0.004$ ) (Figure 4.1).

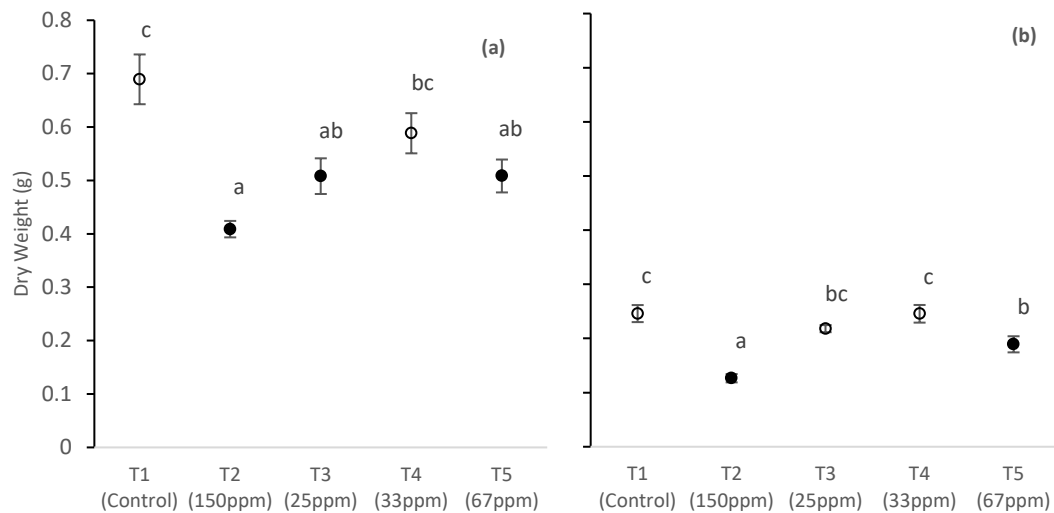


**Figure 4.1 Mean root dry weight (g)(bar) and Root:Shoot (Rt:Sht) ratio (○) at 24 DAP for control (Trt.1) and foliar 6BA treatments 2 to 5, grown under glasshouse conditions in experiment 2; notation indicating treatment differences at the  $P<0.05$  level using Tukey's HSD, error bars are  $\pm 1$  standard error of the mean.**

#### *Plant and crop growth responses*

In experiment 1, no treatment differences from the control (Trt.1) were evident for seedling height ( $P=0.222$ ), stem dry weight ( $P=0.040$ ), leaf dry weight ( $P=0.269$ ) or total dry weight

( $P=0.057$ ). In experiment 2, no treatment differences from the control (Trt.1) occurred for height ( $P=0.001$ ) or stem dry weight ( $P=0.200$ ). Leaf dry weight for treatments 2 and 5, and total dry weight for treatments 2, 3 and 5 were less than the control (Trt.1) in experiment 2 (Figure 4.2).



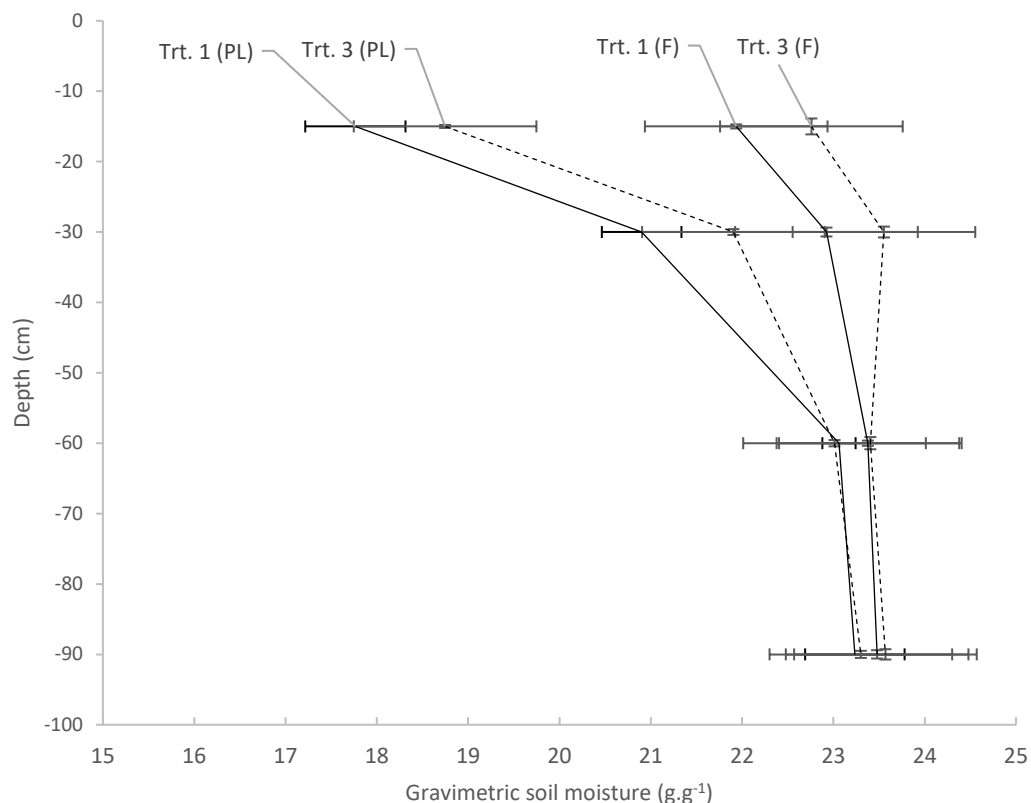
**Figure 4.2. Mean total dry weight (a) and leaf dry weights (b) at 24 DAP for control (Trt.1) and foliar 6BA treatments 2 to 5, grown under glasshouse conditions in experiment 2; notation indicating treatment differences at the  $P<0.005$  level using Tukey's HSD, solid markers indicating treatment differences from the control, error bars are  $\pm 1$  standard error of the mean.**

#### 4.3.2 Evaluation of application methodology; season one field experiments 4 and 5

Experiments 4 and 5 were conducted over the 2016/17 summer production season, located at the ACRI and Bellata sites, respectively; both experiments aimed to evaluate multiple foliar 6BA treatment applications.

##### *Plant root biomass accumulation and access to soil water reserves*

End of season core break sampling in experiment 4 for the binary presence / absence of cotton roots, demonstrated no differences in root growth between the control (Trt.1) and treatment 3; new-season cotton roots being detected at all depths in both the plant line and furrow, for each replicate in both treatments (Trt. 1 and 3). Corresponding gravimetric soil moisture of stratified core samples also showed no differences between the control (Trt.1) and treatment 3, under both the plant line and adjacent furrow, to depth (Figure 4.3, Table 4.3). However, although there were no significant treatment differences, there was a tendency for the 6BA treatment to be wetter across notably the shallower depth profiles (0-60 cm) (Figure 4.3).



**Figure 4.3.** Mean end of season gravimetric soil moisture per location and depth at 237 DAP in experiment 4; control (Trt.1) (—), 6BA treatment 3 (- - -), notation delineating plant line (PL) and furrow (F) locations, error bars being +/- 1 standard error of the mean.

**Table 4.3.** T-test P values for comparison of control (Trt.1) and treatment 3 mean gravimetric soil moisture content per depth and row location, at 237 DAP in experiment 4.

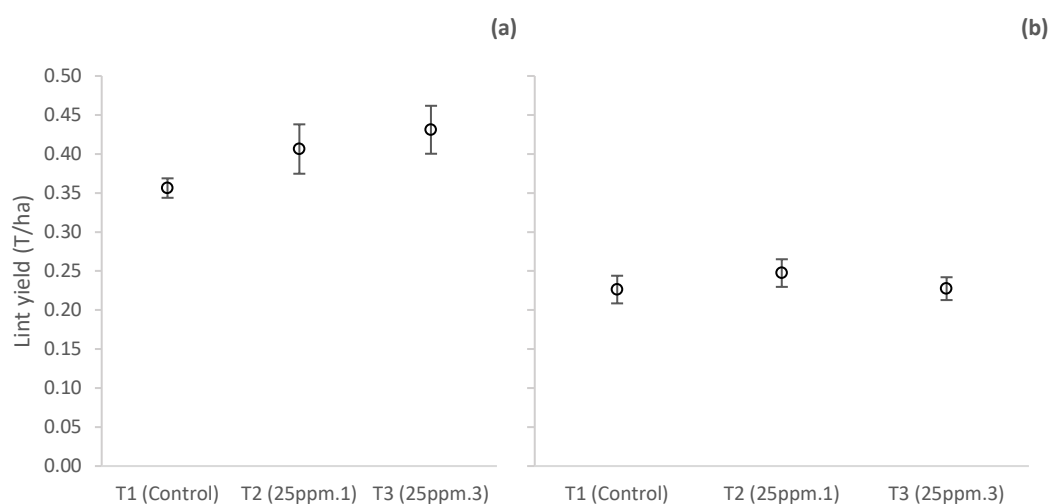
Plant Line	P value	Furrow	P value
15 cm	0.133	15 cm	0.516
30 cm	0.132	30 cm	0.545
60 cm	0.922	60 cm	0.978
90 cm	0.932	90 cm	0.926

### ***Plant and crop growth responses***

Mid-flowering biomass harvest at 124 DAP in experiment 4, demonstrated no treatment differences from the control (Trt.1) for total dry weight (P=0.147), LAI (P=0.169) or the reproductive biomass dry weight proportionate to total dry mater (Fr:Tot) (P=0.945). Moreover, all treatments maintained comparable fruit loads and size, with no treatment differences evident from the control (Trt.1) for mean numbers of squares (P=0.513), green bolls (P=0.522), open bolls (P=0.958), or mean square (P=0.975), green boll (P=0.503) or

open boll ( $P=0.958$ ) weights. Similarly, in experiment 5, mid-flowering biomass harvest at 113 DAP demonstrated no treatment differences from the control (Trt.1) for total dry weight ( $P=0.108$ ), LAI ( $P=0.242$ ) or Fr:Tot ( $P=0.367$ ). Likewise, no differences from the control (Trt.1) in green boll number ( $P=0.242$ ), green boll mean weight ( $P=0.331$ ), open boll number ( $P=0.470$ ) or open boll mean weight ( $P=0.453$ ), were evident.

Consequently, no treatment differences occurred for final open boll numbers ( $m^2$ ) or mean boll weights(g) from hand harvests in both experiment 4 (OB.No;  $P=0.533$ , OB.Size;  $P=0.638$ ) and 5 (OB.No;  $P=0.666$ , OB.Size;  $P=0.388$ ). Both experiments were machine harvested; no treatment differences in lint yield ( $T.ha^{-1}$ ) occurred in either experiment 4 ( $P=0.191$ ) or 5 ( $P=0.222$ ) (Figure 4.4).



**Figure 4.4.** Mean lint yield ( $T.ha^{-1}$ ) of control (Trt.1) and 6BA PGR treatments 2 and 3 in field experiments (a) 4 and (b) 5; error bars are  $\pm 1$  standard error of the mean.

No treatment differences from the control (Trt.1) were found for seed cotton turnout or lint quality parameters in either experiment (Table 4.4).

**Table 4.4.** ANOVA F test  $P$  values for comparison of treatment mean seed cotton turnout (%), lint length, lint strength and micronaire outcomes in field experiments 4 and 5.

	<i>Experiment 4.</i> <i>ACRI ERCF)</i>	<i>(2016.17</i> <i>Experiment 5.</i> <i>BTA ERF)</i>	<i>(2016.17</i>
Seed cotton turnout (%)	$P=0.318$	$P=0.045$	
Lint length	$P=0.567$	$P=0.251$	
Lint strength	$P=0.842$	$P=0.145$	
Micronaire	$P=0.448$	$P=0.037$	

### 4.3.3 6-Benzylaminopurine dosage assessment; season two field experiment 8

Experiment 8 was conducted over the 2017/18 summer production season at the ACRI field site and aimed to evaluate dosage manipulation in a repeated application treatment scenario.

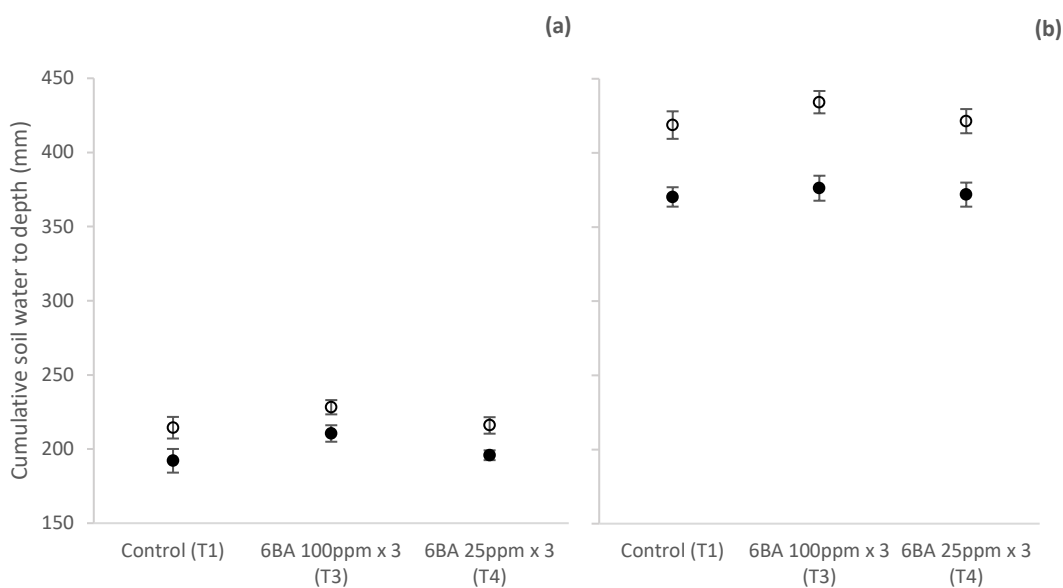
#### *Plant root biomass accumulation and access to soil water reserves*

Mini-rhizotron tubes were installed at 10 DAP in the plant line and skip row of replicates 2,3,4 of the control (Trt.1) and treatments 3 and 4, with the aim of quantifying root growth across the season. Owing to the shrink-swell properties of the Vertosol soil at the experiment site, decreasing soil moisture as the season progressed caused the soil to recede away from the surface of the rhizotron tube and condensation to accumulate in the resultant space between the soil surface and tube. Subsequent camera inspections of root growth at first square (55 DAP) and thereafter, were unsuccessful due to condensation droplets on the outside of the tube surface obscuring imagery capture. Consequently, differences in cumulative soil moisture to depth (mm) both under the plant line and the skip row, were utilised as a proxy for root growth (Wasson et al, 2012); being quantified across the season through electromagnetic induction calibrated to volumetric soil moisture, as detailed in chapter 3, section 4.3.

Analysis of cumulative soil moisture under both the plant line and skip row at early flowering (78 DAP) directly prior to an emergency irrigation (Chapter 3, Section 4.2, Table 3.5), showed no treatment differences from the control (Trt.1) in early season water extraction from the profile to both 0.75 m and 1.5 m depth (Table 4.5, Figure 4.5).

**Table 4.5 Mean cumulative soil moisture (mm) to 0.75 m and 1.5 m depth, under both the plant line and skip row, for 6BA PGR treatments at early flowering (78 DAP) in experiment 8; no significant treatment differences occurred at the P=0.05 level using Tukey's HSD.**

	0.75 m, plant line	0.75 m, skip row	1.5 m, plant line	1.5 m, skip row
	( <i>P</i> =0.081)	( <i>P</i> =0.323)	( <i>P</i> =0.862)	( <i>P</i> =0.600)
Control (Trt.1)	192.1	214.4	370.3 <sup>(a)</sup>	418.8 <sup>(a)</sup>
6BA 100 ppm (Trt. 3)	210.5	228.2	376.2 <sup>(a)</sup>	434.2 <sup>(a)</sup>
6BA 25 ppm (Trt. 4)	195.8	215.9	371.9 <sup>(a)</sup>	421.4 <sup>(a)</sup>

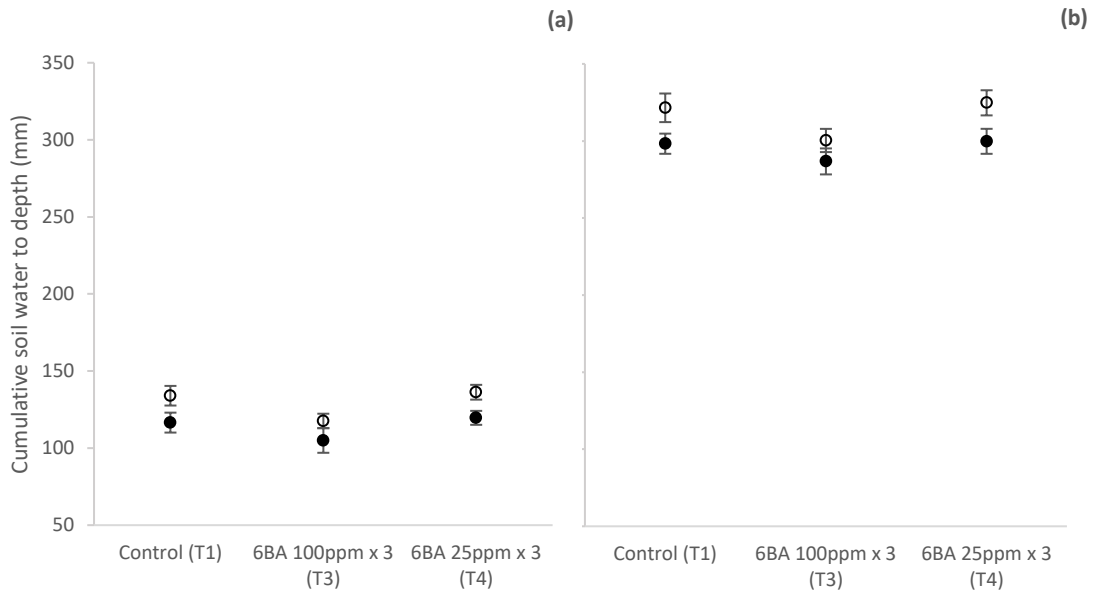


**Figure 4.5. Mean cumulative soil water (mm) to 0.75 m (a) and 1.5 m (b) at early flowering (78 DAP) for control (Trt.1) and 6BA treatments 3 and 4 in experiment 8; plant line (●), skip row (○), error bars being +/- 1 standard error of the mean.**

Cumulative soil moisture at mid flowering (110 DAP, first cracked boll) under both the plant line and skip row showed no treatment differences from the control (Trt.1) in water extraction during reproductive growth from the profile, to both 0.75 m and 1.5 m depth (Table 4.6, Figure 4.6).

**Table 4.6 Mean cumulative soil moisture (mm) to 0.75 m and 1.5 m depth, under both the plant line and skip row, for 6BA PGR treatments at mid-flowering (110 DAP) in experiment 8; no significant treatment differences occurred at the P=0.05 level using Tukey's HSD.**

	0.75 m, plant line	0.75 m, skip row	1.5 m, plant line	1.5 m, skip row
	<i>(P=0.081)</i>	<i>(P=0.058)</i>	<i>(P=0.464)</i>	<i>(P=0.107)</i>
Control (Trt.1)	192	214	298	321
6BA 100 ppm (Trt. 3)	210	228	286	300
6BA 25 ppm (Trt. 4)	195	215	299	324



**Figure 4.6. Mean cumulative soil water (mm) to 0.75 m (a) and 1.5 m (b) at mid flowering (110 DAP) for 6BA PGR treatments in experiment 8; plant line ( ● ), skip row ( ○ ), error bars being +/- 1 standard error of the mean.**

#### ***Plant and crop growth responses***

Early squaring, early and mid-flowering biomass harvests to assess growth and development against the control, were cut for all treatments in experiment 8 within 7 days of 50% population first square (“early squaring”, 55 DAP), first flower (“early flowering”, 81 DAP) and first cracked boll (“mid flowering”, 116 DAP) . No treatment differences from the control (Trt.1) were evident in total dry weight or LAI across all sampling dates (Table 4.7). Treatment 3 proportionate reproductive growth to total biomass was less than the control (Trt.1) at both early and mid-flowering, with no other treatment impacts on Fr:Tot being evident across all sampling dates (Table 4.7).

**Table 4.7 Biomass development effects of 6BA PGR treatments at (a) early squaring 55 DAP, (b) early flowering 81 DAP and (c) mid flowering 116 DAP in experiment 8, as characterised by total dry weight accumulation ( $\text{g}\cdot\text{m}^{-2}$ ), leaf area index (LAI) and reproductive biomass proportionate to total dry weight accumulation (Fr:Tot); notation indicating treatment differences at the  $P=0.05$  level using Tukey's HSD.**

	(a) Early squaring	(b) Early flowering	(c) Mid flowering
<b>Total dry weight (<math>\text{g}\cdot\text{m}^{-2}</math>)</b>			
	( $P=0.462$ )	( $P=0.095$ )	( $P=0.440$ )
Control (Trt.1)	71.81 <sup>(a)</sup>	510.20 <sup>(a)</sup>	1640.00 <sup>(a)</sup>
6BA.3x100 ppm (Trt.3)	64.38 <sup>(a)</sup>	445.50 <sup>(a)</sup>	1526.00 <sup>(a)</sup>
6BA.3x25 ppm (Trt.4)	73.75 <sup>(a)</sup>	473.10 <sup>(a)</sup>	1718.00 <sup>(a)</sup>
<b>Leaf Area Index (LAI)</b>			
	( $P=0.306$ )	( $P=0.531$ )	( $P=0.231$ )
Control (Trt.1)	0.4841 <sup>(a)</sup>	3.031 <sup>(a)</sup>	4.605 <sup>(a)</sup>
6BA.3x100 ppm (Trt.3)	0.4379 <sup>(a)</sup>	3.329 <sup>(a)</sup>	5.378 <sup>(a)</sup>
6BA.3x25 ppm (Trt.4)	0.5325 <sup>(a)</sup>	3.236 <sup>(a)</sup>	5.313 <sup>(a)</sup>
<b>Reproductive biomass / total dry matter (Fr:Tot)</b>			
	( $P=0.732$ )	( $P=0.019$ )	( $P=0.001$ )
Control (Trt.1)	0.0394 <sup>(a)</sup>	0.1502 <sup>(b)</sup>	0.5368 <sup>(b)</sup>
6BA.3x100 ppm (Trt.3)	0.0329 <sup>(a)</sup>	0.0663 <sup>(a)</sup>	0.3902 <sup>(a)</sup>
6BA.3x25 ppm (Trt.4)	0.0354 <sup>(a)</sup>	0.0971 <sup>(ab)</sup>	0.4925 <sup>(b)</sup>

In alignment with early squaring total biomass, LAI and Fr:Tot outcomes, no treatment differences occurred from the control (Trt.1) in the total number of fruiting sites produced at early squaring ( $P=0.585$ ). However, early squaring fruit loads (square count per  $\text{m}^2$ ) and associated total fruit retention rates (retention %), were both reduced in both 6BA treatments (Table 4.8). Squares that were retained did not differ in size between treatments ( $P=0.585$ ).

**Table 4.8. Reproductive growth impacts of 6BA PGR treatments at early squaring (55 DAP) in experiment 8, as characterised by total fruiting sites ( $\text{count}\cdot\text{m}^{-2}$ ), number of squares ( $\text{count}\cdot\text{m}^{-2}$ ), fruit retention (%) and mean square size (g); notation indicating treatment differences at the  $P=0.05$  level using Tukey's HSD.**

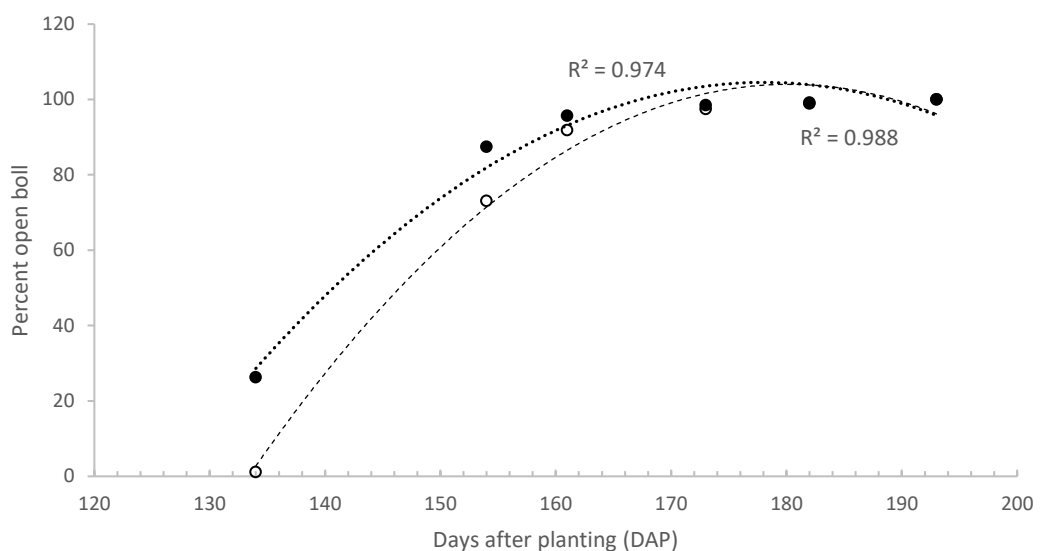
	Total fruiting sites ( $\text{count}\cdot\text{m}^{-2}$ )	Squares ( $\text{count}\cdot\text{m}^{-2}$ )	Retention (%)	Mean square size (g)
	( $P=0.585$ )	( $P<0.001$ )	( $P<0.001$ )	( $P=0.429$ )
Control (Trt.1)	31.18	23.5 <sup>(c)</sup>	79.58 <sup>(c)</sup>	0.1275
6BA.3x100 ppm (Trt. 3)	31.92	2.15 <sup>(a)</sup>	5.6 <sup>(a)</sup>	0.3517
6BA.3x25 ppm (Trt. 4)	25.25	14.52 <sup>(b)</sup>	55.97 <sup>(b)</sup>	0.3062

Resultantly in early flowering, treatment 3 (only) square numbers were reduced compared with the control (Trt.1) ( $P=0.029$ , Trt. 1; 418.2/m<sup>2</sup>, Trt. 3; 245.3/m<sup>2</sup>). No treatment differences were evidenced in either green boll numbers ( $P=0.244$ ) or the mean weight of both squares ( $P=0.371$ ) or green bolls ( $P=0.062$ ), compared with the control at early flowering. The treatment 3 mean green boll mean weight was less than the control (Trt.1) at mid flowering ( $P=0.002$ , Trt. 1; 3.161 g, Trt. 3; 1.634 g), with no other treatment differences evident in green boll weights. Mid flowering fruit loads were otherwise alike, with no treatment differences evident in square counts ( $P=0.073$ ), square mean weight ( $P=0.490$ ), green boll counts ( $P=0.082$ ), open boll counts ( $P=0.510$ ) or open boll mean weights ( $P=0.351$ ).

Generalised linear regression of the square root of sequential boll opening across treatments 3 and 4 in experiment 8, explained treatment delays in maturity (as 60% open boll), for treatment 3 only ( $P=0.019$ ); trend lines were fitted (Figure 4.7) and resulting calendar days to 60% open boll are presented in Table 4.9.

**Table 4.9. Treatment coefficient of determination for fitted trend line and calendar days to maturity as 60% open boll load, for treatment 3 in experiment 8;  $R^2$  all greater than 0.98.**

Treatment	60% open boll (DAP)	Days>Trt.1
Control (Trt.1)	144	-
6BA.3x100 ppm (Trt.3)	149	5

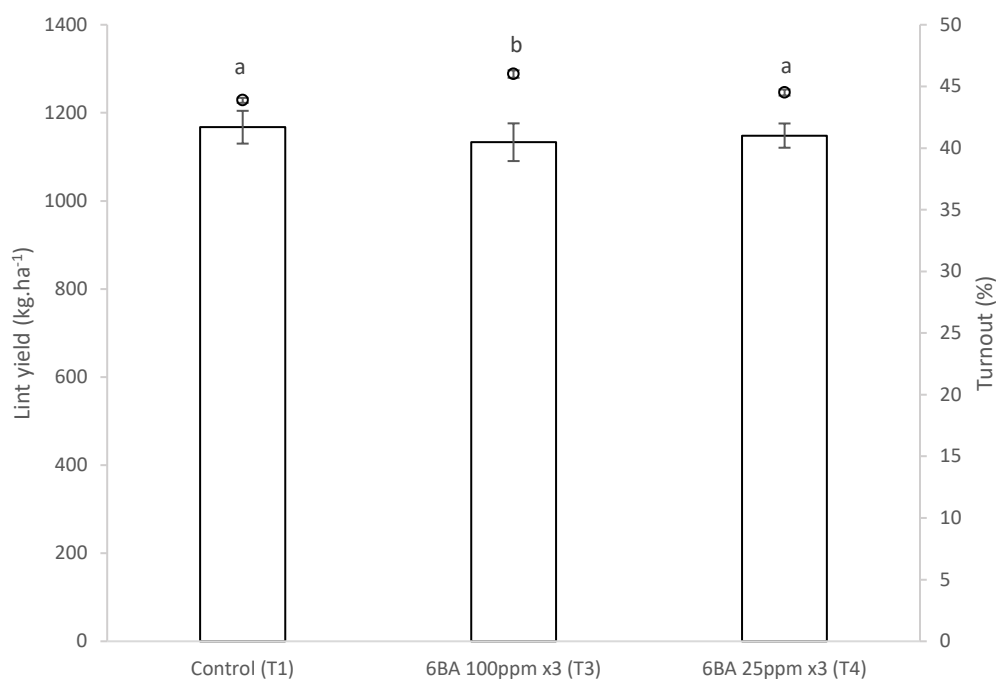


**Figure 4.7. Progression to maturity (60% open boll) of 6BA treatment 3 in experiment 8 ; Control (Trt.1) (...●...), treatment 3 (- - -○- - -).**

No treatment differences occurred for final open boll numbers ( $m^2$ ) ( $P=0.697$ ) or mean open boll weights (g) ( $P=0.095$ ) in experiment 8 (Table 4.10). Moreover, no treatment differences were evident in machine harvested cotton lint yields ( $P=0.772$ ), however treatment 3 had a higher ginning turnout percentage ( $P<0.001$ ) (Figure 4.8).

**Table 4.10. Yield components of 6BA PGR treatments 3 and 4 in experiment 8; no significant differences occurred at the  $P=0.05$  level using Tukey's HSD.**

	Open bolls ( $m^2$ )	Open boll mean weight (g)
	<i>(P=0.697)</i>	<i>(P=0.095)</i>
Control (Trt.1)	130.2	4.047
6BA.3x100 ppm (Trt. 3)	126.4	3.675
6BA.3x25 ppm (Trt. 4)	133.5	4.205



**Figure 4.8. Mean cotton lint yield for 6BA treatments 3 and 4 in experiment 8; lint yield ( $kg \cdot ha^{-1}$ ) (bar), ginning turnout (%) ( $\circ$ ), error bars being  $\pm 1$  standard error of the mean, notation indicating treatment differences at the  $P=0.05$  level using Tukey's HSD.**

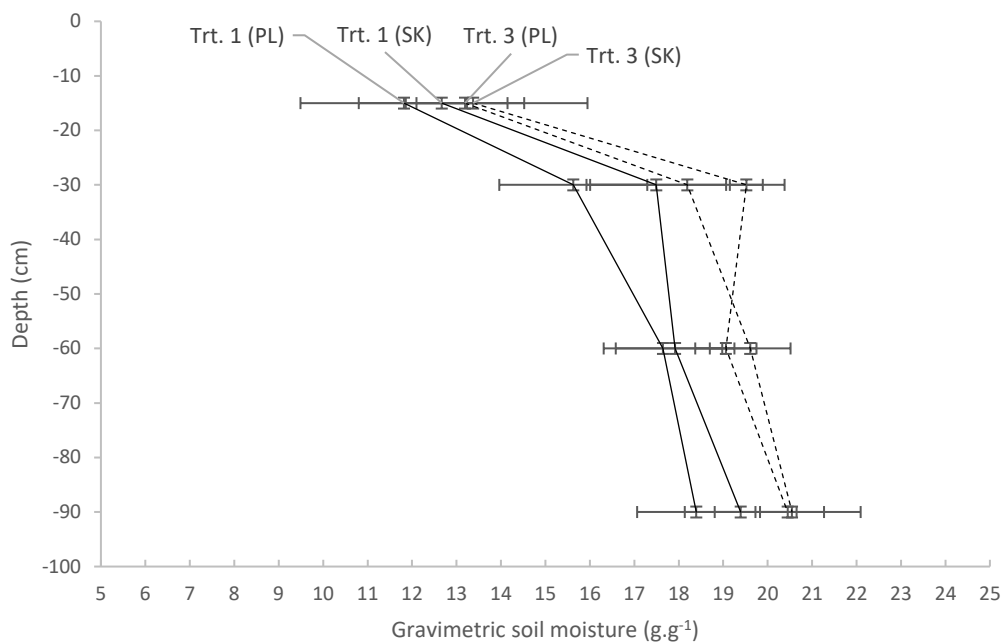
Mean treatment 1 (control) lint quality parameters fell just outside base grade (36, G5) classification; with shorter staple length (Trt. 1; 1.147" or "37"32<sup>nd</sup>) and higher micronaire (Trt. 1; 5.003, G6). Staple strength was classed as "strong" (Trt. 1; 30.05); no treatment differences from the control (Trt.1) occurred for lint length (32nds) ( $P=0.207$ ), lint strength ( $P=0.928$ ) or micronaire ( $P=0.461$ ) in experiment 8.

#### 4.3.4 Concept evaluation; season three field experiments 9 and 10

Experiments 9 and 10 were conducted over the 2018/19 summer production season, being located at the ACRI and Bellata sites, respectively. Outcomes of experiments were shaped by significant environmental events; experiment 9 necessitating a replant in early 2019 following hail on the 22<sup>nd</sup> December 2018 and experiment 10 being planted late (December 2018) on a limited (1/3 PAWC) moisture profile due to prevailing severe drought conditions. Consequently, both experiments had constrained yield potentials and were run with the revised aim of quantifying early season responses to 6BA PGR treatments; being terminated at mid flowering in May 2019.

##### *Plant root biomass accumulation and access to soil water reserves*

Mid flowering (110 DAP) core break sampling in experiment 9 for the binary presence / absence of cotton roots, demonstrated no differences in root growth between the control (Trt.1) and treatment 3; new-season cotton roots being detected at all depths in both the plant line and furrow, for each replicate in both treatments (Trt. 1 and 3). T-test of corresponding gravimetric soil moisture of stratified core samples also demonstrated no differences between the control (Trt.1) and treatment 3, under both the plant line and adjacent furrow, to depth (Figure 4.9, Table 4.11).



**Figure 4.9. Mid flowering gravimetric soil moisture per location and depth at 110 DAP in experiment 8; control (Trt.1) (—), treatment 3 (- - -), notation delineating plant line (PL) and skip row (SK) locations, error bars being +/- 1 standard error of the mean.**

**Table 4.11. T-test P values for comparison of control (Trt.1) and treatment 3 mean gravimetric soil moisture content per depth and row location, at mid-flowering (110 DAP) in experiment 8.**

Plant Line	P value	Skip	P value
15 cm	0.802	15 cm	0.636
30 cm	0.278	30 cm	0.312
60 cm	0.488	60 cm	0.291
90 cm	0.353	90 cm	0.228

Moreover, ANOVA of mid-flowering (109 DAP) mean total electromagnetic conductivity to 1.5 m ( $EC_t$ ), under both the plant line and skip row, showed no treatment differences from the control (Trt.1) (Table 4.12); methodology being detailed in chapter 3, section 4.3.

**Table 4.12 Mean total Electromagnetic Conductivity ( $EC_t$ )( $mS.m^{-1}$ ) of 6BA PGR treatments 2 and 3, at mid-flowering (109 DAP) under the plant line and adjacent skip row to 1.5 m, in experiment 9; no significant treatment differences occurred at the  $P=0.05$  level using Tukey's HSD.**

	<i>ECt Plant Line (<math>mS.m^{-1}</math>)</i>	<i>ECt Skip Row (<math>mS.m^{-1}</math>)</i>
	<i>(P=0.508)</i>	<i>(P=0.260)</i>
Control (Trt.1)	58.52	56.95
6BA 25 ppm (Trt. 2)	56.39	55.82
6BA 25 ppm x3 (Trt. 3)	58.52	57.84

### ***Plant and crop growth responses***

Early squaring and early flowering biomass harvests to assess growth and development against the control, were sampled for all treatments in experiment 9 within 7 days of 50% population first square ("early squaring", 44 DAP), and in both experiment 9 and 10 at first flower ("early flowering", 66 DAP). No treatment differences from the control (Trt.1) were evident in total dry weight, LAI or proportionate reproductive growth to total biomass (Fr:Tot) across both sampling dates in experiment 9 or at early flowering in experiment 10 (Table 4.13).

**Table 4.13 Biomass development effects of 6BA PGR treatments at (a) early squaring and (b) early flowering in experiments 9 and 10, as characterised by total dry weight accumulation ( $\text{g}\cdot\text{m}^{-2}$ ), leaf area index (LAI) and reproductive biomass proportionate to total dry weight accumulation (Fr:Tot); no significant treatment differences occurred at the  $P=0.05$  level using Tukey's HSD.**

	<i>(a) Early squaring</i>	<i>(b) Early flowering</i>	
	<i>Exp 9 (44 DAP)</i>	<i>Exp 9 (66 DAP)</i>	<i>Exp 10 (66 DAP)</i>
<b>Total dry weight (<math>\text{g}\cdot\text{M}^{-2}</math>)</b>			
	<i>(P=0.326)</i>	<i>(P=0.235)</i>	<i>(P=0.995)</i>
Control	235.8	674.9	316.2
6BA.25 ppm	325.0	628.7	-
6BA.25 ppm x3	286.7	688.7	257.18
<b>Leaf Area Index (LAI)</b>			
	<i>(P=0.091)</i>	<i>(P=0.283)</i>	
Control	0.889	3.557	<i>(LAI analysis not completed due to lab biosecurity restrictions)</i>
6BA.25 ppm	1.623	3.195	
6BA.25 ppm x3	1.391	3.456	
<b>Reproductive biomass / total dry matter (Fr:Tot)</b>			
	<i>(P=0.311)</i>	<i>(P=0.756)</i>	<i>(P=0.968)</i>
Control	0.007425	0.07553	0.2010
6BA.25 ppm	0.005641	0.08346	-
6BA.25 ppm x3	0.002647	0.07759	0.1473

Commensurate with early squaring total dry weigh accumulation, LAI and Fr:Tot outcomes in experiment 9, no treatment differences from the control in total fruiting site production were evident ( $P=0.730$ ). Contrasting with similar early squaring analysis in experiment 8, no treatment differences from the control (Trt.1) existed in the number of squares produced per  $\text{m}^2$  ( $P=0.332$ ), the mean weight of squares produced ( $P=0.131$ ) or their subsequent retention ( $P=0.157$ ) (Table 4.14).

**Table 4.14. Reproductive growth impacts of 6BA PGR treatments at early squaring (44 DAP) in experiment 9, as characterised by total fruiting sites ( $\text{count}\cdot\text{m}^{-2}$ ), number of squares ( $\text{count}\cdot\text{m}^{-2}$ ), fruit retention (%) and mean square size (g); no significant treatment differences occurred at the  $P=0.05$  level using Tukey's HSD.**

	<b>Total fruiting sites (<math>\text{count}\cdot\text{m}^{-2}</math>)</b>	<b>Squares (<math>\text{count}\cdot\text{m}^{-2}</math>)</b>	<b>Retention (%)</b>	<b>Mean square size (g)</b>
	<i>(P=0.730)</i>	<i>(P=0.332)</i>	<i>(P=0.157)</i>	<i>(P=0.131)</i>
Control (Trt.1)	79.34	38.66	36.64	0.0455
6BA.25 ppm (Trt. 2)	107.93	20.53	19.8	0.0699
6BA.25 ppm x3 (Trt. 3)	99.55	18.91	13.73	0.0249

Early flowering fruit loads in both experiment 9 and 10, both demonstrated no treatment differences from the control (Trt.1) in square counts (Exp 9; P=0.787, Exp 10; P=0.891), mean square weights (Exp 9; P=0.415, Exp 10; P=0.514), green boll counts (Exp 9; P=0.237, Exp 10; P=0.984) or mean green boll weights (Exp 9; P=0.665, Exp 10; P=0.987).

#### **4.4 Discussion**

Controlled environment and field studies by Burke (2011, 2013) established augmented RSA and associated yield enhancements when cotton seedlings were treated with Adenine-type Cytokinin 6BA at early vegetative growth, in water-limited (rain grown) production settings in the Texas high plains (USA). Leveraging these Texan findings, this study investigated the utility of Adenine-type Cytokinins to promote plant access to soil water reserves, under the differing genetic, environmental and management contexts of Australian rain grown cotton production systems. Prevailing hot and dry climatic conditions (Appendix 3) and the concurrent occurrence of specific extreme climatic events (e.g. hail, 22<sup>nd</sup> December 2018) across all field experiments included within this analysis, adversely influenced crop growth and subsequent yield potentials. However, lint yields in the three field crops examined in this study that were grown to maturity, achieved consistent outcomes commensurate with or above mean annual Australian rain grown cotton yields (CA, 2018b).

##### **4.4.1 Primary 6-Benzylaminopurine treatment impacts on intra-seasonal plant root biomass accumulation and subsequent access to soil water reserves**

The glasshouse and field experiments detailed in this chapter quantified plant root system architecture (RSA) via destructive sampling; for direct measurement (glasshouse) and for binary characterisation of root presence / absence at specific purlieus proximal to the plant (field). Concurrently in field studies, stratified gravimetric soil moisture was measured to similarly characterise plant accession of soil water resources. Appraisal of cumulative volumetric soil moisture via non-injurious apparent bulk soil electrical conductivity ( $EC_a$ ) assessments, to specific depths at key plant growth stages within season, further enabled quantification of plant soil water access as a proxy indicator of root growth at frequent intervals within the growing season.

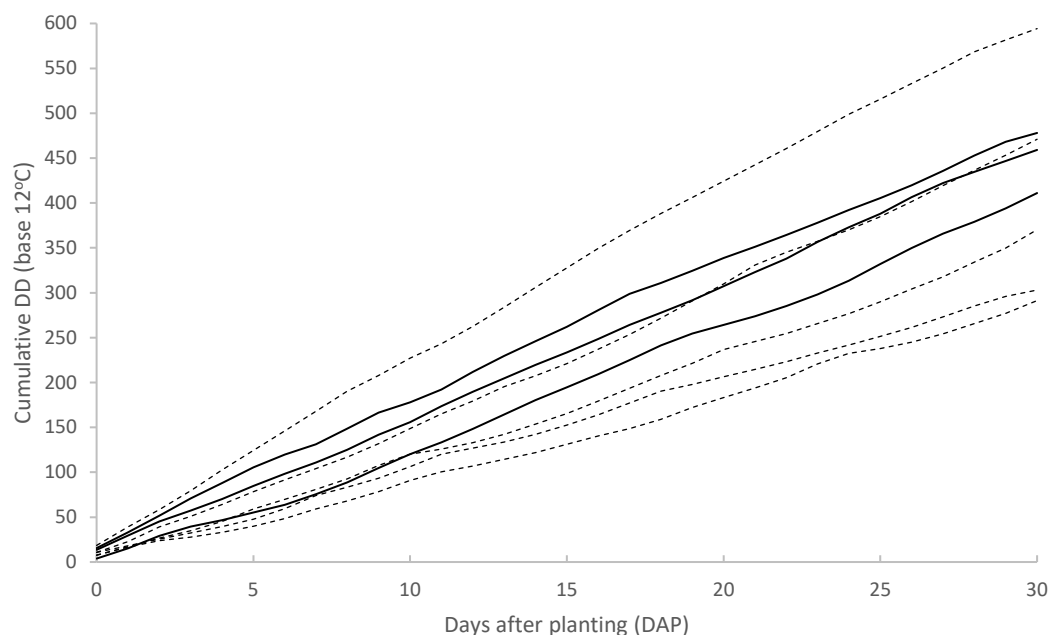
The glasshouse and field experiments showed no evidence of treatment impacts of 6BA PGR applications on root biomass accumulation or associated plant utilisation of soil water reserves, across multiple rate x application method x frequency / timing of application

combinations (Table 4.14, Appendix 4). Early controlled environment experiments to screen a multiplicity of treatment concentrations and application modalities showed no treatment impacts on short term root length (experiment 1 and 2), root dry weight (experiment 1) and proportionate root growth (experiment 1) responses, and negative outcomes for root dry weight accumulation and proportionate root growth (experiment 2). In field studies, cumulative volumetric soil moisture analysis during early and mid-season reproductive growth stages in experiment 8, also indicated no treatment differences from the control in plant utilisation of both shallow and deep soil water reserves; both under the plant line and the adjacent skip row. Core break sampling during mid-season reproductive growth (experiment 9) and at defoliation (experiment 4) again confirmed no treatment differences from the control in the presence of live cotton roots to 1 m depth, both under the plant line and adjacent skip row. Corresponding depth and width stratified gravimetric soil moisture analysis similarly confirmed no associated treatment differences in plant utilisation of soil water reserves. However, a trend toward reduced water extraction in the 6BA treatment could be conceivably indicative of a lesser root biomass presence.

Limited comparative analysis exists for cotton RSA responses to exogenous 6BA treatments; notably early vegetative growth stage applications. Data from this study are in contrast to previous work by Burke (2011, 2013); evidencing both a 2-fold increase in root length at 30 DAP in glasshouse grown cotton seedlings (Burke, 2011) and a corresponding increased lateral root growth at mid flowering (63 DAP) in subsequent field grown cotton evaluations (2013), both instances being sprayed with 25 ppm 6BA at 2-6 leaf stage. These findings are corroborated by (Yuan, Wang, Li, Li, & Tai, 2014) who demonstrated increased early vegetative stage root and shoot dry weight biomass in maize following seed priming application of 6BA. Recently He et al. (2018) has also demonstrated utility of a single early (10 DAP) foliar 30 mg.L<sup>-1</sup> 6BA application to *Phellodendron* spp. seedlings, in promoting increased root biomass (dry weight) at 40 days after treatment. In contrast, M. B. Jackson and Campbell (1979) and recently Tian, Chen, Zhang, and Mi (2005), both noted an inhibitory root biomass accumulation response to comparable early growth 6BA foliar applications, in tomato and maize seedlings, respectively.

Disparity between results in rain grown cotton systems can be accounted for in part by differences in both; the existing environmental settings of field experiments and the methodology utilised to quantify cotton plant root growth and associated plant water uptake. Quantification of treatment RSA response in field grown cotton by Burke (2013) occurred during mid-flowering at 63 DAP commensurate with sampling timing in

experiments 8 and 9. However, the root assay methodology utilised by Burke (2013) was qualitative in nature; random individual plants from a limited number of replicates being sampled to provide a visual indication of phenotypic RSA changes. Suggestion is made that the quantitative RSA analysis conducted in experiments 4, 8 and 9, using stratified core-break root sampling results and accompanying soil profile water extraction patterns, provides greater accuracy of assessment. Comparative differences in soil bulk density between the Lubbock (Tx. USA) Acuff fine loam (USDA classification: Aridic Paleustalfs) and the Narrabri (NSW, Aust.) Grey Vertosol clay (USDA classification: Typic Haplustert) could also account for differences in RSA treatment responses; root growth being restricted as soil strength is increased (Gerard, Sexton, & Shaw, 1982), as in the case of the Narrabri Vertosols. Furthermore, prevalent unseasonal colder early season growing conditions across the Australian field experiments, exceptions being the re-planted experiment 9 and the later planted (due to drought) experiment 10, resulted in reduced cumulative DD at 30 DAP and an increased occurrence of cold shock events in experiments 4,5 and 8, than the three field experiments documented by Burke (2013) (Figure 4.10, Table 4.15). Exogenous 6BA applications have shown utility in invoking cotton plant resilience to cold chill stress via increased sucrose synthase and sucrose phosphate synthase activity in resource sinks, albeit during mid to late reproductive growth (Y. Wang et al., 2011). However, decreased growth rates associated with lower DD accumulation, as well as the occurrence of cold chill stress days, can slow growth and inhibit resultant responses to PGR treatments (Woodward & Stoller, 2017).



**Figure 4.10.** Mean cumulative day degrees (DD) to 30 days after planting in the three field experiments documented in Burke (2013) and experiments 4, 5, 8, 9 and 10; Lubbock field experiments (—), Australian field experiments (- - -), DD calculations from both localities utilised a base temperature of 12°C with climate information sourced from NOAA (NWS:NOAA, 2017) and CottAssist (CSIRO, 2014) for the Lubbock and North West NSW data, respectively.

**Table 4.15.** Cumulative day degree accumulation and occurrence of cold shock days to 30 days after planting, in the three field experiments documented in Burke (2013) and experiments 4, 5, 8, 9 and 10; cold shock occurrence equating to daily minimum temperatures below 11°C, DD calculations from both localities utilised a base temperature of 12°C, climate information sourced from NOAA (NWS:NOAA, 2017) and CottAssist (CSIRO, 2014) for the Lubbock and North West NSW data, respectively.

Experiment	Cumulative DD at 30DAP (with base temp 12°C)	Number cold shock days to 30DAP
Lubbock (7 June 2005)	459.11	0
Lubbock (11 May 2006)	411.06	2
Lubbock (30 May 2008)	478.00	0
Exp. 4 (2016.17 ACRI ERCF)	291.25	15
Exp. 5 (2016.17 BTA ERF)	370.25	8
Exp. 8 (2017.18 ACRI Resil.1)	303.00	9
Exp. 9 (2018.19 ACRI Resil.2b)	594.25	0
Exp. 10 (BTA Resil.)	471.00	0

#### 4.4.2 Corollary plant and crop level responses

Glasshouse and field experiments demonstrated predominantly no intra- and inter-seasonal treatment impacts on total biomass accumulation and leaf area index (LAI) (Table 4.15 and

Appendix 4). Early 6BA induced square shedding in, and proportionate to, the 25 mg.L<sup>-1</sup> and 100 mg.L<sup>-1</sup> triplicate foliar application treatments, resulted in early and mid-flowering fruiting pattern alterations (Table 4.15 and Appendix 4) and progression to maturity impacts for the 100 mg.L<sup>-1</sup> 6BA treatment (Table 4.16 and Appendix 4). Of the crops that were grown to maturity (experiments 4, 5 and 8), principally no treatment differences in yield components, ginning turnouts, lint yields and lint quality parameters were found (Table 4.16 and Appendix 4).

Cotton seedling early vegetative stage dry weight accumulation in controlled environment experiments, demonstrated no response to seed priming / coating treatment methods (experiment 1) and a decrease in total dry weight at 24 DAP from foliar applications in experiment 2. These findings contrast with Burkes (2011) assertions of increased hypocotyl diameter associated with foliar applications and more recently (Fang et al., 2018) findings for comparative seed priming treatments in growth chamber (hydroponically) grown cotton. In field experiments, no further dry weight accumulation differences were shown across all treatment concentrations from early squaring through to mid flowering. Equally, no differences in leaf area index (LAI) were substantiated across all sampling dates.

Biomass assessments demonstrated a reduced number of squares and associated total retention percentages during early squaring, in both the triplicate foliar 25 mg.L<sup>-1</sup> and 100 mg.L<sup>-1</sup> treatments in experiment 8, but not the late season re-planted experiment 9. No corresponding treatment differences occurred in the number of total sites produced, suggesting a treatment induced early square shedding as opposed to delayed reproductive development. Similarly, (Grossmann, 1991) demonstrated exogenous 6BA treatment-induced increased ethylene production and associated leaf abscission, in early vegetative stage cotton plants following 5-leaf foliar applications in growth chamber grown cotton. These outcomes contrast with recent findings by (Fang et al., 2019) who noted increased fruit numbers and associated retention due to improved net photosynthesis ( $P_n$ ) rates, from a similar foliar treatment; however, Fang et al. (2019) work was conducted in a non-resource limited irrigated production setting. Interestingly, these early squaring treatment impacts were not shown in the singular foliar 25 mg.L<sup>-1</sup> 6BA treatment. Woodward and Stoller (2017) noted an approximate 10-day period of efficacy of exogenous Cytokinin PGR treatments in kiwifruit; being dependant not only on the specific PGR compound and application methodology, but also prevailing environmental conditions which determine the pool (resource) size within the plant, via the regulation of compound degradation,

conjugation, transport and perception. It is foreseeable that cumulative treatment applications prolonged PGR efficacy into early reproductive growth with associated (ethylene-induced) fruit abscission responses. Future further time-series exploration of both early square soluble carbohydrate content (indicative of  $P_n$  response), and subtending leaf ethylene levels, post singular and triplicate treatments would be advantageous in quantifying both direct plant responses and time-extent and magnitude of such treatment impacts.

No differences from the control were demonstrated for the proportion of reproductive growth comparative to total biomass, for both the singular and triplicate 25 mg.L<sup>-1</sup> 6BA treatments, from early squaring through to mid-flowering. In experiment 8, due to earlier square shedding, triplicate applications of 100 mg.L<sup>-1</sup> 6BA resulted in reduced proportionate reproductive biomass outcomes at early and mid-flowering; coinciding with a reduced number of squares and consequent green boll mean weight at first flower and mid flowering, respectively. No other treatment differences occurred in square, green boll and open boll counts and mean weights at early and mid-flowering. These outcomes are in contrast with Fang et al. (2019) and Pandey et al. (2003), who both note increased green boll numbers during mid-flowering as a result of early vegetative and first flower 6BA applications, in irrigated field and glasshouse grown cotton, respectively.

No treatment differences in open boll number or mean weights occurred for 6BA treatments in experiments 4, 5 and 8; differing from results evidenced by Burke (2011), Fang et al. (2019), Pandey et al. (2003) and Y. Wang et al. (2011) who all noted increased boll numbers and mean weights from singular applications under (with the exception of Burke, 2011) non-resource limited irrigated cotton. Ginning turnout for the triplicate 100mg.L<sup>-1</sup> foliar treatment in experiment 8 was higher than the control. Paired with no associated lint yield increase, this outcome may be indicative of a treatment induced smaller seed size or reduced seed number; although treatment open boll numbers and mean weights were equal to the control. No other treatment ginning turnout differences were evidenced across the three experiments grown to maturity. Under water limited rain grown production settings, optimising RSA via increases in lateral root growth as documented by Burke (2011, 2013) to increase the effective exploration of the cotton plant root growth area (root space), provided a buffer to the occurrence and impact of water deficit stress, notably in drier seasons; being evidenced by reduced chlorophyll fluorescence levels following prolonged elevated respiratory demand (dark bioassay) (Burke, 2013). Contrasting with experiments documented in this research, such improved resilience to early water deficit stress led to

increased early fruit retention with flow-on later season benefits as both increased overall boll set (open boll numbers), open boll size and resultant lint yield outcomes.

No treatment differences in lint yield occurred in field experiments that were grown to maturity (Exp. 4,5 and 8), supporting outcomes reported by Pandey et al. (2003) in non-water deficit stressed 6BA treated (glasshouse) cotton. Lint yield outcomes contrasted with those of Burke (2013) in rain grown cotton, as well as Fang et al. (2019) and Y.Wang et al. (2011) in irrigated field grown cotton; who all reported lint yield improvements. Interestingly, both Mayeux, Lewis, and Cothren (2011) and Pandey et al. (2003) reported differing (albeit seed cotton) yield outcomes based on the level of water deficit stress; yield improvements being proportionate to the intensity of stress incurred. Both noted an advantageous 6BA yield response in water deficit stressed cotton compared to the untreated, but water stressed control, under glasshouse conditions. Future quantification of cotton absolute (water deficit) stress levels via continuous and detailed plant-based stress measurements, such as canopy temperature accumulated stress hours from early vegetative growth, would be a beneficial covariate.

#### **4.5 Conclusions**

This study investigated; the impacts of specific Adenine-type Cytokinin 6-Benzylaminopurine treatment scenarios on early root biomass accumulation and the consequential effects thereafter of plant accessibility to soil water resources, as explained by yield, lint quality, biomass and LA accumulation, as well as fruit development, retention and maturity outcomes. In contrast to findings of Burke (2011, 2013), no root growth was promoted nor increased accessibility to soil water reserves evidenced. Moreover, suspected 6BA treatment-induced increased ethylene levels (notably in higher concentration triplicate applications) in early reproductive growth decreased early fruit retention, resulting in flow on morphological impacts on maturity. In summary, although applied in a culturally suitable / appropriate manner, 6BA treatments were not effective in increasing root biomass accumulation, subsequent access to soil water reserves and follow on lint yield or quality outcomes, in north west NSW. While the 6BA component and application regimes failed to induce the pursued physiological changes, it is possible that geographical, climate and procedural differences may have been contributing factors to the successful tests of 6BA by Burke (2011, 2013) in rain grown cotton.

## **Chapter 5. “Fitness for Environment”: Bioactive Gibberellin Plant Growth Regulators to Build Early Canopy Capacity to Increase Access to Soil Water Reserves**

### **5.1 Introduction**

Water is a significant constraint of rain grown cotton production systems. Extreme variability of crop water supply (as stored soil reserves and in-crop rainfall) characterises year to year variations in yield and quality (Jordan & Ritchie, 1971). Current climate risk management strategies such as the use of skip row configurations and early nitrogen management, provide access to additional soil water reserves and regulate vegetative growth, to buffer against in-season variability of climatic conditions and manage downside yield risk. However, such strategies are relatively inflexible in their application in season and resultantly, in mean or above seasonal rainfall years, can incur yield penalties due to lost yield potential (CRDC, 2002). Leveraging principles underpinning these existing strategies, can inform the development of novel adaptive management approaches using plant growth regulators (PGRs), that enable in-season agile management of system challenges and limitations under variable climatic conditions (Gunderson, 1999).

Research in other agricultural crops and non-resource limited cotton, has demonstrated utility of Gibberellin PGRs to promote early season above and below ground growth. Employing such findings to develop optimal plant root / shoot structure and canopy biomass characteristics in rain grown cotton, may afford water deficit adaptation opportunities during the critical early flowering to cut out stage. Such climate risk mitigation being achieved through increased access to soil water reserves via augmenting canopy net photosynthetic ( $P_n$ ) capacity and reproductive (number of sites produced and associated retention) scope during vegetative growth. Accordingly, understanding cotton plant and crop responses to specific Gibberellin PGR treatment scenarios, can enable development of framework criteria for application within rain grown cotton systems that are tailored to defined production settings.

Following a sigmoidal curve pattern, cotton’s early season above ground vegetative growth is comparatively slow to other commercial fibre and oilseed crops; evidencing an initial lag phase prior to early squaring (Robertson, Bednarz, & Burmester, 2007). Subsequent vegetative growth increases rapidly through early flowering, plateauing post physiological cut-out due to the redirection of carbohydrates and nutrients associated with boll filling (Guthrie, Burmester, Edmisten, & Wells, 1995). Such protracted initial above ground

vegetative growth (“lag phase”) results from both an early priority for assimilate diversion toward development of root architecture, as well as reduced light interception by a limited leaf area, on a small plant (Guthrie et al., 1995; Hansen et al., 1996; Robertson et al., 2007). Further growth reductions can be incurred by impacts of sub-optimal air and soil temperatures, disease, insect pests and wind damage / sand blasting, on leaf area accumulation of young plants (Larsen & Cannon, 1966; Robertson et al., 2007). Impacts are cumulative in nature, with amalgamations of these individually sub-lethal abiotic stressors having capability to compromise crop viability (CSD, 2019; Robertson et al., 2007).

Solar radiation drives both growth and development; the extent of utilisation determining the plant biomass production capacity (Pearce, Brown, & Blaser, 1965). Increases in crop light interception, as characterized by leaf area index (LAI, ratio of leaf to soil area as  $m^2.m^{-2}$ ), correspond with increasing canopy net photosynthesis ( $P_n$ ) and resultant plant growth rates, up to a maximal  $P_n$  level and corresponding “optimal” LAI value (Pearce et al., 1965). Specific crop optimum LAIs are determined by multiple biotic and abiotic variables, via impacts on patterns of canopy light interception and reflection (Pearce et al., 1965). Leaf arrangement, shape and age, as well as petiole crotch angle, and incoming light intensity (as influenced by climate, geographic location, diurnal fluctuations) all having significant influence (Pearce et al., 1965).

In an Australian context, maximum solar radiation interception and subsequent crop growth rates occur at LAI of approximately 3.5 (Constable, 1977; Constable & Gleeson, 1977) to 4 (Heitholt, Pettigrew, & Meredith, 1992). Emerging cotton seedlings have a LAI of approximately 0.01, expanding to 1.0 at 6-8 weeks post planting in early squaring and increasing rapidly, commensurate with vegetative growth rates, to 5.0 during the following 6-week period in early flowering to physiological cut-out (Ashley, Doss, & Bennett, 1965; Guthrie et al., 1995). Consequently, in early vegetative growth only a small proportion of available radiation is being harvested and utilised by the plant, for assimilate production to increase plant vegetative framework (above and below ground) and leaf biomass accumulation (Guthrie et al., 1995).

Vigorous early vegetative development maximises the crops potential for improved yield and quality outcomes; seed cotton yields being evidenced to increase proportionate to expanding plant size in early season cotton plants (Ashley et al., 1965; Hansen et al., 1996). Promotion of simultaneous early plant above and below ground biomass growth and

canopy development, to increase the plant “carrying capacity” and promote increased crop resilience to episodic water deficits (Biles & Cothren, 2001), is consistent with the nutritional hypothesis of fruit survival in cotton (Ehlig & LeMert, 1973; Mauney, 1986a; Turner, 1986). Coincidentally, low yields obtained in rain grown cotton production systems reflect a reduced total above ground biomass (height-yield association) between early squaring and cut out; vegetative (node) growth, canopy expansion and the development of fruiting branches and resultant sites being proportionate to crop water availability (Bruce & Shipp, 1962. ; Rijks, 1965 ). Additional water deficit induced yield reductions originate from decreased fruit retention (resulting in a decreased boll number) and a reduced mean open boll size (Larson et al., 1997; Rijks, 1965 ).

The relationship between LAI and cotton plant fruiting patterns indicate that formation of new fruit is dependent on concurrent vegetative growth producing leaf area, until LAI reaches 5.0 (Ashley et al., 1965). Importantly, earlier establishment of optimum leaf area results in higher rates of early fruit production as well as increased retention of those fruit (R. Wells & Meredith, 1984). Opportunity exists to influence this stage effectively because developing squares are not yet dominant sinks for carbohydrates, minerals and water (Guthrie et al., 1995). Moreover, evidenced limitations in seasonal skip row soil water extraction patterns (Goynes, 2005) are compounded by the metabolic costs associated with increased root (vegetative) growth. Reductions in root elongation and sloughing of older roots post physiological cut-out, are associated with assimilate diversion toward boll-loading and result in reductions in soil space exploration and a net-decrease in total root absorption potential and consequent water and nutrient uptake (Robertson et al., 2007) . Therefore, due to cotton’s genetic predominance of early root biomass accumulation (Robertson et al., 2007), the most opportune time to augment root system architecture and facilitate exploration of a larger soil-space for water (and nutrient) capital, is during early vegetative growth.

Endogenous plant hormones are innately involved in structural morphology, fruiting and shedding in cotton (Guinn, 1986), either directly or as a message carrier during growth and development (Hearn & Constable, 1984a). Consequently, exogenous application of plant growth regulators can be utilised to influence endogenous hormone concentrations, transport and / or associated receptor sites; plant responses being dependant on varietal, management and environmental contexts (Livingstone & Parker, 1994; Rademacher, 2000). Gibberellins are involved in a high proportion of plant growth and development processes; promoting germination, cell elongation and growth, flower and fruit

development (Rademacher, 2000). Limited references regarding the physiological role of Gibberellins in root growth exist due to evidence suggesting a lack of promotion of root elongation (Tanimoto & Hirano, 2013). However, molecular mechanisms of Gibberellin signal transduction in shoots have recently been substantiated in roots; evidence suggesting Gibberellins control root growth at very low concentrations compared to shoot regulation (Tanimoto & Hirano, 2013).

Promotion of early root growth utilising Gibberellins as a limited water adaption strategy, has been demonstrated in other crops including maize (Silverman et al., 2017), okra (Jasmine & Merina, 2012), tomato (Choudhury, Islam, Sarkar, & Ali, 2013) and in cotton as a proportionate component of a proprietary preparation PGR-IV (Zhao & Oosterhuis, 1995); Gibberellins have also been utilised to promote root elongation in horticultural plant *Lemna minor* (Inada & Shimmen, 2000). Early vegetative stage foliar applications of Gibberellin A<sub>3</sub>, has demonstrated utility in cotton in promoting increased LAI under irrigated international production environs (Hansen et al., 1996; Larson et al., 1997; Stuart & Cathey, 1961). However, a scarcity of detailed information exists regarding cotton crop and plant (notably RSA) response to Gibberellin A<sub>3</sub> and especially Gibberellin A<sub>4,7</sub> applications, under rain grown production contexts (Appendix 2); location, proximity and method of exogenous Gibberellin application having implication for plant phenological response (Oosterhuis & Zhao, 1994).

This chapter details a series of glasshouse and field experiments, conducted to determine the capability of Gibberellin PGRs to increase plant structure (viz. vegetative framework of roots, stems, branches, petioles) and photosynthetic source biomass in early vegetative stage cotton plants, with the objective of increasing plant canopy capacity and access to soil water reserves to improve rain grown cotton productivity.

These experiments aimed to:

- Quantify the impact of Gibberellin PGR treatment choice, rate, application type, frequency and timing combinations, on plant above and below ground biomass accumulation and resultant canopy capacity and access to soil water reserves; and
- Understand consequent plant and crop level responses to treatment impacts, as explained by; fruit development and retention, lint yield and lint quality outcomes.

Specifically, the hypothesis tested was;

H<sub>0</sub>: Gibberellin PGR treatments applied in early vegetative growth stage to Australian cotton cultivar Sicot 746B3F under optimal (glasshouse) and field growing conditions, induce no detectable plant phenological response on plant above and below ground vegetative frameworks and leaf biomass accumulation, as well as resultant plant canopy capacity and access to soil water reserves.

## **5.2 Materials and methods**

A stepped approach to experiments was used to test aims and respond to the null hypothesis across three production seasons (Table 4.1). Sourcing singular Gibberellin A<sub>47</sub> (GA<sub>4,7</sub>) product into Australia, that was fermented under alike production methodologies to the Gibberellin A<sub>3</sub> (GA<sub>3</sub>), necessitated an initial emphasis toward evaluating GA<sub>3</sub> in experiments 1, 2, 4, 5 and 8. Subsequent availability of GA<sub>4,7</sub> enabled a direct comparisons of treatment effects between GA<sub>3</sub> and GA<sub>4,7</sub> in experiments 8 and 9. Successively, further GA<sub>4,7</sub> treatments were validated in field experiments 8, 9, 10 and 11.

### **5.2.1 Site description**

To enable hypothesis testing of Gibberellin treatments on rain grown cotton plants, 8 experiments were grown under both controlled environment (spring 2016) and rain grown field conditions across multiple locations during the 2016-17, 2017-18 and 2018-19 cotton seasons. Specific site locations, climatic conditions, experimental design, crop and plot management protocols are detailed in Chapter 3, Sections 3.4.1 and 3.4.2.

### **5.2.2 Treatments**

For the purpose of this analysis, treatments utilised per experiment are detailed in Table 5.1. For brevity, PGR treatment acronyms referencing the active ingredient, are utilised throughout and explained in the Abbreviations section, with full nomenclature, structural descriptions and proprietary sources detailed in Appendix 1.

**Table 5.1 Treatments per experiment, as included in chapter 5 analysis; preliminary screening under controlled environment conditions in experiments 1 and 2, dosage assessments in field experiments 4, 5, 10 and 11 and comparative efficacy appraisal field experiments 8 and 9.**

<b>Exp. No., name</b>	<b>Trt. No.</b>	<b>Compound</b>	<b>Conc. (mg.L<sup>-1</sup>)</b>	<b>Application</b>	<b>Timing</b>	<b>Frequency</b>
<b>Experiment 1. (GH Seed Ckga)</b>						
	1	Untreated control				
	8	GA <sub>3</sub>	200.00	Seed prime, 20 h submergence	Seed	Once
	9	GA <sub>3</sub>	500	Seed prime, 10 sec. submergence	Seed	Once
<b>Experiment 2. (GH Foliar Ckga)</b>						
	1	Untreated control				
	8	GA <sub>3</sub>	80.00	Foliar, to drip	2 leaf	Once
	9	GA <sub>3</sub>	160.00	Foliar, to drip	2 leaf	Once
<b>Experiment 4. (2016.17 ACRI ERCF)</b>						
	1	Untreated control, water spray only				
	8	GA <sub>3</sub>	80.00	Foliar, to drip	2 leaf	3 x 10 d <sup>(1)</sup>
<b>Experiment 5. (2016.17 BTA ERF)</b>						
	1	Untreated control, water spray only				
	6	GA <sub>3</sub>	80.00	Foliar, to drip	2 leaf	3 x 10 d <sup>(1)</sup>
<b>Experiment 8. (2017.18 ACRI Resil. 1)</b>						
	1	Untreated control, water spray only				
	5	GA <sub>3</sub>	80.00	Foliar, to drip	2 leaf	3 x 10 d <sup>(1)</sup>
	6	GA <sub>4,7</sub>	80.00	Foliar, to drip	2 leaf	3 x 10 d <sup>(1)</sup>
<b>Experiment 9. (2018.19 ACRI Resil. 2b)</b>						
	1	Untreated control, water spray only				
	4	GA <sub>4,7</sub>	80.00	Foliar, to drip	2 leaf	3 x 10 d <sup>(1)</sup>
	5	GA <sub>3</sub>	80.00	Foliar, to drip	2 leaf	3 x 10 d <sup>(1)</sup>
<b>Experiment 10. (2018.19 BTA Resil. Agron)</b>						
	1	Untreated control, water spray only				
	3	GA <sub>4,7</sub>	80.00	Foliar, to drip	2 leaf	3 x 10 d <sup>(1)</sup>
<b>Experiment 11. (2018.19 THH Resil. Agron)</b>						
	1	Untreated control, water spray only				
	6	GA <sub>4,7</sub>	80.00	Foliar, to drip	2 leaf	3 x 7 d <sup>(2)</sup>

<sup>(1)</sup> Nomenclature is 3 applications in 10-day intervals

<sup>(2)</sup> Nomenclature is 3 applications in 7-day intervals

### 5.2.3 Measurements

Quantification of cotton plant response to PGR treatment applications varied contingent on individual experiment rationale (Table 5.1) and prevailing climatic conditions. Crop measurements per experiment are detailed in Table 5.2, with referenced sampling and processing methodologies being detailed in Chapter 3, Sections; 3.4.2 plot sampling protocol, 3.4.3 soil moisture (EC<sub>a</sub>, volumetric, gravimetric), 3.4.4 root growth (core break sampling, minirhizotron, soil pit), 3.5.1 growth (biomass, leaf area), 3.6.1 fruit mapping and retention, 3.6.2 maturity, 3.6.3 lint yield (hand and machine harvest), 3.6.4 ginning and 3.6.5 quality parameters.

**Table 5.2 Plant and crop physiological measurements per experiment, as included in Chapter 5 analysis; unless otherwise specified all treatments and replicates being sampled.**

Exp	Root growth		Soil moisture (proxy, root gth.)		Growth and development (above ground)				Yield components		Yield	Quality	
	Soil pit	Coreface	Soil Cores	EC <sub>a</sub>	VGR	Biomass	Leaf area	Fruit retention	Maturity	Seed cotton	OB no. and Lint size	Ginning	HVI
1.	Via biomass at 13 DAP		-	-	Early vegetative, 13 DAP								
2.	Via biomass at 24 DAP		-	-	Early vegetative, 24 DAP								
4.	-	-	-	-	>25 DAP 10-day intv. 6 weeks	Mid flowering, 124 DAP			-	1 m <sup>2</sup> hand harvest, 182 DAP	10 m machine harvest, 231 DAP		
5.	-	-	-	-	>25 DAP 10-day intv. 6 weeks	Mid flowering, 113 DAP			-	1 m <sup>2</sup> hand harvest, 152 DAP	15 m machine harvest, 181 DAP		
8.		-	-	78 DAP (FF), 110 DAP (mid flowering)	>25 DAP 10-day intv. 12 weeks	55 DAP (FSqu), 81 DAP (FF), 116 DAP (mid flowering)			Maturity	1 m <sup>2</sup> hand harvests from 134 DAP, 10-day intervals	10 m machine harvest, 210 DAP	Obtained from machine harvest	
9.	-	Mid flowering, 108 DAP		109 DAP (mid flowering)	>25 DAP 10-day intv. 6 weeks	66 DAP (FF)							
10.	-	Mid flowering, 91 DAP		-	>25 DAP 10-day intv. 6 weeks	66 DAP (FF)							
11	At defoliation, 168 DAP				>50 DAP 10-day intv. 6 weeks	74 DAP (FSqu), 96 DAP (FF)			Maturity	1 m <sup>2</sup> hand harvests from 134 DAP, 10-day intervals	-	Obtained from maturity hand harvest	

#### 5.2.4 Data analysis

One-way analysis of variance (ANOVA) with Tukey's post-hoc HSD was used to determine treatment differences in:

- Root length, root dry weight and Rt:Sht ratio in experiments 1 and 2.
- Height, leaf dry weight, stem dry weight and total dry weight in experiments 1 and 2.
- Total dry weight, LAI and Fr:Tot; at early squaring in experiment 8, at early flowering in experiments 8 and 9 and at mid flowering in experiment 8.
- Total number of fruiting sites, square counts and mean weights, total fruit retention at early squaring in experiment 8.
- Square and green boll counts and mean weights at early flowering in experiments 8 and 9.
- Square, green and open boll counts and mean weights at mid flowering in experiment 8.
- Yield components as final open boll counts and mean weights in experiment 8.
- Lint yield, turnout in experiment 8.
- Lint length, strength and micronaire in experiment 8.
- Volumetric soil moisture, cumulative to depth; at early and mid-flowering in experiment 8.
- Mean  $EC_t$  to depth at mid flowering in experiment 9.

A two-sided t-test (two sample, one variate with group factor) was utilised to determine treatment differences in;

- Total dry weight LAI and Fr:Tot; at early squaring in experiment 11, at early flowering in experiments 10 and 11 and at mid flowering in experiment 4.
- Total number of fruiting sites, square counts and mean weights, total fruit retention at early squaring in experiment 11.
- Square and green boll counts and mean weights at early flowering in experiments 10 and 11.
- Square, green and open boll counts and mean weights at mid flowering in experiment 4.

- Yield components as final open boll counts and mean weights in experiments 4, 5 and 11.
- Lint yield and turnout in experiments 4, 5 and 11.
- Lint length, strength and micronaire in experiments 4, 5 and 11.
- Stratified gravimetric soil moisture at mid flowering in experiments 9 and 10.

Generalised linear regression was used to determine treatment difference from the control in experiments 8 and 11, for progression to maturity (60% open boll). Descriptive methodologies of data analysis are described in Chapter 3, Section 8.

### **5.3 Results**

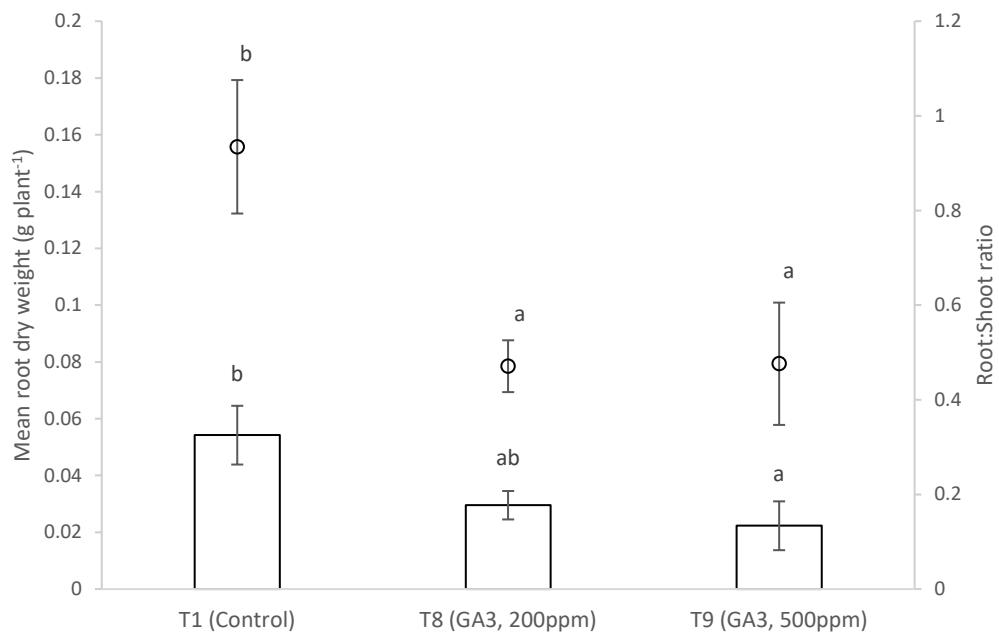
On account of prevailing climatic conditions, the presentation and analysis of results is sequential across seasons; being apportioned into a) direct above and below ground plant biomass response(s) to Gibberellin PGR treatments, with examination of resultant canopy capacity and access to soil water reserves and b) consequent plant and crop level responses to treatment impacts, as explained by; fruit development and retention, lint yield and lint quality outcomes. A similarly apportioned synopsis of results by specific Gibberellin is provided in Appendix 5.

#### **5.3.1 Glasshouse preliminary screening; experiments 1 and 2**

Experiments 1 and 2 were conducted under glass house conditions for the purpose of rapid screening of seed priming (experiment 1) and foliar (experiment 2) PGR treatment applications.

##### ***Gibberellin A<sub>3</sub> PGR impacts on root growth***

Experiment 1 (seed priming) was terminated with a biomass harvest at 13 days after planting (DAP) and demonstrated no treatment differences from the control in root length (P=0.199). The root dry weight of treatment 9 (P=0.014) and Root:Shoot ratios (P=0.004) of both GA<sub>3</sub> treatments being less than the control (Figure 5.1).

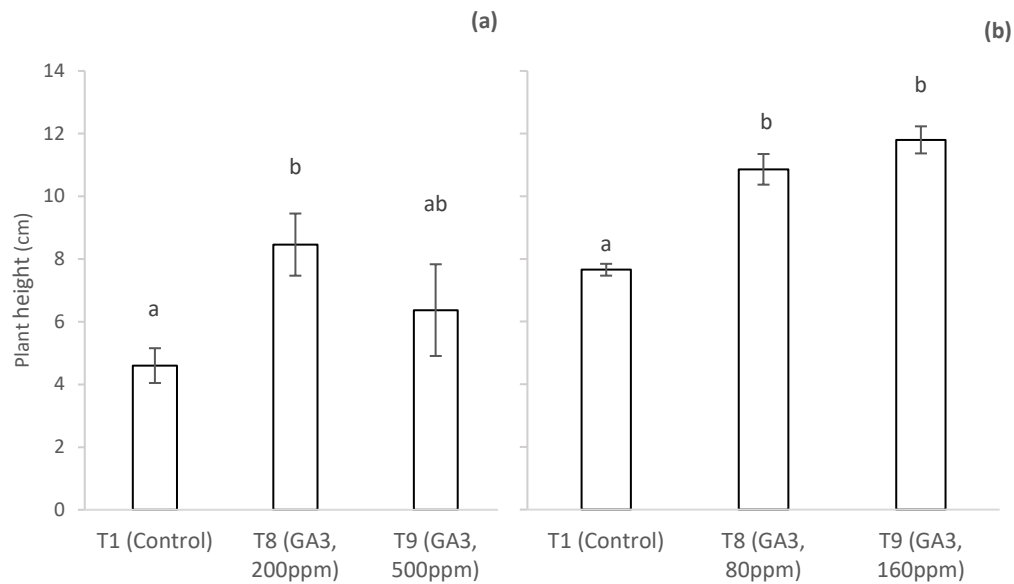


**Figure 5.1** Mean root dry weight (g) (bar) and Root:Shoot (Rt:Sht) ratio (○) at 13 DAP for seed priming GA<sub>3</sub> PGR treatments, grown under glasshouse conditions in experiment 1; notation indicating treatment differences at the P<0.05 level, error bars are +/-1 standard error of the mean.

Experiment 2 (foliar) was terminated with a biomass harvest at 24 DAP (8 days post treatment) and showed no treatment differences from the control (Trt. 1) in root length (P=0.720), root dry weight (P=0.729).

#### ***Gibberellin A<sub>3</sub> PGR impacts on above ground growth***

In experiment 1, height of treatment 8 at biomass harvest was higher than the control (P=0.038), however no consequent PGR treatment effect was evident in total dry matter accumulation (P=0.103) (Figure 5.2). Similar differences were evidenced in experiment 2, with both PGR treatments having mean heights higher than the control (Trt. 1) (P<0.01), with no differences in total dry weight (P=0.845) being evidenced at 24 DAP (Figure 5.2).



**Figure 5.2** mean seedling height (cm) at (a) 13 DAP for seed priming GA<sub>3</sub> PGR treatments grown under glasshouse conditions in experiment 1 and (b) 24 DAP for foliar GA<sub>3</sub> PGR treatments grown under glasshouse conditions in experiment 2; notation indicating treatment differences at the P<0.05 level, error bars are +/-1 standard error of the mean.

No treatment differences were evident for the consequent Root:Shoot ratio (P=0.500).

### 5.3.2 Gibberellin A<sub>3</sub> dosage assessment; season one field experiments 4 and 5

Experiments 4 and 5 were conducted over the 2016/17 summer production season at the ACRI and Bellata field sites, respectively, for the preliminary assessment of dosage in repetitive treatment applications.

#### *Gibberellin A<sub>3</sub> PGR impacts on above ground growth*

Mid flowering biomass harvest at 124 DAP in experiment 4 demonstrated no treatment differences from the control for foliar gibberellin applications (Trt. 8) in total dry weight accumulation (P=0.543), leaf area index (LAI) (P=0.196) or proportionate reproductive biomass accumulation (Fr:Tot) (P=0.499). Moreover, no treatment differences from the control were evidenced in square counts (P=0.746), square mean weights (P=0.835), green boll counts (P=0.409), or green boll mean weights (P=0.509).

#### *Plant and crop level responses*

End of season yield components for both experiment 4 and 5 demonstrated no differences from the control (Trt.1) in open boll counts (Table 5.3). Mean open boll weights in experiment 4 did not differ from the control (Trt.1) but were comparatively reduced in

experiment 5 (Table 5.3). Resultant machine harvest lint yields and ginning turnout in both experiment 4 and 5 did not differ from the control (Trt. 1) (Table 5.3).

**Table 5.3 Mean yield components, ginning and lint yield outcomes of Gibberellin PGR treatments in experiments 4 and 5; notation (\*) indicating significantly different means at the P=0.05 level using t-test.**

	Exp. 4 (2016.17 ACRI ERCF)	Exp. 5 (2016.17 BTA ERF)
<b>Open bolls (m<sup>2</sup>)</b>	(P=0.206)	(P=0.166)
Control	50.60	67.75
GA <sub>3</sub> , 2 L foliar, 80 mg.L <sup>-1</sup> , 3x 10 d	65.20	53.25
<b>Open boll mean weight (g)</b>	(P=0.764)	(P=0.041)
Control	2.6031	3.012
GA <sub>3</sub> , 2 L foliar, 80 mg.L <sup>-1</sup> , 3x 10 d	2.6640	2.329*
<b>Ginning turnout (%)</b>	(P=0.859)	(P=0.476)
Control	37.94	41.78
GA <sub>3</sub> , 2 L foliar, 80 mg.L <sup>-1</sup> , 3x 10 d	38.18	41.35
<b>Lint yield (kg lint/ha, machine harvested)</b>	(P=0.899)	(P=0.829)
Control	356.3	226.3
GA <sub>3</sub> , 2 L foliar, 80 mg.L <sup>-1</sup> , 3x 10 d	361.3	221.9

In experiment 4, mean treatment 1 (control) lint quality parameters fell just outside base grade (36, G5) classification (ACPM, 2019) due to a shorter staple length (Trt.1; 1.18” or “37”32<sup>nd</sup>) and higher micronaire (Trt.1; 5.186, G6). Staple strength of treatment 1 was classed as “very strong” (Trt.1; 34.06); no treatment differences from the control (Trt.1) occurred for lint length (P=0.381), lint strength (P=0.618) or micronaire (P=0.315). Treatment 1 (control) lint quality in experiment 5 also fell outside base grade classification, owing to shorter fibre length (Trt.1; 1.107” or 36 32<sup>nd</sup>); micronaire was within base grade classification (Trt.1; 4.990, G5) and strength was classed as “very strong” (Trt.1; 34.10). No treatment differences in lint length (P=0.153), strength (P=0.188) or micronaire (P=0.079) were evident in experiment 5.

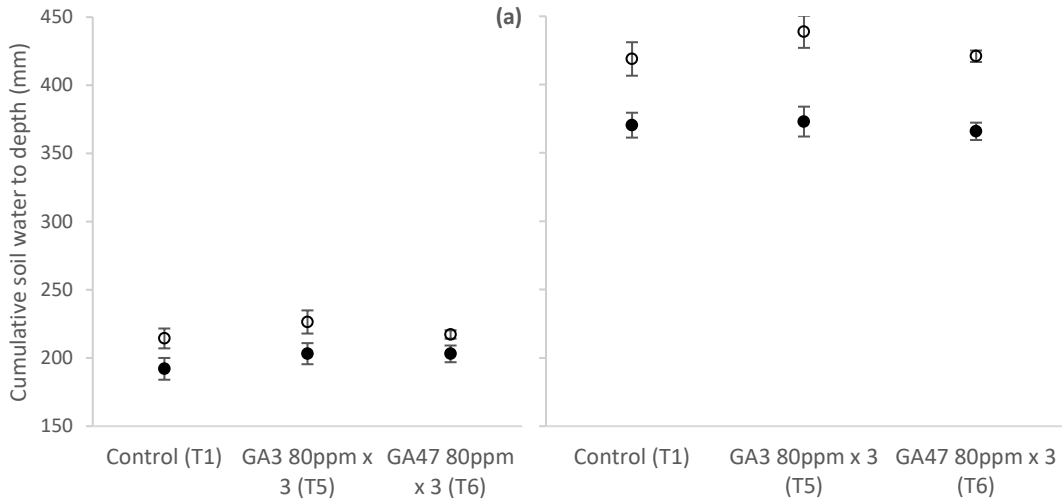
### 5.3.3 Direct comparison of GA<sub>3</sub> and GA<sub>4,7</sub> treatments; season two field experiment 8

Based at the Narrabri (ACRI) research site, experiment 8 evaluated both GA<sub>3</sub> and GA<sub>4,7</sub> PGR treatments during the 2017/18 summer production season.

#### ***Gibberellin A<sub>3</sub> and A<sub>4,7</sub> PGR impacts on root growth***

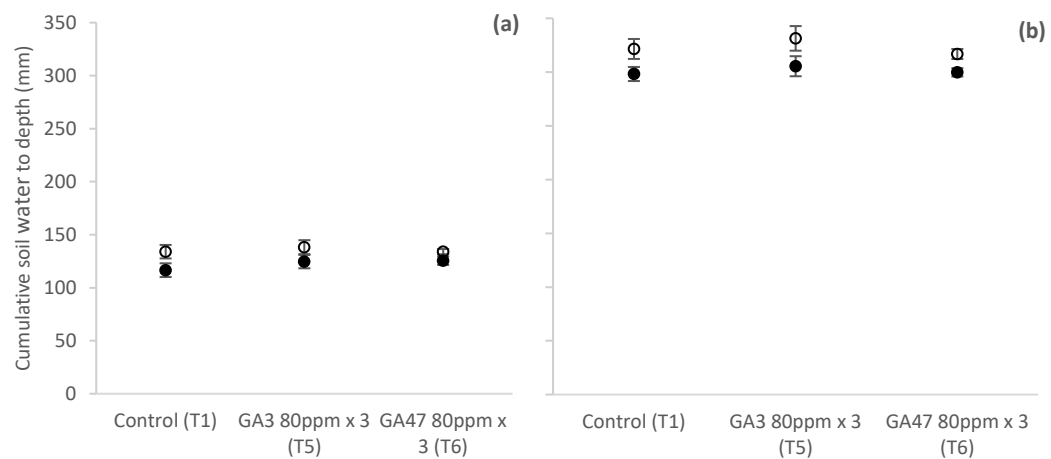
Due to logistical limitations associated with the temporal and spatial scale of investigation, cumulative soil moisture (mm) to depth was used as a proxy assessment of root growth. Prior calibration of weighted total apparent bulk soil electrical conductivity (EC<sub>t</sub>) to volumetric soil moisture at the experiment site, facilitated the use of non-destructive and time-efficient electromagnetic induction, to quantify cumulative volumetric soil moisture

(mm) to depth in experiment 8, as detailed in Chapter 3, Section 3.4.3. Analysis of cumulative soil moisture under both the plant line and skip row at early flowering (78 DAP) directly prior to a salvage irrigation (Chapter 3, Section 3.4.2, Table 3.5), showed no treatment differences from the control (Trt.1) in early season water extraction from the profile to both 0.75 cm and 1.5 m depth (Figure 5.3).



**Figure 5.3** mean cumulative soil water (mm) to (a) 0.75 m and (b) 1.5 m at early flowering (78 DAP) for Gibberellin PGR treatments in experiment 8; plant line (●), skip row (○), error bars being +/-1 standard error of the mean, no significant treatment differences being detected at the P=0.05 level using Tukey's HSD.

Cumulative soil moisture at mid flowering (110 DAP, first cracked boll) under both the plant line and skip row showed no treatment differences from the control (Trt. 1) in water extraction during reproductive growth from the profile, both to 0.75 m and 1.5 m depth (Figure 5.4).



**Figure 5.4** mean cumulative soil water (mm) to (a) 0.75 m and (b) 1.5 m at mid flowering (110 DAP) for Gibberellin PGR treatments in experiment 8; plant line (●), skip row (○), error bars being +/-1 standard error of the mean, no significant treatment differences being detected at the P=0.05 level using Tukey's HSD.

***Gibberellin A<sub>3</sub> and A<sub>4,7</sub> PGR impacts on above ground growth***

Early squaring, early and mid-flowering biomass harvests to assess growth and development against the control, were cut for all treatments in experiment 8 within 7 days of 50% population first square (“early squaring”, 55 DAP), first flower (“early flowering”, 81 DAP) and first cracked boll (“mid flowering”, 116 DAP). No treatment differences from the control (Trt. 1) were evident in total dry weight accumulation across the early squaring (P=0.843), early flowering (P=0.092) and mid flowering (P=0.683) sampling dates. Leaf area index (LAI) analyses showed no treatment differences from the control (Trt. 1) at early squaring or early flowering; Gibberellin A<sub>3</sub> (Trt. 5) having a higher LAI than the control (Trt. 1) at mid flowering (P=0.013) (Table 5.4).

**Table 5.4 mean LAI at early squaring (55 DAP), early flowering (81 DAP) and mid flowering (116 DAP), for Gibberellin PGR treatments in experiment 8; notation indicating treatment differences at the P=0.05 level using Tukey’s HSD.**

	Early squaring LAI	Early flowering LAI	Mid flowering LAI
	<i>(P=0.843)</i>	<i>(P=0.076)</i>	<i>(P=0.013)</i>
Control (Trt.1)	0.4841 <sup>(a)</sup>	3.031 <sup>(a)</sup>	4.605 <sup>(a)</sup>
GA <sub>3</sub> 80 ppm (Trt. 5)	0.4792 <sup>(a)</sup>	2.631 <sup>(a)</sup>	5.965 <sup>(b)</sup>
GA <sub>4,7</sub> 80 ppm (Trt. 6)	0.4593 <sup>(a)</sup>	3.093 <sup>(a)</sup>	5.428 <sup>(ab)</sup>

Reproductive growth proportionate to total biomass accumulation (Fr:Tot) at early squaring was reduced (compared to the control, Trt.1), in the Gibberellin A<sub>3</sub> treatment (Trt. 5) only. Both treatments produced a higher number of total sites (also indicative of more nodes), but without associated increases in biomass had no capacity to support the additional sites, resulting in reduced retention. No total biomass differences from the control (Trt. 1) were evidenced at early squaring, Gibberellin A<sub>3</sub> (Trt. 5) grew an increased number of total sites, however the majority of squares (presumably pinhead or immature) were then shed with resultant decreased square numbers and retention percentage being evident (Table 5.7). Gibberellin A<sub>4,7</sub> (Trt. 6) had similar (to Trt. 1) numbers of total sites developed at early squaring, comparable square shedding and resultant number of retained squares, although retention was lower than the control (Trt. 1) (Table 5.5). No differences from the control (Trt. 1) in mean square weight (g) were evidenced (P=0.438).

**Table 5.5 Reproductive growth impacts of Gibberellin PGR treatments at early squaring (55 DAP) in experiment 8, as characterised by total fruiting sites (count.m<sup>-2</sup>), number of squares (count.m<sup>-2</sup>), early square shedding (count.m<sup>-2</sup>) and total fruit retention rates (%); notation indicating treatment differences at the P=0.05 level using Tukey's HSD.**

	Total fruiting sites (count.m <sup>-2</sup> )	Squares (count.m <sup>-2</sup> )	Shed sites (count.m <sup>-2</sup> )	Retention (%)
	<i>(P=0.006)</i>	<i>(P=0.017)</i>	<i>(P&lt;0.001)</i>	<i>(P&lt;0.001)</i>
Control (Trt.1)	31.18 <sup>(a)</sup>	23.5 <sup>(b)</sup>	7.68 <sup>(a)</sup>	79.58 <sup>(c)</sup>
GA <sub>3</sub> 80 ppm (Trt. 5)	60.22 <sup>(b)</sup>	8.29 <sup>(a)</sup>	51.94 <sup>(b)</sup>	14.16 <sup>(a)</sup>
GA <sub>4,7</sub> 80 ppm (Trt. 6)	24.95 <sup>(a)</sup>	15.75 <sup>(ab)</sup>	9.2 <sup>(a)</sup>	59.37 <sup>(b)</sup>

No differences in Fr:Tot were evident from the control (Trt. 1) at early flowering (P=0.061) (81 DAP). Resultantly no Gibberellin treatment differences from the control (Trt. 1) occurred in square (P=0.402) or green boll (P=0.279) counts, or square (P=0.200) or green boll (P=0.150) mean weights, at early flowering. Although mid-flowering (116 DAP) reproductive proportionate biomass (Fr:Tot) was reduced for both Gibberellin treatments, mean square numbers of the Gibberellin A<sub>3</sub> (Trt. 5) treatment was higher than the control (Trt. 1) (Table 5.6). No corresponding differences in mean square weight occurred (P=0.139). Moreover, no statistical differences in treatment mean green boll counts occurred, although both Gibberellin treatments had smaller mean green boll weights; the weight of evidence suggesting a possible delay in (notably) the Gibberellin treatments (notably Trt. 5) fruit development due to earlier shedding (Table 5.8). No differences occurred from the control (Trt. 1) in either mean open boll counts (P=0.459) or mean weights (P=0.401).

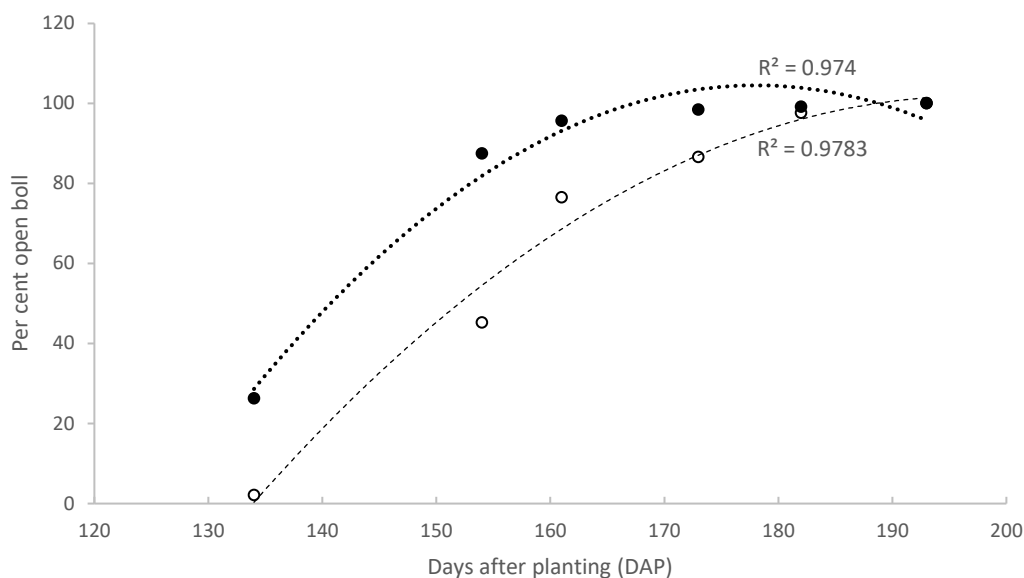
**Table 5.6 Reproductive growth impacts of Gibberellin PGR treatments at mid flowering (116 DAP) in experiment 8, as characterised by reproductive biomass accumulation proportionate to total (Fr:Tot), mean number of squares (count.m<sup>-2</sup>), mean square weight (g), mean number of green bolls (count.m<sup>-2</sup>) and mean green boll weight (g); notation indicating treatment differences at the P=0.05 level using Tukey's HSD.**

	Fr:Tot	Squares (count.m <sup>-2</sup> )	Avg square weight (g)	Green bolls (count.m <sup>-2</sup> )	Avg green boll weight (g)
	<i>(P&lt;0.001)</i>	<i>(P&lt;0.001)</i>	<i>P=0.139</i>	<i>(P=0.057)</i>	<i>(P&lt;0.001)</i>
Control (Trt.1)	0.5368 <sup>(c)</sup>	41 <sup>(a)</sup>	0.1662 <sup>(a)</sup>	274.3 <sup>(a)</sup>	3.161 <sup>(c)</sup>
GA <sub>3</sub> 80 ppm (Trt. 5)	0.2638 <sup>(a)</sup>	355.9 <sup>(b)</sup>	0.1233 <sup>(a)</sup>	400.9 <sup>(a)</sup>	0.895 <sup>(a)</sup>
GA <sub>4,7</sub> 80 ppm (Trt. 6)	0.4710 <sup>(b)</sup>	109 <sup>(a)</sup>	0.1448 <sup>(a)</sup>	305.4 <sup>(a)</sup>	2.4296 <sup>(b)</sup>

Generalised linear regression of the square root of sequential boll opening across treatments 5 and 6 in experiment 8, explained treatment delays in maturity (as 60% open boll), for treatment 5 only (P<0.001); trend lines were fitted (Figure 5.5) and resulting calendar days to 60% open boll are presented in Table 5.7.

**Table 5.7 Mean calendar days to maturity as 60% open boll load for treatment 5 in experiment 8; all R<sup>2</sup> values being greater than 0.974.**

Treatment	60% open boll (DAP)	Days>Trt.1
Control (Trt.1)	144.32	-
GA <sub>3</sub> 80 ppm (Trt. 5)	155.98	11.65



**Figure 5.5 Progression to maturity (60% open boll) in experiment 8 of treatment 3; Control (Trt. 1) (...●....), treatment 5 (- - -○- - -).**

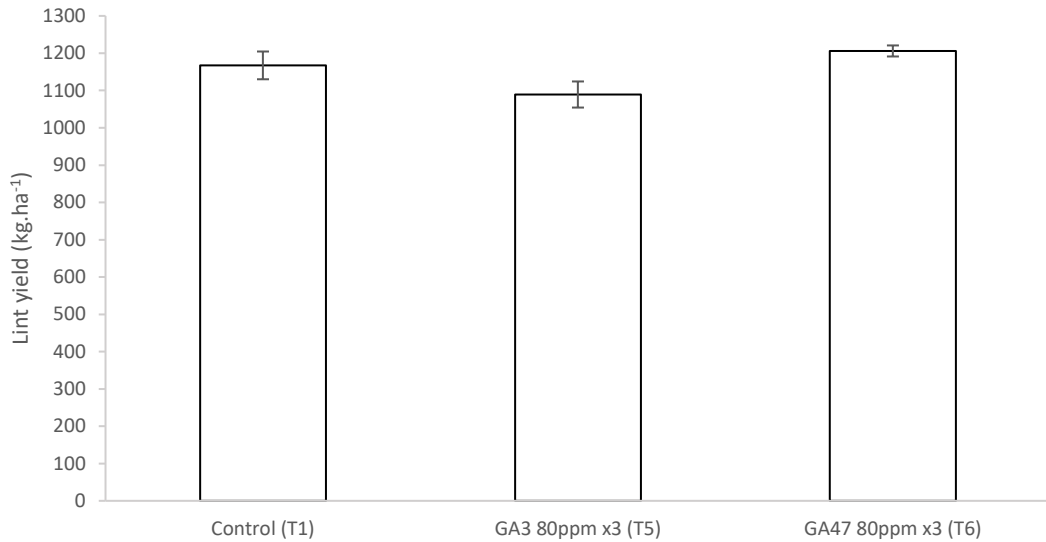
### *Plant and crop level responses*

No treatment differences occurred for final open boll numbers (m<sup>2</sup>) (P=0.677) although mean boll weights were decreased for both Gibberellin treatments (P=0.002) (Table 5.8)

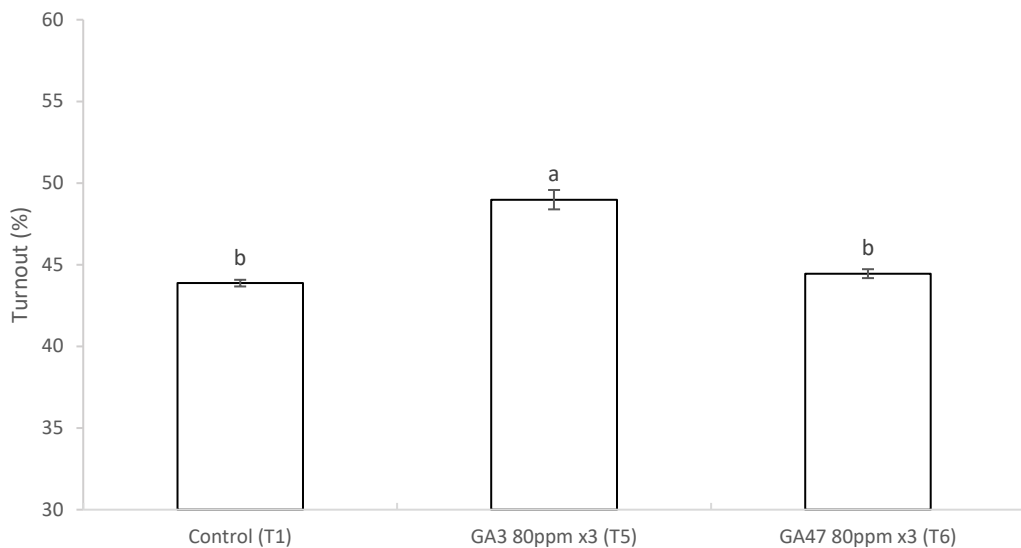
**Table 5.8 yield components of Gibberellin PGR treatments 5 and 6 in experiment 8; notation indicating treatment differences at the P=0.05 level using Tukey's HSD.**

	Open bolls (m <sup>2</sup> )	Open boll mean weight (g)
	(P=0.677)	(P=0.002)
Control (Trt.1)	130 <sup>(a)</sup>	4.047 <sup>(b)</sup>
GA <sub>3</sub> 80 ppm (Trt. 5)	131.8 <sup>(a)</sup>	3.498 <sup>(a)</sup>
GA <sub>4,7</sub> 80 ppm (Trt. 6)	136 <sup>(a)</sup>	3.705 <sup>(a)</sup>

No statistically significant treatment differences were evidenced in machine harvested cotton yields (P=0.076) (Figure 5.6), however Gibberellin A<sub>3</sub> (Trt. 5) had a higher ginning turnout (P<0.001) than both the control (Trt. 1) and Gibberellin A<sub>4,7</sub> treatment (Trt. 6) (Figure 5.7).



**Figure 5.6 Mean machine harvested cotton lint yield for Gibberellin PGR treatments 5 and 6 in experiment 8; error bars being +/- standard error of the mean, no significant treatment differences occurring at the P=0.05 level using Tukey's HSD.**



**Figure 5.7 Mean ginning turnout (%) for Gibberellin PGR treatments 5 and 6 in experiment 8; error bars being +/- standard error of the mean, notation indicating treatment differences at the P=0.05 level using Tukey's HSD.**

Mean treatment 1 (control) lint quality parameters fell just outside base grade (36, G5) classification; with shorter staple length (Trt. 1; 1.147: or “37” 32<sup>nd</sup>) and higher micronaire (Trt. 1; 5.003, G6); staple strength was classed as “strong” (Trt. 1; 30.05). Gibberellin A<sub>3</sub> treatment (Trt. 5) mean staple length was shorter (P=0.035) than the control (Trt. 5; 1.102 or “33” 32<sup>nd</sup>); no staple length difference occurred between Gibberellin A<sub>4,7</sub> (Trt. 6) and the control (Trt. 1). No treatment differences occurred from the control for lint strength (P=0.436) or micronaire (P=0.152) in experiment 8.

### 5.3.4 Gibberellin treatment comparison and GA<sub>4,7</sub> dosage assessment; season three field experiments 9, 10 and 11

Experiments 9, 10 and 11 were conducted over the 2018/19 summer season, being located at the ACRI, Bellata and Terry Hie Hie sites, respectively. As described in Chapter 4, experiments were conducted under challenging environmental conditions; requiring replants (experiment 9), requiring late plantings in less than optimal soil moisture conditions (experiment 10) and in purposefully-early-planted experiment 11, experiencing unseasonal yield-limiting and season-ending lack of January / February / March precipitation (drought conditions). Experiments 9 and 10 were deliberately terminated at mid-flowering in May 2019.

#### *Gibberellin A<sub>3</sub> and A<sub>4,7</sub> PGR impacts on root growth*

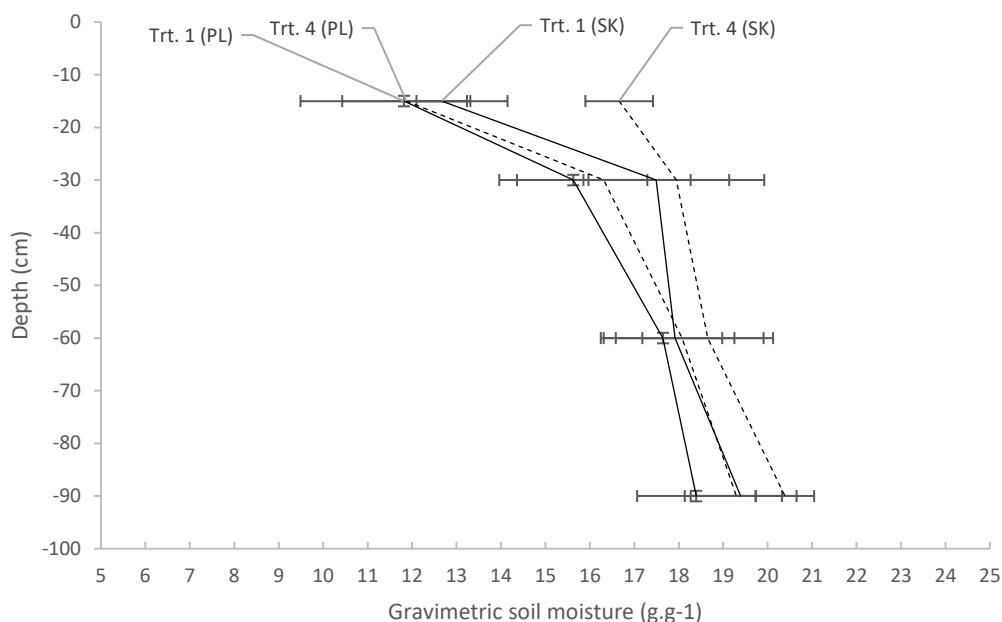
As in season two, EC<sub>t</sub> was used as a proxy assessment of root growth under both the plant line and skip row, across all treatments and reps. Analysis of mean EC<sub>t</sub> to 1.5 m, at mid-flowering (109 DAP) both under both the plant line and skip row, showed no treatment differences from the control (Trt.1) (Table 5.9); methodology being detailed in Chapter 3, section 3.4.2.

**Table 5.9 Mean total Electromagnetic Conductivity (EC<sub>t</sub>) (mS.m<sup>-1</sup>) of Gibberellin PGR treatments 4 and 5, at mid flowering (109 DAP) under the plant line and adjacent skip row to 1.5m depth, in experiment 9; notation indicating treatment differences at the P=0.05 level using Tukey's HSD.**

	EC <sub>t</sub> Plant line (mS.m <sup>-1</sup> )	EC <sub>t</sub> Skip row (mS.m <sup>-1</sup> )
	( <i>P</i> =0.897)	( <i>P</i> =0.713)
Control (Trt.1)	58.52	56.95
GA <sub>4,7</sub> 80 ppm (Trt. 4)	57.49	57.67
GA <sub>3</sub> 80 ppm (Trt. 5)	57.59	58.18

As volumetric soil moisture had not been previously calibrated to EC<sub>t</sub> at the replant site used in experiment 9, soil cores (gravimetric soil moisture) and core break sampling (root presence) were used to further validate EC<sub>t</sub> results. Mid-flowering (110 DAP) core break sampling in experiment 9 for the binary presence / absence of cotton roots, demonstrated no differences in root growth between the control (Trt. 1) and Gibberellin A<sub>4,7</sub> (Trt. 4); new season cotton roots being detected at both the plant line and skip row, for each replicate in both treatments (Trt. 1 and 4). T-test analysis of corresponding gravimetric soil moisture of stratified core samples, demonstrated differences in shallow (0-15 cm) skip row soil moisture; the control (Trt. 1) being drier than the Gibberellin A<sub>4,7</sub> treatment (Trt. 4) mean

(Figure 5.8, Table 5.10). Otherwise, no gravimetric soil moisture differences from the control (Trt.1) were evidenced under both the plant line and adjacent skip row, to depth (Figure 5.8, Table 5.10).



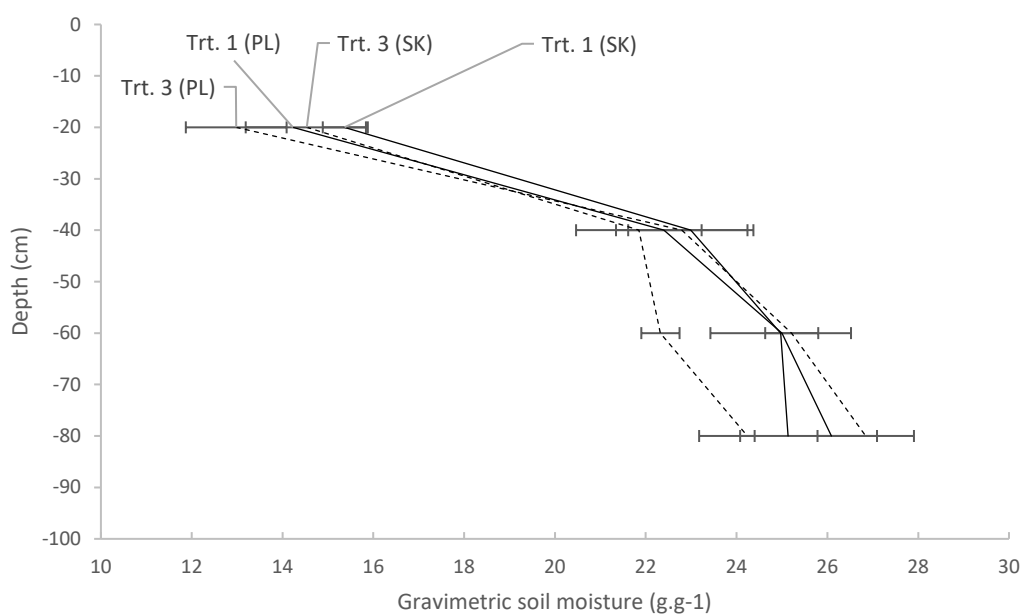
**Figure 5.8 Mid flowering gravimetric soil moisture per location and depth at 110 DAP in experiment 9; control (Trt. 1) (—), treatment 4 (- - -), error bars are +/- SE of the mean, notation delineating plant line (PL) and skip row (SK) locations.**

**Table 5.10 T-Test P values for comparison of control (Trt. 1) and treatment 4 mean gravimetric soil moisture content per depth and row location, at mid flowering (110 DAP) in experiment 8.**

Plant Line	P value	Skip	P value
15 cm	0.986	15 cm	0.014
30 cm	0.802	30 cm	0.867
60 cm	0.858	60 cm	0.730
90 cm	0.620	90 cm	0.520

As with experiment 9, no calibration existed for  $EC_t$  to volumetric soil moisture for the Bellata location of experiment 10; resultantly, soil cores and core break sampling were utilised to determine stratified gravimetric soil moisture and quantify the presence / absence of roots at mid-flowering (91 DAP). No differences were found in root growth between the control (Trt. 1) and Gibberellin A4,7 (Trt. 3); new season cotton roots being detected at both the plant line and skip row, for each replicate in both treatments (Trt. 1 and 3). T-test of corresponding gravimetric soil moisture of stratified core samples demonstrated differences plant line soil moisture at 60 cm; the Gibberellin A4,7 treatment (Trt. 3) being drier than the control (Trt. 1) mean (Figure 5.9, Table 5.11). Otherwise, no gravimetric

soil moisture differences from the control (Trt. 1) were evidenced under both the plant line and adjacent skip row, to depth (Figure 5.9, Table 5.11).

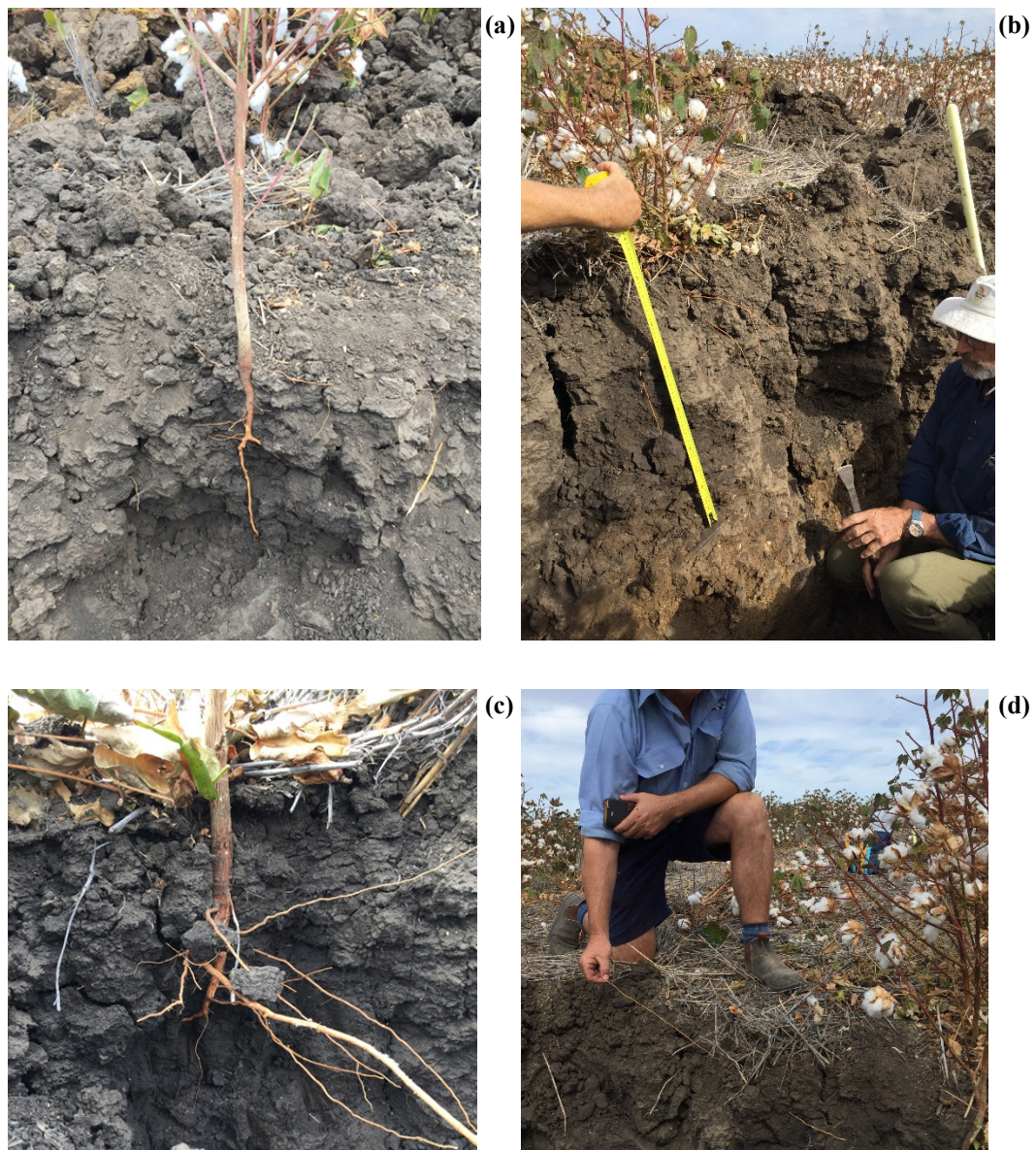


**Figure 5.9** Mid flowering gravimetric soil moisture per location and depth at 91 DAP in experiment 9; control (Trt. 1) (—), treatment 3 (- - -), error bars being +/- 1 SE of the mean, notation delineating plant line (PL) and skip row (SK) locations.

**Table 5.11** T-Test P values for comparison of control (Trt. 1) and treatment 3 mean gravimetric soil moisture content per depth and row location, at mid flowering (91 DAP) in experiment 10.

Plant Line	P value	Skip	P value
20 cm	0.892	20 cm	0.120
40 cm	0.743	40 cm	0.931
60 cm	0.020	60 cm	0.890
80 cm	0.399	80 cm	0.486

Qualitative examination of root architecture via excavation of soil pits occurred post defoliation (168 DAP) in experiment 11; methodology being explained in Chapter 3, Section 3.4.3. Additional to an increased stem diameter (cotyledon) of the Gibberellin A<sub>4,7</sub> treatment (Trt. 6) relative to the control (Trt. 1), examination of root architecture showed an increased tap root length and significant lateral root exploration into the skip row, of the Gibberellin A<sub>47</sub> treatment (Trt. 6), not being evident in the control (Trt. 1) plot (Figure 5.10).



**Figure 5.10** Post defoliation soil pit exploration in experiment 11; qualitative evaluation of root architecture comparative to (a) the control (Trt. 1) plot, demonstrated (b) an increased tap root length and (c, d) significant lateral root exploration of the skip row.

***Gibberellin A<sub>3</sub> and A<sub>4,7</sub> PGR impacts on above ground growth***

An early-flowering (66 DAP) biomass harvest was cut to assess growth and development of gibberellin treatments (Trt. 4 and 5) against the control (Trt. 1) in experiment 9. No treatment differences from the control (Trt. 1) were evident for total biomass accumulation (P=0.864), leaf area index (LAI) (P=0.401) or proportionate reproductive biomass (Fr:Tot) (P=0.116). No treatment differences in square counts were evident (P=0.186), although Gibberellin A<sub>3</sub> treatment (Trt. 5) had a reduced mean square weight compared to the control

(Trt. 1) ( $P=0.019$ ). No treatment differences were evident in either green boll (including white flower) counts ( $P=0.671$ ) or green boll mean weight ( $P=0.287$ ) at early flowering.

Early flowering biomass assessments in experiment 10 (66 DAP) also evidenced no treatment differences in total dry weight accumulation ( $P=0.719$ ) or proportionate reproductive biomass accumulation ( $P=0.176$ ); no LAI was assessed due to biosecurity limitations. Furthermore, no treatment differences were evident in square counts ( $P=0.184$ ), mean square size ( $P=0.134$ ), green boll counts ( $P=0.782$ ) or mean green boll weights ( $P=0.092$ ). In (early planted) experiment 11, the Gibberellin A<sub>47</sub> treatment (Trt. 6) demonstrated no differences from the control in total biomass accumulation, LAI or Fr:Tot at both early squaring (74 DAP) or early flowering (96 DAP) (Table 5.12).

**Table 5.12 Mean total dry weight (g), Leaf Area Index (LAI) and proportionate reproductive dry weight (Fr:Tot) for Gibberellin A<sub>47</sub> treatment (Trt. 6) in experiment 11; notation (\*) denoting treatment differences at the  $P=0.05$  level using t-test.**

	Early squaring (74 DAP)	Early flowering (96 DAP)
<b>Total dry weight (g)</b>	<i>(P=0.179)</i>	<i>(P=0.268)</i>
Control (Trt.1)	140.8	315.7
GA <sub>4,7</sub> 80 ppm (Trt. 6)	126.5	248.5
<b>LAI</b>	<i>(P=0.151)</i>	<i>P=0.426)</i>
Control (Trt.1)	0.8411	2.512
GA <sub>4,7</sub> 80 ppm (Trt. 6)	0.7487	2.271
<b>Fr:Tot</b>	<i>(P=0.099)</i>	<i>(P=0.474)</i>
Control (Trt.1)	0.0068	0.1424
GA <sub>4,7</sub> 80 ppm (Trt. 6)	0.0021	0.1145

At early squaring, no treatment differences occurred in either square counts ( $P=0.065$ ) or mean square weights ( $P=0.957$ ); however, the Gibberellin A<sub>47</sub> treatment (Trt. 6) grew a greater amount of fruiting sites, had a higher rate of shedding and had a lower rate of total fruit retention than the control (Trt. 1) (Table 5.13). By early flowering (96 DAP) in experiment 11, no treatment differences were evident in square counts ( $P=0.973$ ), although the Gibberellin A<sub>47</sub> (Trt. 6) mean square weight (0.0775 g) was less than the control (Trt. 1; 0.0952 g) ( $P=0.004$ ). No treatment differences were evident in green boll counts ( $P=0.090$ ) or mean boll weights ( $P=0.057$ ).

**Table 5.13 Reproductive growth impacts of Gibberellin PGR treatment (Trt. 6) at early squaring (74 DAP) in experiment 11, as characterised by the mean number of squares (count.m<sup>-2</sup>), mean square weight (g), total number of fruiting sites (count.m<sup>-2</sup>), aborted fruit (count.m<sup>-2</sup>) and total fruit retention (%); asterix (\*) denoting treatment differences at the P=0.05 level using t-test.**

	Squares (count.m <sup>-2</sup> )	Avg square weight (g)	Total sites (count.m <sup>-2</sup> )	Shed sites (count.m <sup>-2</sup> )	Retention (%)
	(P=0.065)	(P=0.957)	P=0.028	(P=0.012)	(P=0.042)
Control (Trt.1)	10.918	0.5403	62.95	51.33	19.47
GA <sub>4,7</sub> 80 ppm (Trt. 6)	1.556	0.5667	91.32*	89.77*	1.67*

Generalised linear regression of the square root of sequential boll opening of Gibberellin A<sub>47</sub> treatment (Trt. 6) suggested no treatment causal effect of any delay in progression to maturity (taken as 60% open boll) (P=0.162).

#### ***Plant and crop level responses***

No treatment differences occurred for final open boll numbers (m<sup>2</sup>) (P=0.172) or mean boll size (weight, g) (P=0.757). No treatment differences were evident in lint yield, ginning turnout, lint length, strength or micronaire (Table 5.14)

**Table 5.14 Mean yield components, cotton lint yield and lint quality outcomes for Gibberellin A<sub>47</sub> PGR treatment (Trt. 6) in experiment 11; asterix (\*) denoting treatment differences at the P=0.05 level using t-test.**

	Open bolls (No.m <sup>-2</sup> )	Avg. open boll weight (g)	Lint yield (kg.ha <sup>-1</sup> )	Ginning turnout (%)	Lint length (" , 32 <sup>nd</sup> )	Lint strength (g.tex <sup>-1</sup> )	Micronaire
	(P=0.172)	(P=0.757)	(P=0.400)	(P=0.602)	(P=0.213)	(P=0.715)	(P=0.127)
Control (Trt.1)	174.7	3.336	702.8	48.37	1.062, 34	27.05	3.333
GA <sub>4,7</sub> 80 ppm (Trt. 6)	159.0	3.381	656.4	48.68	1.035, 33	26.72	3.700

## **5.4 Discussion**

Prevailing hot and dry climatic conditions (Appendix 3) and the impact of isolated extreme climatic events across experiments included within this analysis, adversely influenced crop growth and subsequent yield potentials. However, lint yields achieved in the four crops grown to harvest in this study, were at or above the respective mean annual Australian rain grown cotton yields (ABARES, 2019; CA, 2018b). Discussion of the resulting outcomes is partitioned in line with overall experiment aims; primary Gibberellin PGR treatment impacts on above and below ground growth, with corollary plant and crop level responses.

#### **5.4.1 Primary Gibberellin PGR treatment impacts on plant above and below ground biomass accumulation, resultant canopy capacity and access to soil water resources.**

##### ***Root biomass accumulation and accession of soil water reserves***

Early controlled environment experiments to screen multiple GA<sub>3</sub> treatment concentration and application modalities verified no impacts on root length for both seed priming and 2 leaf foliar applications (experiments 1 and 2, respectively). Root dry weight was reduced by high concentration seed priming (experiment 1) with a similar magnitude reduction (although not statistically significant) demonstrated by the lower concentration, longer exposure seed priming treatment. No changes in root dry weight were caused by foliar applications. Consequent root to shoot proportionate dry weight ratios were reduced in both seed priming treatments (experiment 1), with no such differences from the control evident for foliar treatments in experiment 2. Under field conditions in experiment 8 at both early and mid-flowering, no GA<sub>3</sub> or GA<sub>4,7</sub> treatment differences were evidenced for cumulative volumetric soil moisture to both 0.75 m and 1.5 m depth, under both the plant line and skip row. These findings were supported by comparative EC<sub>t</sub> assessments in experiment 9, indicating no differences in electrical conductivity (as a proxy for soil water) between control, GA<sub>3</sub> and GA<sub>4,7</sub> treatments, across all replicates. Additionally, comparative assessment of gravimetric soil moisture at mid flowering in experiments 9 and 10, demonstrated no overall increased accession of soil water reserves by Gibberellin A<sub>4,7</sub> treatments both under the plant line and adjacent skip row.

Qualitative assessment of root growth and lateral root architecture across comparative soil pits in experiment 11 indicated increased lateral root exploration of the skip row soil space comparative to the control. However quantitative binary characterisation by core-break sampling of GA<sub>4,7</sub> treatments during mid-season reproductive growth in experiments 9 and 10 confirmed no treatment differences from the control in the presence of live cotton roots to 1 m depth, both under the plant line and adjacent skip row. Later season growth in experiment 11 being possibly attributable increased mid-late-season water availability associated with early fruit shedding (comparatively decreased retention to experiments 9 and 10) decreasing early growth stage water use.

Weight of evidence from quantitative analysis of both glasshouse and field experiments indicated no positive treatment impacts of either GA<sub>3</sub> or GA<sub>4,7</sub> PGR treatment applications on root biomass accumulation or associated plant utilisation of soil water reserves; across multiple rate x application method x frequency / timing application combinations. These

outcomes substantiate previous findings by Ergle (1958), demonstrating no treatment differences in root dry weight accumulation for consecutive, early vegetative growth stage foliar applications of GA<sub>3</sub> under glasshouse conditions. However, these investigations contrast to previous research in cotton by Zhao and Oosterhuis (1997) who noted increased root dry weight under water limited conditions following foliar treatment with composite proprietary product PGR-IV, containing GA<sub>3</sub>. Such differences in outcomes are conceivably attributable to the stimulatory impact of the concurrent product component in PGR-IV, indole butyric acid (IBA), on cotton root growth (Urwiler & Oosterhuis, 1986). These findings also differ from those of Onanuga et al. (2012) who noted increased root absorption area and volume at 30 days post treatment, following triplicate 20-day consecutive foliar sprays of comparatively lower concentration GA<sub>3</sub>, in hydroponically grown cotton. Apart from differences in treatment timing and dosage concentration, plausible reasons for lack of correlation between plant responses to these GA treatments being significant physical, chemical and biological differences between glasshouse / field and hydroponic (growth chamber) production environments; having influence on the rhizosphere and consequent plant growth and response to PGR treatment (Hedden, Rademacher, & Phillips, 2010; Rademacher, 2015; Watt, Silk, & Passioura, 2006).

#### ***Shoot biomass accumulation and canopy capacity***

Significant GA<sub>3</sub> PGR treatment increases in plant height to both foliar and low concentration seed priming applications, were evidenced in early controlled environment experiments 1 and 2. Increased mean seedling height at two weeks post application was also evident in the higher concentration seed priming treatment, but was not significant via Tukey's HSD. All controlled environment GA<sub>3</sub> foliar and seed priming treatments in experiments 1 and 2 demonstrated no effect on total dry matter accumulation. Likewise in field experiments, no GA<sub>3</sub> foliar treatment differences in dry weight accumulation were evidenced at early flowering in experiments 8 and 9, or mid flowering in experiments 4 and 8. Similarly, GA<sub>4,7</sub> treatments evidenced no effects on total dry weight accumulation; at early squaring in experiments 8 and 11, at early flowering in experiments 8, 9, 10 and 11, and at mid flowering in experiment 8.

No gibberellin A3 PGR treatment impacts on canopy capacity, as described by leaf area index (LAI), were verified at early squaring (experiment 8) or early flowering (experiment 8 and 9). However, GA<sub>3</sub> PGR treatment increased LAI at mid flowering in experiment 8, with no treatment difference in LAI evidenced at the same time in experiment 4.

Correspondingly, GA<sub>4,7</sub> treatments showed no effects on LAI accumulation; at early squaring in experiments 8 and 11, at early flowering in experiments 8, 9 and 11, and at mid flowering in experiment 8. Synopsis of findings for both total dry weight accumulation and corresponding differences in LAI suggest no consistent contributory impact of the specific Gibberellin PGR treatments scenarios evaluated. Height impacts of GA<sub>3</sub> treatments demonstrated in controlled environment experiments, agree with the findings of Ergle (1958), Hansen et al. (1996) and Larson et al. (1997) also being foliar treatments under controlled environment conditions. Ergle's (1958) findings also correspond with the overall lack of both GA<sub>3</sub> and GA<sub>4,7</sub> treatment differences in total dry weight accumulation and leaf area index.

Documented benefits associated with Gibberellin PGR treatment impacts in cotton, occur due to improvements in both the scale and efficiency of plant assimilate production. Contrasting to findings reported in this chapter, Hansen et al. (1996) notes an increased LAI and corresponding canopy P<sub>n</sub> rate in response to a single foliar treatment of GA<sub>3</sub> at 7-leaf stage in glasshouse grown cotton. Similar findings were reported by Larson et al. (1997) under irrigated field conditions, with a corresponding increase in lint yield following a single vegetative stage foliar application. Recently, Fang et al. (2019) has also demonstrated the utility of (lower concentration and duration) GA<sub>3</sub> seed priming to induce a positive leaf (as opposed to canopy) P<sub>n</sub> effect in irrigated field grown cotton, with associated increase in leaf soluble carbohydrate content, translocation and floral bud starch content; leading to boll size, boll retention and lint yield benefits. J. C. F. da Costa et al. (2017) also notes increased leaf photosynthetic pigment (Chlorophyll a and b, Carotenoid) content in irrigated field grown cotton at 90 days post the first vegetative stage foliar GA<sub>3</sub> treatment.

#### **5.4.2 Gibberellin PGR treatment impacts on reproductive development, lint yield and quality outcomes.**

Early squaring Gibberellin PGR treatment responses in experiments 8 and 11, both demonstrated an increased development of fruiting sites with contrasting decrease in fruit retention and subsequent total square counts. No changes in mean square weights are evident for either the GA<sub>3</sub> treatment in experiment 8 or GA<sub>4,7</sub> treatments in experiments 8 and 11. Flow on gibberellin 4,7 treatment impacts on reproductive growth at early flowering in experiments 8, 9, 10 and 11 show no differences in proportionate reproductive biomass (Fr:Tot) accumulation, square number or size, or green boll number or size.

However, a decreased mean green boll weight at mid flowering in experiment 8, accompanied by no other changes to square, green or open boll numbers or weights, indicated a delay in fruit development consequent from reduced early squaring retention. Similarly, early flowering Gibberellin A3 flow on treatment impacts, in experiments 8 and 9, show no differences in square or green boll counts, with reduced mean square and green boll mean weights. This delay in maturity due to decreased early retention is further evidenced as a smaller mid flowering green boll size in experiment 8.

These findings are supported by earlier outcomes reported by Bornman, Addicott, and Spurr (1966), Bornman, Spurr, and Addicott (1967), Lyon and Smith (1966) and Mathur and Mittal (1964); associating Gibberellin (notably GA<sub>3</sub>) treatments with an increased cell proliferation and cellular starch content in the abscission zone, with consequent increased abscission rates following exogenous PGR treatment. However, Mathur and Mittal (1964), as well as recently, Fang et al. (2018), substantiated exogenous GA<sub>3</sub> induction of an increased number of fruiting sites, that despite decreased retention rates, also (in contrast) resulted in a net increase in the number of flowers following a later application timing. Fang et al. (2019) noting the role of increased supply of soluble carbohydrates (via increased leaf Pn) in retaining bolls and increasing lint yield. Walhood (1958) also validated early abscission findings, noting a later maturation and decreased open boll size following exogenous GA<sub>3</sub> application in irrigated cotton; suggesting impacts of earlier abscission events.

Resulting yield component outcomes of no differences in open boll numbers for both GA<sub>4,7</sub> and GA<sub>3</sub> treatments concurred with Subbiah and Mariakulandia (1972) but differed from recent work by Fang et al. (2019) which was associated with the afore mentioned increase in leaf net photosynthesis in irrigated cotton. Decreases in GA<sub>3</sub> open boll size in experiments 5 and 8 corroborate with later maturity outcomes documented by Walhood (1958) and the relative increased efficacy of gibberellin A3 compared to both A7 and A4 as noted by Lyon and Smith (1966). Bhatt and Ramanujam (1971) demonstrate an increased fibre length associated with exogenous GA<sub>3</sub> in irrigated cotton, being in direct contrast to the lack of length difference quantified in both GA<sub>3</sub> and GA<sub>4,7</sub> rain grown experiments. Relative water deficits associated with rain grown production may account for differences in plant and crop level responses to Gibberellin PGR treatments. Primarily, cell turgor (associated with water availability) impacts the plant's capacity to capitalise on Gibberellin PGR induced increased cell wall plasticity (Rademacher, 2000) and resultant elongation that facilitates increased biomass and LAI.

## 5.5 Conclusions

No evidence was found from quantitative analysis of both field and controlled environment experiments, of root biomass accumulation or associated increased plant utilisation of soil water reserves. Moreover, demonstrated PGR treatment impacts on plant height was not associated with any changes in total above ground dry matter accumulation or canopy capacity (as LAI). However, increased height was associated with an increased production of nodes and associated fruiting sites, which on account of lack of canopy capacity and presumably resultant resources, shed their early squares, resulting in decreased square numbers and retention percentages during early reproductive growth. This is in contrast to results from some non-resource limited irrigated scenarios where increased node and fruiting site production was associated with increased canopy capacity and leaf Pn rates, resulting in increased floral bud starch content and despite decreased retention, a net benefit in square numbers. Decreased retention of early fruit in the experiments covered in this body of work caused maturity delays (and presumably associated WUE disadvantages), which resulted in smaller boll sizes at harvest.

The efficacy of gibberellin PGRs to promote canopy capacity and improve access to soil water reserves in rain grown cotton appears to be influenced by the water resource-limited nature of the production context. PGR treatments did not invoke the early canopy capacity and root biomass changes pursued, demonstrating an inability to override a lack of resources to achieve the increased Pn documented in irrigated systems. This resulted in early reproductive growth changes that had flow on maturity and in some cases negative lint yield and quality consequences.

## **Chapter 6 “Dynamic Fruiting”: Gibberellin Biosynthesis Inhibiting Plant Growth Regulators to Shift Timing of Crop Peak Resource Demand**

### **6.1 Introduction**

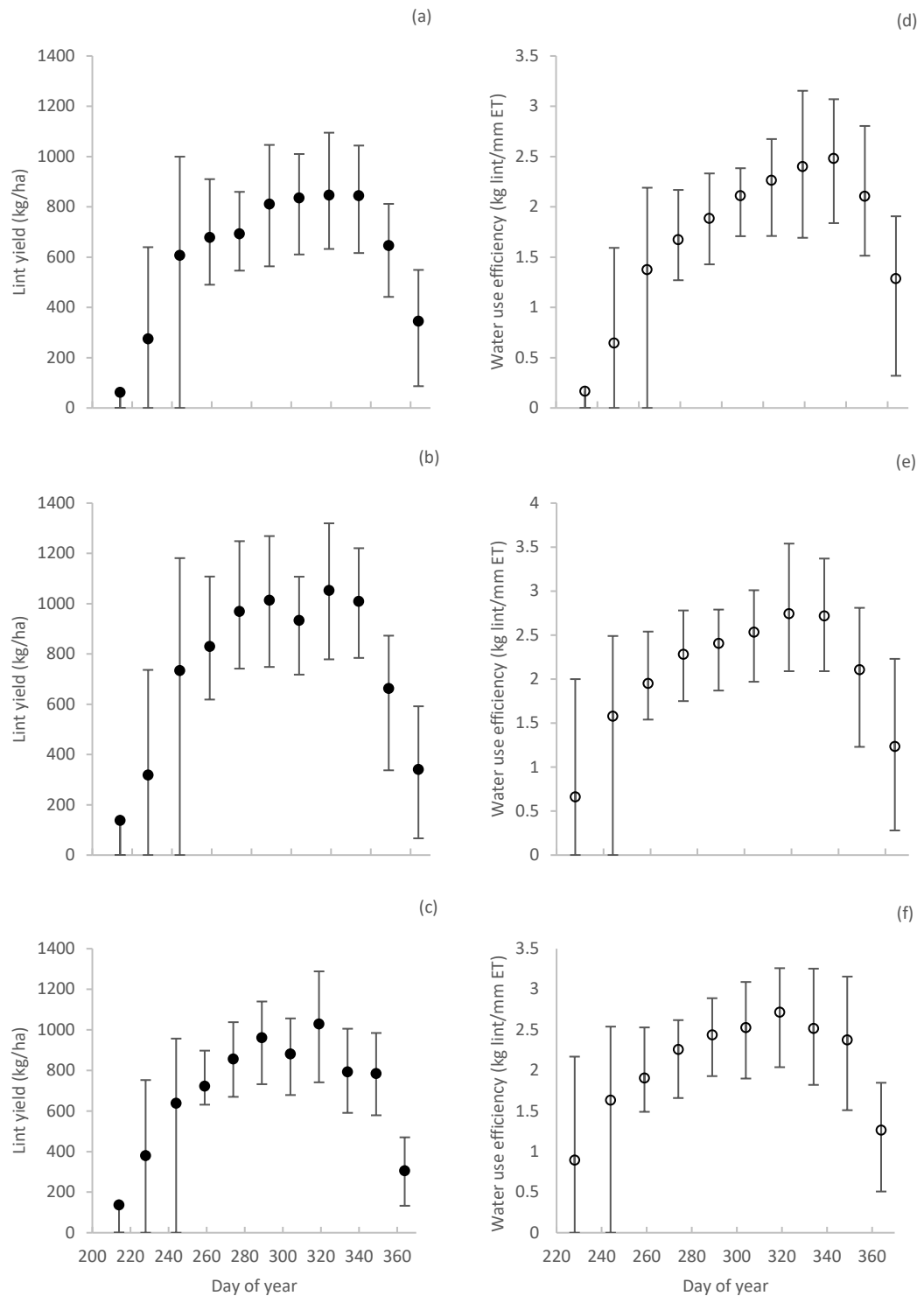
Exploiting the rationale basis to multiple planting dates in the development of adaptive management strategies using Gibberellin biosynthesis inhibiting PGRs, can potentially manage climate risk by delaying the timing of crop peak resource demand to take advantage of potentially advantageous climatic conditions later in the season. Consequently, understanding cotton plant and crop responses to specific gibberellin biosynthesis inhibitor treatment scenarios, can enable future development of criteria frameworks for strategic, predictive applications within Australian rain grown cotton systems, that stabilise or improve annualised yield outcomes and improve downside risk associated with crop establishment outside the optimum window.

Lint yields are strongly correlated with both stored soil moisture at planting as well as the prevailing rainfall and temperature patterns; principally during the reproductive growth phase (Bange et al., 2005; Turner, 1986). Resultantly, lint yields are impacted by timing of establishment; simulation modelling of mean crop yield as impacted by time of planting, across current rain grown cotton production areas, defines an optimal planting window on mean between the 15<sup>th</sup> October and 15<sup>th</sup> November (day of year: 289-320) annually (Bange, 2019). Congruently, physiological crop response simulations (Chapter 3, section 3.7) of mean lint yields as influenced by time of planting, specific to the Narrabri (ACRI), Bellata (BTA) and Terry Hie Hie (THH) field sites, also demonstrated both increased lint yield variability and notably downside risk (being the potential to incur a decline in yield associated with changing environmental conditions) associated with crop establishment outside the optimum window (Figure 6.1).

Manipulating or staggering cotton crop planting date is a current systems-level strategy to manage climate risk and promote system resilience by proportionately transitioning phases of crop maximum water use (peak flowering) into alternate climate time-periods of lower temperature and vapour pressure deficits (Braunack et al., 2012). However, the climatically sensitive nature of rain grown cotton production can disrupt opportunities for implementation of multiple planting dates (Darbyshire, Crean, & Anwar, 2018). Rain grown cotton planting opportunities (in Australia) are both legislation and climate dependant; being shaped by overarching stewardship requirements (for transgenic cotton), existing soil profile water reserves, soil temperature and most importantly, the occurrence

of planting rainfall events, where timing varies across production regions and between seasons. Any delay in planting increases the reliance on additional rainfall to provide subsequent crop establishment opportunities; the risk of failing to obtain a defined planting opportunity being at best three out of ten years across Australian rain grown production areas (Baird et al., 2018; Bange, 2019).

Consequent to the rain-dependant nature of the production system, an imperative exists to utilise the earliest (indeed, any) possible rainfall occurrence to plant the crop and avoid the chance of missing establishment (Gourley, O'Neil, & Christie, 2016; Grundy & Klepper, 2016); often resulting in planting earlier than the optimal window. The impact and risks of early planting are associated with the occurrence of flowering during late December and January (opposed to February and early March), resulting in maximum crop water requirements coinciding with peak evaporative demand and the probable occurrence of extreme heat events (Braunack et al., 2012; CSD, 2018a); as evidenced by water use efficiency simulation outcomes manipulated by planting date, for the Narrabri (ACRI), Bellata (BTA) and Terry Hie Hie (THH) field sites (Figure 6.1). It is proposed that temporarily delaying peak flowering (crop maximum water requirements) into a later season period of potentially lower mean temperatures and vapour pressure deficits can improve or stabilise annualised yield outcomes and improve the downside risk associated with crop establishment outside the optimum window.



**Figure 6.1** Fifty year historical mean lint yields ( $\text{kg}\cdot\text{ha}^{-1}$ ) at (a) Narrabri (ACRI), (b) Bellata (BTA) and (c) Terry Hie Hie (THH) field sites, as well as fifty year historical mean water use efficiency (WUE) ( $\text{kg lint} / \text{mm ET}$ ) at (d) Narrabri (ACRI), (e) Bellata (BTA) and (f) Terry Hie Hie (THH) field sites; obtained from crop response simulations using OzCOT (Hearn, 1994), per 15-day establishment intervals (1-Aug to 29-Dec), range is 80% (lower) and 20% (upper) probability of exceedance.

As a perennial, short-day woody shrub, that has been domesticated as a day-neutral, self-inductive annual crop; cotton possesses a complex and simultaneously developing vegetative and reproductive morphology (V. A. da Costa & Cothren, 2012; Mauney, 2012). Temperature is a paramount environmental determinant influencing growth (Constable & Shaw, 1988; R. Wells & Stewart, 2010); the primary axis remaining vegetative, with axillary branches differentiating at the base of each leaf (Mauney, 1986b). The time between planting and the emergence of the first square is a complex interaction between the vegetative growth rate (rate of node development) and the node at which the first fruiting branch occurs (NFFB) (Mauney, 1986b, 2012). Axillary buds of the first 4 nodes remain dormant or form monopodial vegetative branches (R. Wells & Stewart, 2010). Sympodial (reproductive) development thereafter comprises a series of independent shoot initiations. Each axillary primordium receives its own environmental signal for differentiation and development (Mauney, 1966, 2012; R. C. McGarry et al., 2016); the exact genetic, hormonal and environmental mechanisms of which are unclear, although temperature and leaf area (resultantly) are significant determinants, with little or no photoperiodic response evident (V. A. da Costa & Cothren, 2012; Fang et al., 2018; Guinn, 1986; Mathur & Mittal, 1964; Mauney, 1963, 1966; Mauney & Phillips, 1963; R. C. McGarry et al., 2016; Moraghan, Hesketh, & Low, 1968). Fundamentally, the production of fruiting forms is dependent on growth (i.e. cell division and expansion); actual numbers of potential fruiting forms, whether during initial squaring or in development of new squares during mid-flowering, depend on the rate of production of successive nodes (Jordan, 1986).

In-crop PGR applications enable active regulation of such developmental processes through “fine-tuning according to need”; both by affecting the optimal growth or development stage via treatment timing and / or the intensity of manipulation via treatment dosage (Hedden et al., 2010). Gibberellin biosynthesis inhibitor (GaBI) PGR, Mepiquat chloride, is currently widely utilised in Australian cotton production systems for the control of excessive vegetative growth during flowering and to limit growth and indirectly induce physiological cut-out in high-input irrigated systems (S. Williams et al., 2019). As detailed prior (Chapter 2), GaBI PGRs reduce the bioactive Gibberellin content of treated plants by disrupting specific stages of the Gibberellin biosynthesis pathway, particular to the individual chemical (Rademacher, 2000, 2015). This results in a reduction of cell division in the shoot apical meristem, a reduction in cell growth (expansion) and decreased cell wall plasticity (Rademacher, 1991); being evidenced at a plant level as reduced shoot elongation

and key to cotton plant reproductive development, a decreased number of main-stem nodes (V. A. da Costa & Cothren, 2012; Rademacher, 2000).

Significant variation exists between GaBI PGR chemicals in cotton plant phenotypic response to treatment (Appendix 2), many of which are unquantified in early vegetative growth stage cotton or rain grown production contexts. Resultantly, opportunity exists to explore the application of both Mepiquat chloride and atypical GaBI PGRs for the purpose of temporally delaying crop early vegetative growth and subsequent progression into simultaneous vegetative and reproductive growth, under Australian rain grown production conditions. This chapter details a series of glasshouse and field experiments, conducted to determine the capability of a selection of gibberellin biosynthesis inhibiting PGRs to slow growth or induce a physiological stasis, in early vegetative stage cotton plants, with the objective of delaying crop-level reproductive phase change (taken as progression to 50% plant population first square (FSqu)) and subsequent crop peak resource demand. These experiments aimed to:

- Quantify the impact of specific gibberellin biosynthesis inhibiting PGR treatment choice, rate, application type, frequency and timing combinations, on time to FSqu; and
- Understand consequent treatment flow-on impacts on yield, lint quality, biomass and leaf area accumulation and fruit development, retention and maturity outcomes.

Specifically, the hypothesis tested was;

H<sub>0</sub>: gibberellin biosynthesis inhibiting PGR treatments applied in early vegetative growth stage, induce no detectable plant phenological response in the timing of crop-level reproductive phase change and consequent peak resource demand.

## **6.2 Materials and methods**

A staged approach to both test aims and provide a binary hypothesis response was employed; preliminary screening of treatments occurring in experiments 3, 6 and 7, with further concept evaluation of treatments in Experiments 8, 9 and 11 (Table 6.1). Initial broader-scope screening experiments (Exp. 3, 6 and 7) facilitated contraction of treatment selections based on discernible response mechanisms; being direct impacts and/or responses otherwise indicative of sought-after phenology. Resultant concept evaluation experiments (Exp. 8, 9 and 11) confirmed (or otherwise) treatment consistency and further

characterised the treatment response, via more detailed assessments notably of fruit growth, retention, and maturity under early planting scenarios.

### **6.2.1 Site description**

To enable screening and subsequent evaluation of gibberellin biosynthesis inhibitor treatments on rain grown cotton plants, seven experiments were grown under both controlled environment (spring 2016), and rain grown field conditions across multiple locations during the 2016-17, 2017-18 and 2018-19 cotton seasons. The respective site locations, climatic conditions, experimental design, crop and plot management protocols are detailed in Chapter 3, Sections 3.1 and 3.2.

### **6.2.2 Treatments**

For the purpose of this analysis, treatments utilised per experiment are detailed in Table 6.1. For brevity, PGR treatment acronyms referencing the active ingredient, are utilised throughout and explained in the Abbreviations section, with full nomenclature, structural descriptions and proprietary sources detailed in Appendix 1.

**Table 6.1 Treatments utilised per experiment, as included in chapter 6 analysis; all treatments being applied at 4 leaf stage, preliminary screening of treatments in experiments 3, 6 and 7, further concept evaluation of treatments in 8, 9 and 11.**

<b>Experiment</b> <i>No, name</i>	<b>Treatment</b> <i>Number</i>	<i>Compound</i>	<i>Conc. (mg.L<sup>-1</sup>)</i>	<i>Application</i>	<i>Frequency</i>
<b>Experiment 3. (2016 GH GaBI)</b>					
	1	Untreated control, water spray only			
	2	CCC	125.00	Foliar, to drip	Once
	3	MC	60.00	Foliar, to drip	Once
	4	Paclo	375.00	Foliar, to drip	Once
	5	Paclo	0.1 g ai.m <sup>-3</sup>	Soil drench	Once
	6	Unicon	300.00	Foliar, to drip	Once
	7	Unicon	0.015 g ai.m <sup>-3</sup>	Soil drench	Once
	8	TEP	2000.00	Foliar, to drip	Once
	9	PhxCa	100.00	Foliar, to drip	Once
<b>Experiment 6. (2016.17ACRI GaBI)</b>					
	1	Untreated control, water spray only			
	2	CCC	125.00	Foliar, to drip	3 x7d
	3	MC	60.00	Foliar, to drip	3 x7d
	4	Paclo	375.00	Foliar, to drip	3 x7d
	5	Paclo	0.1 g ai.m <sup>-3</sup>	Soil drench	2 x20d
	6	Unicon	300.00	Foliar, to drip	3 x7d
	7	Unicon	0.015 g ai.m <sup>-3</sup>	Soil drench	2 x20d
	8	TEP	2000.00	Foliar, to drip	3 x7d
	9	PhxCa	100.00	Foliar, to drip	3 x7d
<b>Experiment 7. (2016.17 THH GaBI)</b>					
	1	Untreated control, water spray only			
	2	CCC	125.00	Foliar, to drip	3 x7d
	3	MC	60.00	Foliar, to drip	3 x7d
	4	Paclo	375.00	Foliar, to drip	3 x7d
	5	Paclo	0.1 g ai.m <sup>-3</sup>	Soil drench	2 x20d
	6	Unicon	300.00	Foliar, to drip	3 x7d
	7	Unicon	0.015 g ai.m <sup>-3</sup>	Soil drench	2 x20d
	8	TEP	2000.00	Foliar, to drip	3 x7d
	9	PhxCa	100.00	Foliar, to drip	3 x7d
<b>Experiment 8. (2017.18 ACRI Resil.)</b>					
	1	Untreated control, planting 1 (S1), water spray only			
	2	Untreated control, planting 2 (S2), water spray only			
	12	Paclo	375.00	Foliar, to drip	3 x7d
	13	Paclo_S2	375.00	Foliar, to drip	3 x7d
	14	TEP	2000.00	Foliar, to drip	3 x7d
	15	TEP_S2	2000.00	Foliar, to drip	3 x7d
	16	PhxCa	100.00	Foliar, to drip	3 x7d
	17	PhxCa_S2	100.00	Foliar, to drip	3 x7d
<b>Experiment 9. (2018.19 ACRI Resil. 2b)</b>					
	1	Untreated control, water spray only			
	7	Paclo	375.00	Foliar, to drip	3 x7d
	8	TEP	2000.00	Foliar, to drip	3 x7d
<b>Experiment 11. (2018.19 THH Resil.)</b>					
	1	Untreated control, water spray only			
	2	TEP	2000.00	Foliar, to drip	3 x7d
	3	Paclo	375.00	Foliar, to drip	3 x7d
	4	Unicon	300.00	Foliar, to drip	3 x7d
	5	PhxCa	100.00	Foliar, to drip	3 x7d

### **6.2.3 Measurements**

Measurements of growth and development varied in scope and intensity across experiments, commensurate with experiment rationale as preliminary screening or subsequent concept evaluation. Measurements provided phenotypic snapshots from which to compare PGR treatment responses with standardised control (Trt.1) growth and development parameters. Crop measurements per experiment are described in Table 6.2, with referenced sampling and processing methodologies being detailed in Chapter 3, sections; 3.4.2 plot sampling protocol, 3.5.1 growth (VGR, biomass, leaf area), 3.5.2 development (FSqu, flowering and physiological cut out), 3.6.1 fruit mapping and retention, 3.6.2 maturity and lint yield (hand and machine harvests), 3.6.3 ginning and quality parameters.

**Table 6.2 Details of plant and crop measurements taken per experiment as included in Chapter 6 analysis; all replicates being sampled.**

<i>Exp</i>	<i>Growth</i>			<i>Development</i>			<i>Yield components</i>		<i>Yield</i>		<i>Quality</i>		
	<i>VGR</i>	<i>Biomass</i>	<i>Leaf area</i>	<i>Fruit retention</i>	<i>TTFSq</i>	<i>TTFE</i>	<i>Cut out (NAWF)</i>	<i>Maturity</i>	<i>Seed cotton</i>	<i>OB no. and size</i>	<i>Lint</i>	<i>Gin Turnout</i>	<i>HVI</i>
3	>19 DAP	Early flowering, 59 DAP		All Trt.	Control								
6.	>27 DAP 10-day intervals, 6 weeks	Mid flowering, 100 DAP		Control	Control	Snapshot, 7 DAP	Maturity 1 m <sup>2</sup> hand harvest from 146 DAP, 10 day intervals			10 m machine harvest, 217 DAP		Not recorded	
7.	>26 DAP 10-day intervals, 6 weeks	Mid flowering, 130 DAP, reps 1-3		Not recorded <sup>(1)</sup>	All treatments and reps	Snapshot, 90 and 94 DAP	Maturity 1 m <sup>2</sup> hand harvest from 116 DAP, 10 day intervals			Obtained from maturity hand harvests			
8.	>25 DAP 10-day intervals, 12 weeks	55 DAP (S1 FSqu), 81 DAP (S1 FF), 96 DAP (S2 FSqu), 116 DAP (S1 mid flowering, S2 FSqu + 10 d), 124 DAP (S2 FF)		Control	Control	91, 98, 105, 116, 124, 132 DAP	Maturity 1 m <sup>2</sup> hand harvest from 134 DAP, 10 day intervals			10 m machine harvest, 210 DAP		Obtained from machine harvest	
9.	>19 DAP	45 DAP (Fsqu), 66 DAP (FF)		Control	Control								
11.	>50 DAP 10-day intervals, 6 weeks	74 DAP (Fsqu), 96 DAP (FF)		Control	Control	106, 116, 119, 123, 128, 134 DAP	Maturity 1 m <sup>2</sup> hand harvest from 134 DAP, 10 day intervals			Obtained from maturity hand harvests			

<sup>(1)</sup> Square shedding event associated with sucking insect pest (*Thrips tabaci*, *Frankliniella schultzei*, *F. occidentalis*) infestation.

## 6.2.4 Data analysis

One-way Analysis of Variance (ANOVA) with Tukey's post-hoc HSD test was used to determine treatment differences in:

- FSqu in experiment 3.
- FF in experiment 7.
- Number of nodes above white flower (NAWF) in experiments 6 and 7.
- Number of squares, green bolls, open bolls, mean square size, mean green boll size, mean open boll size, leaf area index (LAI), total dry weight and reproductive biomass/total dry weight, at mid-flowering in experiments 3, 6 and 7.
- Number of pinhead squares, number of squares, mean square size, retention (number of squares / total fruit sites) and reproductive biomass/total dry weight, at control first square in experiments 8, 9 and 11.
- Number of green bolls, mean green boll size, retention, reproductive biomass/total dry weight, total biomass and LAI, at control FF in experiments 8, 9 and 11.
- Seed cotton yield, ginning turnout, lint yield, micronaire, length and strength for experiments 6, 7, 8 and 11.

Nodes above white flower data from experiments 8 and 11 were pooled and generalised linear regression utilised to determine treatment differences from the control in progression to physiological cut out (defined as 4 NAWF). In two separate analysis, maturity harvest data from experiments 6 and 7, and experiments 8 and 11, were pooled and generalised linear regression utilised to determine treatment differences from the control in progression to maturity (60% open boll). Methodologies of data analysis referenced are detailed in Chapter 3, Section 8.

## 6.3 Results

### 6.3.1 Glasshouse and early field screening; experiments 3, 6 and 7

#### *Delaying crop-level reproductive phase change*

In experiment 3, under glasshouse conditions and with single applications of PGR treatments (Table 6.1), mean days to 50% population first square (FSqu) of group B biosynthesis inhibitor treatments 4 (Paclo(f)), 5 (Paclo(s)) and 6 (Unicon(f)) were later than treatment 1 (control) ( $P < 0.001$ ) (Table 6.3). In experiment 7, obtaining time to population

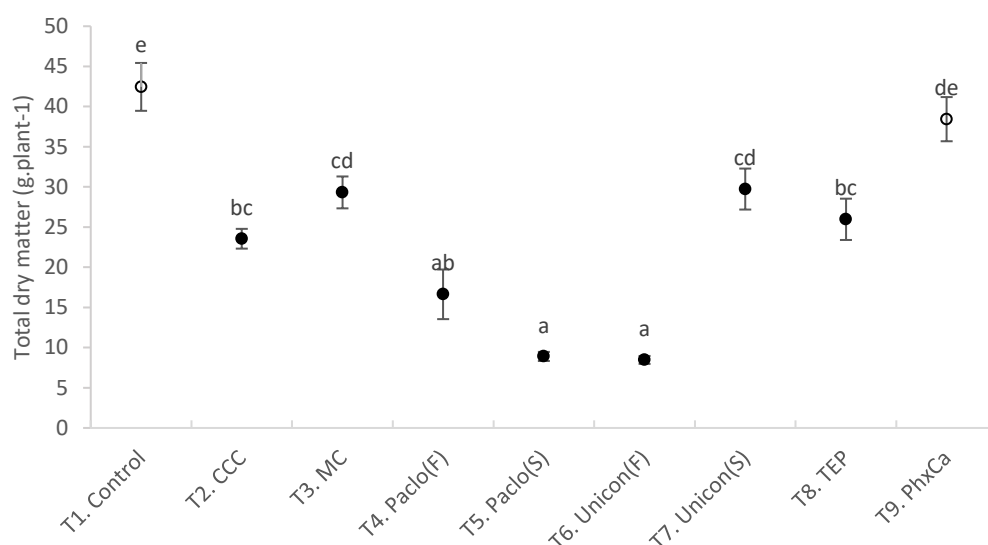
50% first square was impeded by an early insect-induced square shedding event; under field conditions and with multiple consecutive applications of each PGR treatment (Table 6.1), no differences from the control (Trt.1) occurred for mean days to 50% population first flower (FF) (P=0.024) (Table 6.3).

**Table 6.3 Mean days to population first square and first flower for Gibberellin biosynthesis inhibitor PGR treatments in experiments 3 and 7, respectively; notation indicating treatment differences at the P<0.05 level using Tukey's HSD.**

Treatment	Experiment 3. (2016 GH GaBI) FSqu DAP (P<0.001)	Experiment 7. (2016.17 THH GaBI) FF DAP (P=0.024)
1. Control	25 <sup>(a)</sup>	81 <sup>(ab)</sup>
2. CCC	28 <sup>(a)</sup>	83 <sup>(ab)</sup>
3. MC	28 <sup>(a)</sup>	81 <sup>(ab)</sup>
4. Paclo(f)	42 <sup>(b)</sup>	81 <sup>(ab)</sup>
5. Paclo(s)	41 <sup>(b)</sup>	80 <sup>(a)</sup>
6. Unicon(f)	36 <sup>(b)</sup>	81 <sup>(ab)</sup>
7. Unicon(s)	27 <sup>(a)</sup>	79 <sup>(a)</sup>
8. TEP	28 <sup>(a)</sup>	87 <sup>(b)</sup>
9. PhxCa	27 <sup>(a)</sup>	81 <sup>(ab)</sup>

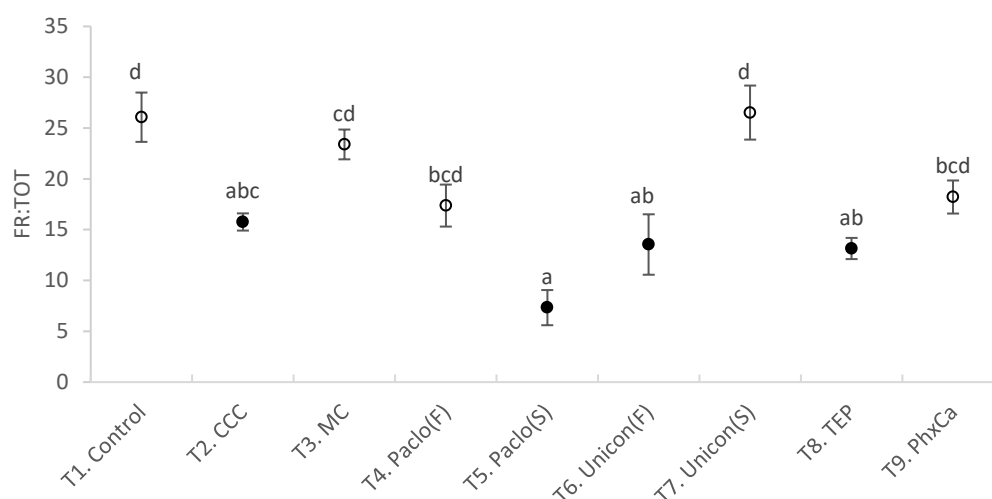
### ***Consequent treatment flow-on impacts***

Early to mid-flowering biomass harvests to assess growth and development against the control (Trt.1) were cut at 59 DAP (28/11/2016), 100 DAP (1/03/17) and 130 DAP (28/02/17) for experiments 3, 6 and 7, respectively. No differences in specific leaf area (SLA) (m<sup>2</sup>.kg<sup>-1</sup>) occurred between the Control (Trt.1) and all other treatments in experiment 3; however, treatment 5 (Paclo(s)) SLA was less than in treatments 7 (Unicon(S)) and 8 (TEP) (P<0.001). No differences in leaf area index (LAI) from the control were found for all treatments in either experiment 6 (P=0.117) or experiment 7 (P=0.366). In experiment 3, the mean total dry matter (g.plant<sup>-1</sup>) of treatments 7 (Unicon(s)), 3 (MC), 8 (TEP), 2 (CCC), 4 (Paclo(f)), 5 (Paclo(s)) and 6 (Unicon(f)) were less than treatment 1 (control) (P<0.001) (Figure 6.1). No differences in total dry matter accumulation existed in experiment 6 (P=0.161), however; treatment 2 (CCC) was 17% less than the control ( $\mu(\text{Trt.1})=1635.09$ ,  $\mu(\text{Trt.2})=1389.70$ , P=0.007) in experiment 7, with no other treatment differences evident.



**Figure 6.1 Mean total dry matter (g/plant) at 59 DAP for Gibberellin biosynthesis inhibitor PGR treatments grown under glasshouse conditions in experiment 3; notation indicating treatment differences at the  $P < 0.05$  level using Tukey's HSD, solid markers indicating treatment differences from control (Trt.1), error bars are  $\pm 1$  standard error of the mean.**

In experiment 3, delay in reproductive development was evidenced as reduced (compared to control, Trt. 1) mean reproductive biomass dry weight (squares, white flowers, green bolls) proportionate to total dry matter, for treatments 2 (CCC), 6 (Unicon(F)), 8 (TEP) and 5 (Paclo(S)) ( $P < 0.001$ ) (Figure 6.2). There was no treatment differences associated with reproductive biomass proportionate to total dry matter (FR:TOT) in field experiment 6 ( $P = 0.089$ ); however experiment 7, treatment 2 (CCC) was half the value of control ( $\mu(\text{Trt.1}) = 0.4400$ ,  $\mu(\text{Trt.2}) = 0.2204$ ), with no other treatment differences evident.



**Figure 6.2 Mean FR:TOT for Gibberellin biosynthesis inhibitor PGR treatments grown under glasshouse conditions in experiment 3; notation indicating treatment differences at the  $P < 0.05$  level using Tukey's HSD, solid markers indicating treatment differences from the control (Trt.1), error bars are  $\pm 1$  standard error of the mean.**

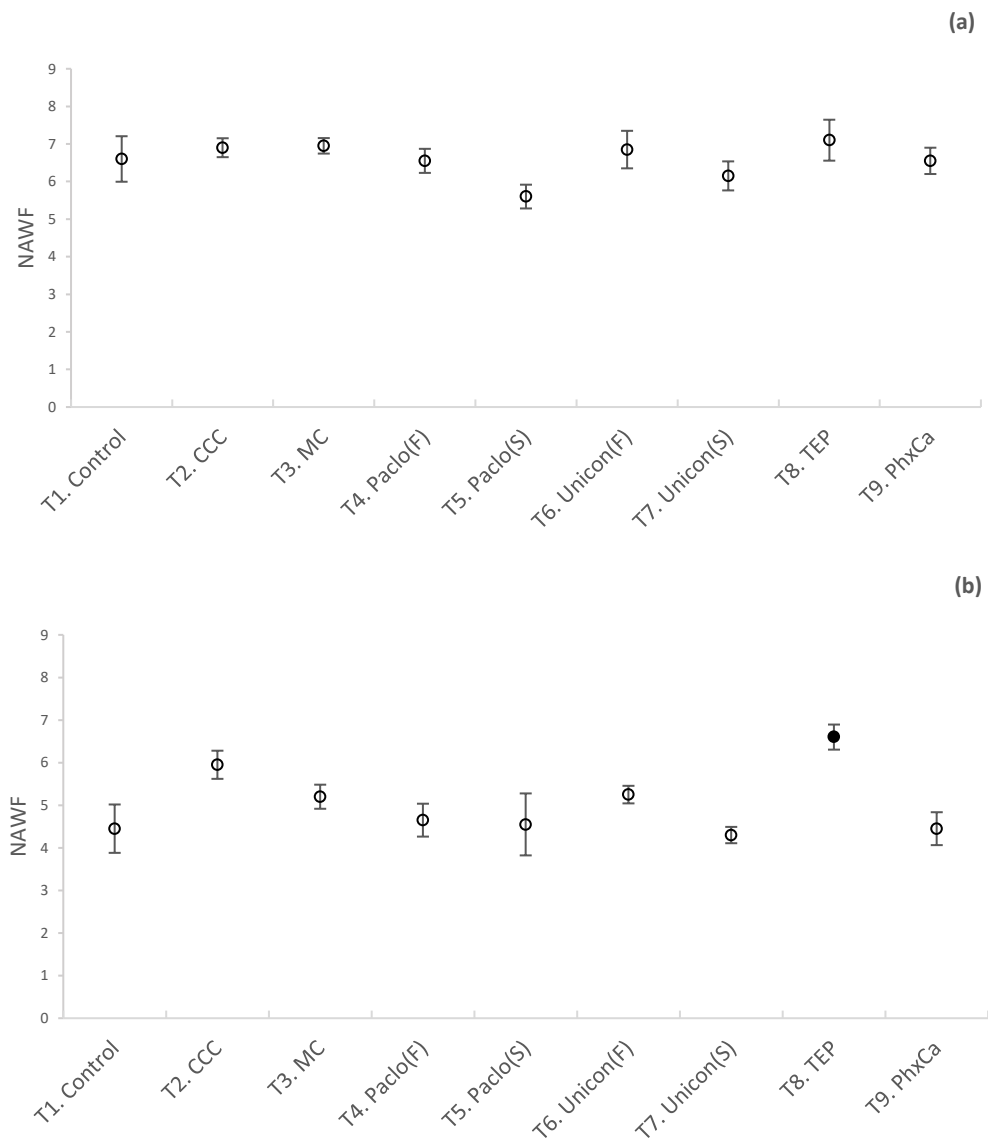
In experiment 3, all treatments maintained comparable square loads and size, with no treatment differences evident from the control (Trt.1) for mean number of squares per plant ( $P=0.014$ ) or mean square size ( $P=0.166$ ). Both green boll numbers (white flowers and small green bolls) per plant and mean green boll weight, were reduced in treatments 5 (Pacl(s)), 6 (Unicon(F)), 4 (Pacl(F)), 2 (CCC) and 8 (TEP), compared with the control (Trt.1) ( $P<0.001$ ). No treatment differences from the control (Trt.1) were evident for the number of squares ( $m^2$ ) ( $P=0.193$ ), mean square weight (g) ( $P=0.155$ ), number of green bolls ( $m^2$ ) ( $P=0.600$ ), mean green boll weight (g) ( $P=0.023$ ), number of open bolls ( $P=0.013$ ) or mean open boll weight ( $P=0.261$ ) in experiment 6. In experiment 7, treatment 2 (CCC) had an increased mean number of squares (per  $m^2$ ) compared to all other treatments ( $P=0.006$ ); there were no differences in mean square size ( $P=0.126$ ) (Table 6.4). Treatment 6 (Unicon(F)) and 8 (TEP) had an increased mean number of green bolls (per  $m^2$ ) ( $P=0.003$ ); there were no differences from the control (Trt.1) between mean green boll size ( $P=0.032$ ) (Table 6.4). There were no differences from the control (Trt.1) in number of open bolls (per  $m^2$ ) ( $P=0.003$ ); treatments 8 (TEP) and 2 (CCC) had smaller mean open boll sizes compared with the control (Trt.1) ( $P<0.001$ ) (Table 6.4).

**Table 6.4 Mean mid-flowering (130 DAP) fruit load (count. $m^{-2}$ ) and mean fruit size (g) for Gibberellin biosynthesis inhibitor PGR treatments grown under rain grown field conditions in experiment 7; notation indicating treatment differences at the  $P=0.05$  level using Tukey's HSD.**

	Sq.No ( $P=0.006$ )	SquSize ( $P=0.126$ )	GB.No ( $P=0.003$ )	GBSize ( $P=0.032$ )	OB.No ( $P=0.003$ )	OBSize ( $P<0.001$ )
1. Control	0.00 <sup>(a)</sup>	0.0000 <sup>(a)</sup>	86.50 <sup>(a)</sup>	4.0210 <sup>(ab)</sup>	65.28 <sup>(ab)</sup>	5.4840 <sup>(b)</sup>
2. CCC	74.52 <sup>(b)</sup>	0.0997 <sup>(a)</sup>	128.60 <sup>(ab)</sup>	2.6340 <sup>(a)</sup>	0.00 <sup>(a)</sup>	0.0000 <sup>(a)</sup>
3. MC	3.63 <sup>(a)</sup>	0.0833 <sup>(a)</sup>	140.60 <sup>(abc)</sup>	4.2850 <sup>(ab)</sup>	41.63 <sup>(ab)</sup>	6.0670 <sup>(b)</sup>
4. Pacl(f)	0.00 <sup>(a)</sup>	0.0000 <sup>(a)</sup>	114.50 <sup>(ab)</sup>	4.5910 <sup>(ab)</sup>	89.70 <sup>(b)</sup>	5.7180 <sup>(b)</sup>
5. Pacl(s)	0.00 <sup>(a)</sup>	0.0000 <sup>(a)</sup>	140.40 <sup>(abc)</sup>	4.6900 <sup>(b)</sup>	45.74 <sup>(ab)</sup>	5.6970 <sup>(b)</sup>
6. Unicon(f)	0.00 <sup>(a)</sup>	0.0000 <sup>(a)</sup>	163.50 <sup>(bc)</sup>	4.4400 <sup>(ab)</sup>	37.72 <sup>(ab)</sup>	6.1230 <sup>(b)</sup>
7. Unicon(s)	4.11 <sup>(a)</sup>	0.0500 <sup>(a)</sup>	117.80 <sup>(ab)</sup>	3.6820 <sup>(ab)</sup>	79.73 <sup>(b)</sup>	5.3340 <sup>(b)</sup>
8. TEP	2.28 <sup>(a)</sup>	0.0667 <sup>(a)</sup>	209.10 <sup>(c)</sup>	3.3050 <sup>(ab)</sup>	2.22 <sup>(a)</sup>	1.7670 <sup>(a)</sup>
9. PhxCa	8.13 <sup>(a)</sup>	0.1750 <sup>(a)</sup>	141.20 <sup>(abc)</sup>	3.6190 <sup>(ab)</sup>	45.87 <sup>(ab)</sup>	6.0130 <sup>(b)</sup>

Assessments for differences in nodes above the highest first position white flower (NAWF), occurred at 77 and 94 DAP in experiments 6 and 7, respectively; control (Trt.1) in experiment 6 was approaching physiological cut out at 6 NAWF and had cut out (4 NAWF) in experiment 7. No differences from the control (Trt.1) in NAWF occurred in experiment 6 ( $P=0.078$ ). However, treatment 8 (TEP) had a higher mean NAWF count than

the control (Trt.1) in experiment 7, with no other treatment differences occurring ( $P < 0.001$ ) (Figure 6.3).



**Figure 6.3 Mean nodes above the highest first position white flower (NAWF) for Gibberellin biosynthesis inhibitor PGR treatments in (a) experiment 6 just prior to control (Trt.1) cut out and (b) at control (Trt.1) cut out in experiment 7; solid markers indicating treatment differences from control (Trt.1) at the  $P < 0.05$  level using Tukey's HSD, error bars are +/- 1 standard error of the mean.**

Generalised linear regression analysis of sequential boll opening across all treatments, for aggregated data from experiments 6 and 7, explained treatment delays in maturity (as 60% open boll), for treatments 2 (CCC) ( $P = 0.022$ ), 8 (TEP) ( $P = 0.004$ ) and at  $P < 0.10$  level, treatment 6 (Unicon(F)) ( $P = 0.067$ ); polynomial trend lines were fitted and resulting calendar days to 60% open boll load are presented in Table 6.5.

**Table 6.5 Treatment coefficient of determination for fitted polynomial trend line and calendar days to maturity as 60% open boll load, for Gibberellin biosynthesis inhibitor PGR treatments in field experiments 6 and 7; R<sup>2</sup> all greater than 0.98.**

Experiment	Treatment	60% open boll (DAP)	Days>Trt1
6	1. Control	145	0
7	1. Control	143	0
6	2. CCC	148	3
7	2. CCC	154	11
6	6. Unicon(f)	154	9
7	6. Unicon(f)	149	6
6	8. TEP	157	12
7	8. TEP	155	12

No treatment differences occurred for final open boll numbers (per m<sup>2</sup>) or mean open boll weights (g) in both experiment 6 (OB.No; P=0.570, OB.Size; P=0.100) and 7 (OB.No; P=0.954, OB.Size; P=0.222). Both experiments were defoliated and managed for harvest according to control (Trt.1) maturity, resulting in residual green bolls on later maturing treatments in both experiments. However no treatment differences occurred in either the number or mean weight of residual green bolls in experiment 6 (GB.No; P=0.588, GB.Size; P=0.615) or 7 (GB.No; P=0.654, GB.Size; P=0.447).

No treatment differences occurred for seed cotton yield in experiment 6 (P=0.328). Similarly, no treatment differences were found for seed cotton turnout (P=0.183) and lint yield (P=0.882) for experiment 7. All treatments had a higher lint strength (g force per tx) than the control (Trt.1) (P<0.001), with all treatments being classed as “strong” to “very strong”. Treatments 2 (CCC) and 8 (TEP) had higher micronaire than the control (Trt.1) (P<0.001). However, both means were still within base range (G5) of 3.5-4.9. Lint length (32nds) was increased from the control (Trt.1) in treatments 5 (Paclo(S)), 6 (Unicon(F), 7 (Unicon(S)), 9 (PhxCa), 3 (MC) and 2 (CCC) (P<0.001), to above base grade.

### 6.3.2 Concept evaluation in field experiments 8, 9 and 11

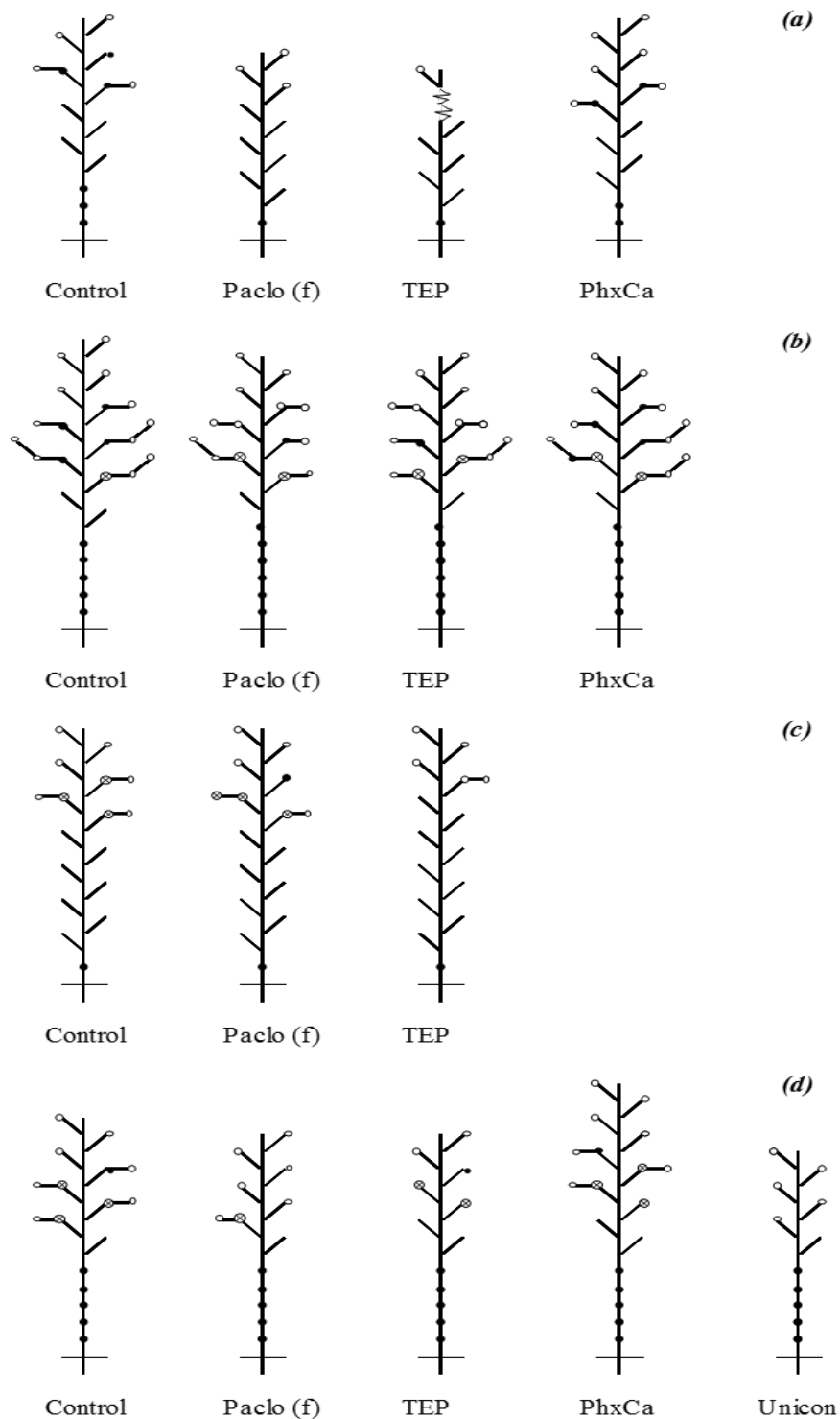
#### *Delaying crop-level reproductive phase change*

Crop-level phase change from vegetative to reproductive growth, taken as 50% population first square, occurred at 55, 42, 40 and 74 DAP for the control treatments in experiments 8 planting 1, 8 planting 2, 9 and 11, respectively. Biomass assays to quantify treatment differences in early reproductive development occurred within 7 days thereafter (“early

squaring”) (Table 6.6, Figure 6.4). Compared with the control (Trt.1), across all experiments the TEP treatments induced a delay in reproductive development, evidenced through both a reduced number of total fruiting sites and a reduction in proportionate retention of squares to those lesser numbers of sites. Paclo treatments evidenced similar effects across the earlier planted experiments 8 (planting 1) and 11 only. In experiment 11, the Unicon treatment caused a reduction in total fruiting site production with no corresponding significant decrease in square numbers or proportionate retention (although both evidenced a decreased trend); having potential system benefits but being counter intuitive to the current experiment aims (Figure 6.4). PhxCa treatments demonstrated no impacts on early reproductive growth across the three experiments sampled.

**Table 6.6 Mean square counts (per m<sup>2</sup>), total reproductive sites (per m<sup>2</sup>), square retention (%) and mean square size (g) of Gibberellin biosynthesis inhibitor PGR treatments at early squaring in field experiments 8, 9 and 11; notation indicating treatment differences at the P=0.05 level using Tukey’s HSD.**

	<i>Experiment 8; planting 1</i>	<i>Experiment 8; planting 2</i>	<i>Experiment 9</i>	<i>Experiment 11</i>
<b><i>Square count (per m<sup>2</sup>)</i></b>				
	<i>(P&lt;0.001)</i>	<i>(P=0.021)</i>	<i>(P=0.168)</i>	<i>(P=0.002)</i>
Control	5.87 <sup>(b)</sup>	16.09 <sup>(b)</sup>	9.30 <sup>(a)</sup>	2.73 <sup>(b)</sup>
Paclo	0.00 <sup>(a)</sup>	7.97 <sup>(ab)</sup>	4.58 <sup>(a)</sup>	0.000 <sup>(a)</sup>
Unicon	-	-	-	0.63 <sup>(ab)</sup>
TEP	0.00 <sup>(a)</sup>	4.18 <sup>(a)</sup>	1.82 <sup>(a)</sup>	0.00 <sup>(a)</sup>
PhxCa	6.45 <sup>(b)</sup>	12.90 <sup>(ab)</sup>	-	2.13 <sup>(ab)</sup>
<b><i>Total fruiting sites (per m<sup>2</sup>)</i></b>				
	<i>(P&lt;0.001)</i>	<i>(P&lt;0.039)</i>	<i>(P=0.389)</i>	<i>(P&lt;0.001)</i>
Control	7.79 <sup>(b)</sup>	22.86 <sup>(b)</sup>	20.11 <sup>(b)</sup>	15.5625 <sup>(b)</sup>
Paclo	0.18 <sup>(a)</sup>	13.82 <sup>(ab)</sup>	15.03 <sup>(ab)</sup>	8.34 <sup>(a)</sup>
Unicon	-	-	-	5.073 <sup>(a)</sup>
TEP	0.00 <sup>(a)</sup>	8.53 <sup>(a)</sup>	11.44 <sup>(a)</sup>	7.578 <sup>(a)</sup>
PhxCa	7.65 <sup>(b)</sup>	18.47 <sup>(ab)</sup>	-	19.58 <sup>(b)</sup>
<b><i>Retention (%)</i></b>				
	<i>(P&lt;0.001)</i>	<i>(P=0.015)</i>	<i>(P=0.257)</i>	<i>(P=0.020)</i>
Control	79.58 <sup>(b)</sup>	68.12 <sup>(b)</sup>	34.97 <sup>(b)</sup>	19.47 <sup>(b)</sup>
Paclo	0.00 <sup>(a)</sup>	57.84 <sup>(ab)</sup>	30.21 <sup>(b)</sup>	0.00 <sup>(a)</sup>
Unicon	-	-	-	8.33 <sup>(ab)</sup>
TEP	0.00 <sup>(a)</sup>	46.88 <sup>(a)</sup>	15.59 <sup>(a)</sup>	0.00 <sup>(a)</sup>
PhxCa	71.50 <sup>(b)</sup>	68.04 <sup>(b)</sup>	-	11.05 <sup>(ab)</sup>
<b><i>Mean square size (g)</i></b>				
	<i>(P&lt;0.001)</i>	<i>(P=0.088)</i>	<i>(P=0.760)</i>	<i>(P=0.021)</i>
Control	0.54 <sup>(b)</sup>	0.02 <sup>(a)</sup>	0.052 <sup>(a)</sup>	0.054 <sup>(ab)</sup>
Paclo	0.00 <sup>(a)</sup>	0.01 <sup>(a)</sup>	0.09 <sup>(a)</sup>	0.00 <sup>(a)</sup>
Unicon	-	-	-	0.02 <sup>(ab)</sup>
TEP	0.05 <sup>(a)</sup>	0.03 <sup>(a)</sup>	0.06 <sup>(a)</sup>	0.00 <sup>(a)</sup>
PhxCa	0.33 <sup>(b)</sup>	0.01 <sup>(a)</sup>	-	0.07 <sup>(b)</sup>



**Figure 6.4 Schematic diagram of plant architecture at early squaring, showing the mean number and position of nodes (nd), clustered nodes (clustered), vegetative branches (vege), pinhead squares (ph), squares (sq) and shed sites (sh) for all treatments in (a) experiment 8, planting 1, (b) experiment 8, planting 2, (c) experiment 9 and (d) experiment 11; legend specifies individual vegetative and reproductive structures. Clustered nodes being evidenced as extremely short internode lengths (<0.5 cm).**

### *Consequent treatment flow-on impacts*

Early square and early flowering biomass harvests to assess growth and development against the control were taken for all treatments in experiments 8, 9 and 11 within 7 days of 50% population first square (“early squaring”) and first flower (“early flowering”) dates as detailed in Table 6.2. Treatment responses in total dry weight accumulation, LAI and reproductive biomass proportionate to total dry weight are described in Table 6.7. TEP treatments decreased total biomass accumulation at both early squaring and flowering, with no consistent corresponding differences from the control (Trt.1) being evident for proportionate reproductive biomass accumulation. Decreases in LAI being evident in early planted experiments 8 (planting 1) and 11 only. The Unicon treatment in experiment 11 decreasing total biomass and LAI at early flowering only, with no other differences being evident. Paclo and PhxCa treatments gave no consistent differences from the control (Trt.1) in biomass, LAI or FR:TOT accumulation at either early squaring or flowering across the four planting times.

**Table 6.7 Biomass development effects of Gibberellin biosynthesis inhibitor PGR treatments at (a) early squaring and (b) early flowering in field experiments 8, 9 and 11, as characterised by total dry weight accumulation (g.m<sup>-2</sup>), leaf area index (LAI) and FR:TOT; notation indicating treatment differences at the P=0.05 level using Tukey's HSD.**

	<i>(a) early squaring</i>				<i>(b) early flowering</i>			
	<i>Exp 8, S1</i>	<i>Exp 8, S2</i>	<i>Exp 9</i>	<i>Exp 11</i>	<i>Exp 8, S1</i>	<i>Exp 8, S2</i>	<i>Exp 9</i>	<i>Exp 11</i>
<b>Total dry weight (g.M<sup>-2</sup>)</b>								
	<i>(P&lt;0.001)</i>	<i>(P=0.170)</i>	<i>(P=0.205)</i>	<i>(P&lt;0.001)</i>	<i>(P&lt;0.001)</i>	<i>(P&lt;0.001)</i>	<i>(P&lt;0.001)</i>	<i>(P=0.062)</i>
Control	71.81 <sup>(c)</sup>	125.4 <sup>(b)</sup>	254.8	140.8 <sup>(c)</sup>	425.6 <sup>(b)</sup>	1521 <sup>(b)</sup>	674.9 <sup>(b)</sup>	315.7
Pacló	49.41 <sup>(b)</sup>	125.3 <sup>(b)</sup>	295.8	113.3 <sup>(bc)</sup>	327.4 <sup>(b)</sup>	1527 <sup>(b)</sup>	648.3 <sup>(b)</sup>	239.7
Unicon	-	-	-	99.4 <sup>(b)</sup>	-	-	-	160.2
TEP	22.00 <sup>(a)</sup>	70.3 <sup>(a)</sup>	182.9	42.5 <sup>(a)</sup>	158.7 <sup>(a)</sup>	1124 <sup>(a)</sup>	437.2 <sup>(a)</sup>	180.2
PhxCa	65.76 <sup>(c)</sup>	102.2 <sup>(ab)</sup>	-	137.1 <sup>(c)</sup>	381.6 <sup>(b)</sup>	1641 <sup>(b)</sup>	-	238.6
<b>Leaf Area Index (LAI)</b>								
	<i>(P&lt;0.001)</i>	<i>(P=0.029)</i>	<i>(P=0.720)</i>	<i>(P&lt;0.001)</i>	<i>(P&lt;0.001)</i>	<i>(P=0.010)</i>	<i>(P=0.119)</i>	<i>(P&lt;0.001)</i>
Control	0.4841 <sup>(c)</sup>	1.795 <sup>(ab)</sup>	1.0210	0.8411 <sup>(c)</sup>	3.031 <sup>(b)</sup>	4.754 <sup>(ab)</sup>	3.557	2.466 <sup>(b)</sup>
Pacló	0.2831 <sup>(b)</sup>	1.857 <sup>(b)</sup>	1.0759	0.5898 <sup>(bc)</sup>	2.614 <sup>(b)</sup>	4.630 <sup>(ab)</sup>	3.217	3.293 <sup>(c)</sup>
Unicon	-	-	-	0.5125 <sup>(b)</sup>	-	-	-	2.631 <sup>(bc)</sup>
TEP	0.1038 <sup>(a)</sup>	1.014 <sup>(a)</sup>	0.8904	0.2009 <sup>(a)</sup>	1.336 <sup>(a)</sup>	3.850 <sup>(a)</sup>	2.399	1.296 <sup>(a)</sup>
PhxCa	0.4948 <sup>(c)</sup>	1.584 <sup>(ab)</sup>	-	0.7631 <sup>(bc)</sup>	2.989 <sup>(b)</sup>	5.313 <sup>(b)</sup>	-	2.492 <sup>(b)</sup>
<b>Reproductive biomass / total dry matter (Fr/Tot)</b>								
	<i>(P=0.281)</i>	<i>(P=0.584)</i>	<i>(P=0.024)</i>	<i>(P=0.024)</i>	<i>(P=0.002)</i>	<i>(P=0.381)</i>	<i>(P=0.054)</i>	<i>(P=0.015)</i>
Control	0.0394	0.0069	0.0285 <sup>(b)</sup>	0.0068 <sup>(b)</sup>	0.1502 <sup>(b)</sup>	0.4433	0.0755	0.1424 <sup>(ab)</sup>
Pacló	0.0205	0.0036	0.0208 <sup>(b)</sup>	0.00 <sup>(a)</sup>	0.0761 <sup>(a)</sup>	0.4205	0.0503	0.0881 <sup>(ab)</sup>
Unicon	-	-	-	0.0027 <sup>(ab)</sup>	-	-	-	0.0906 <sup>(ab)</sup>
TEP	0.0165	0.0048	0.0069 <sup>(a)</sup>	0.00 <sup>(a)</sup>	0.0724 <sup>(a)</sup>	0.4323	0.0562	0.0218 <sup>(a)</sup>
PhxCa	0.0379	0.0060	-	0.0045 <sup>(ab)</sup>	0.1235 <sup>(b)</sup>	0.4286	-	0.1776 <sup>(b)</sup>

Generalised linear regression analysis of progression to physiological cut out across all treatments, for aggregated data from experiments 8 (planting 1 and 2) and 11, explained treatment delays in cut out, taken as 4 nodes above the first position white flower (4 NAWF), for the Trinexapac ethyl (TEP) treatment only ( $P < 0.001$ ); trend lines were fitted (Figure 6.5) and resulting calendar days to 4 NAWF are presented in Table 6.8.

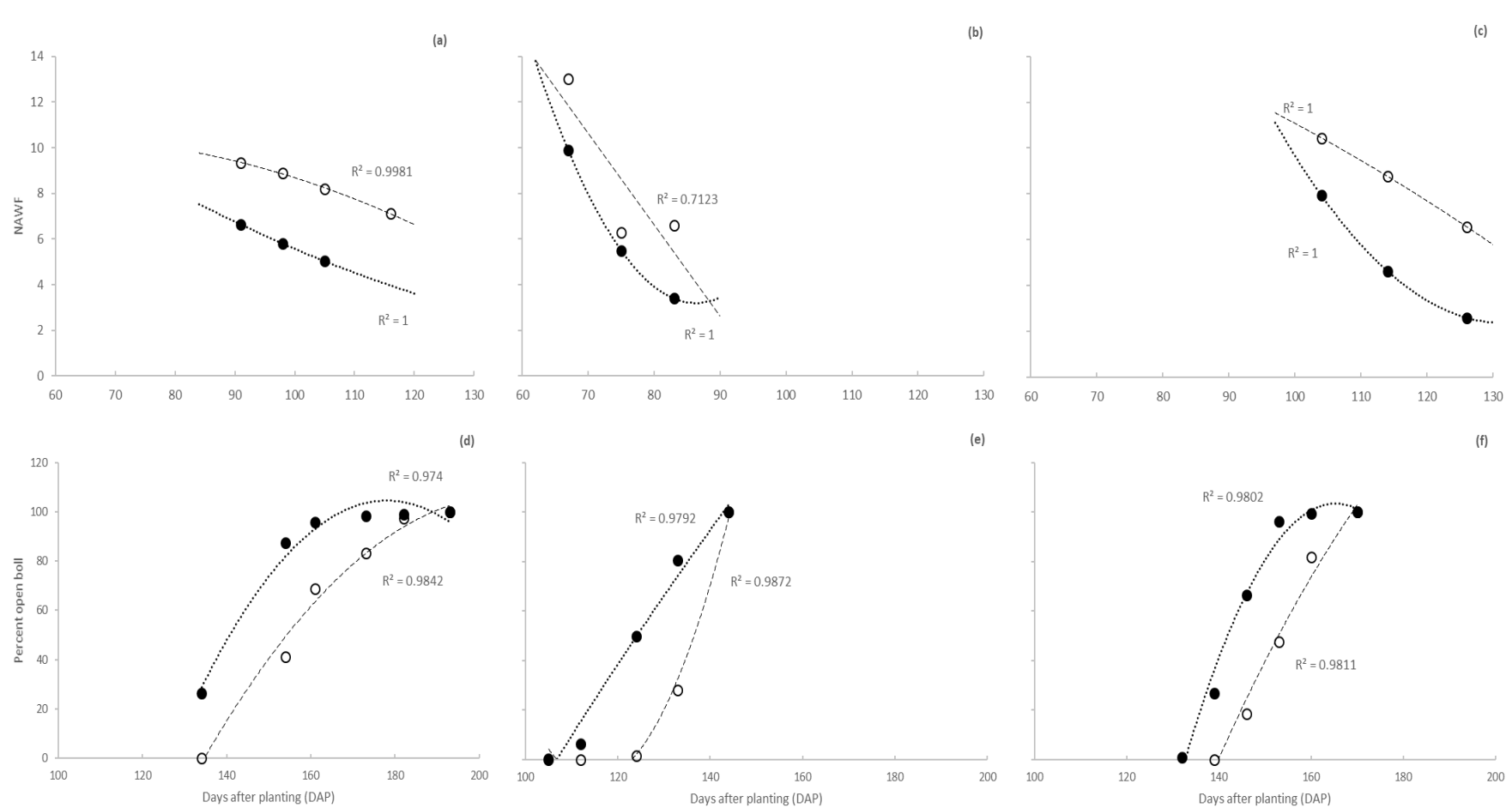
**Table 6.8 Treatment calendar days to physiological cut out (4 NAWF), for Gibberellin biosynthesis inhibitor PGR treatments in field experiments 8 and 11;  $R^2$  all greater than 0.71.**

Exp.	Treatment	4 NAWF (DAP)	Days>Trt1
8_S1	Control (Trt.1)	118.7696	0
8_S2	Control (Trt. 2)	80.3188	0
11	Control (Trt.1)	116.7794	0
8_S1	TEP (Trt. 14)	136.955	18.1854
8_S2	TEP (Trt. 15)	86.565	6.2462
11	TEP (Trt. 2)	139.4551	22.6752

Generalised linear regression analysis of sequential boll opening across all treatments, for aggregated data from experiments 8 (planting 1 and 2) and 11, explained treatment delays in maturity (as 60% open boll), for the Trinexapac ethyl (TEP) treatment only ( $P = 0.002$ ); trend lines were fitted (Figure 6.5) and resulting calendar days to 60% open boll load are presented in Table 6.9.

**Table 6.9 Treatment calendar days to maturity as 60% open boll load, for Gibberellin biosynthesis inhibitor PGR treatments in field experiments 8 and 11;  $R^2$  all greater than 0.94.**

Exp.	Treatment	60% open boll (DAP)	Days>Trt1
8_S1	Control (Trt.1)	144.3267	0
8_S2	Control (Trt. 2)	127.6093	0
11	Control (Trt.1)	144.248	0
8_S1	TEP (Trt. 14)	159.4896	15.1629
8_S2	TEP (Trt. 15)	138.2772	10.6679
11	TEP (Trt. 2)	155.686	11.4380



**Figure 6.5 Progression to physiological cut out (4 nodes above white flower (NAWF)) in experiment 8, planting 1 (a), planting 2 (b), experiment 11 (c) and progression to maturity (60% open boll) in experiment 8, planting 1 (d), planting 2 (e), experiment 11 (f), of gibberellin biosynthesis inhibiting PGR treatment Trinexapac ethyl (TEP); Control (....●....), TEP (----○----).**

Experiments 8 (planting 1 and 2) and 11 were managed for harvest and defoliated according to the control (Trt.1) maturity, resulting in residual unopen (green) bolls on later maturing treatments in all experiments. Final open boll numbers (per m<sup>2</sup>), mean open boll weights (g) and residual green boll counts (per m<sup>2</sup>) are presented in Table 6.10. Paclo, Unicon and PhxCa treatments demonstrating no differences from the control (Trt.1) in final OB numbers or size, with no residual unopened bolls present. TEP treatments demonstrated no differences from the control (Trt.1) in final OB size across the three experiments, with reduced OB counts demonstrated in the earlier planted experiments 8 (planting 1) and 11. Corresponding elevated numbers of residual unopened (green) bolls compared with the control (Trt.1), were sampled in experiment 11.

**Table 6.10 Yield components of Gibberellin biosynthesis inhibitor PGR treatments in experiments 8 and 11; notation indicating treatment differences at the P=0.05 level using Tukey's HSD.**

	<i>Exp 8, S1</i>	<i>Exp 8, S2</i>	<i>Exp 11</i>
<b><i>Open bolls (m<sup>2</sup>)</i></b>			
	<i>(P=0.003)</i>	<i>(P=0.488)</i>	<i>(P&lt;0.001)</i>
Control	260.5 <sup>(b)</sup>	224.2	174.7 <sup>(b)</sup>
Paclo	250.3 <sup>(ab)</sup>	204.7	159.8 <sup>(b)</sup>
Unicon	-	-	153 <sup>(b)</sup>
TEP	226.5 <sup>(a)</sup>	215.3	107 <sup>(a)</sup>
PhxCa	279.3 <sup>(b)</sup>	225	155.3 <sup>(b)</sup>
<b><i>Open boll mean weight (g)</i></b>			
	<i>(P=0.017)</i>	<i>(P=0.161)</i>	<i>(P=0.514)</i>
Control	4.047 <sup>(ab)</sup>	4.058	3.336
Paclo	3.895 <sup>(ab)</sup>	4.832	3.427
Unicon	-	-	3.535
TEP	4.189 <sup>(b)</sup>	3.978	3.329
PhxCa	3.692 <sup>(a)</sup>	4.064	3.263
<b><i>Residual green bolls (m<sup>2</sup>)<sup>(1)(2)</sup></i></b>			
			<i>(P&lt;0.001)</i>
Control	0.00	0.00	0.00 <sup>(a)</sup>
Paclo	0.00	0.00	0.00 <sup>(a)</sup>
Unicon	0.00	0.00	0.00 <sup>(a)</sup>
TEP	0.00	0.00	8.667 <sup>(b)</sup>
PhxCa	0.00	0.00	0.00 <sup>(a)</sup>
<b><i>Residual green boll mean weight (g)</i></b>			
			<i>(P&lt;0.001)</i>
Control	0.00	0.00	0.00 <sup>(a)</sup>
Paclo	0.00	0.00	0.00 <sup>(a)</sup>
Unicon	0.00	0.00	0.00 <sup>(a)</sup>
TEP	0.00	0.00	2.467 <sup>(b)</sup>
PhxCa	0.00	0.00	0.00 <sup>(a)</sup>

(1) Green bolls in experiment 8 (both plantings) eventually ripened and were harvested.

(2) Due to being situated within a commercial cotton crop with impending crop rotation constraints, residual unopened bolls in experiment 11 were harvested green at the last maturity harvest and processed for dry weights.

No treatment differences occurred for hand harvested cotton lint yields in experiment 8 (planting 1; P=0.910, planting 2; P=0.542). Machine harvested cotton lint yields for experiment 8 (planting 1 and 2), hand harvested cotton lint yield for experiment 11 (no machine harvesting occurred) and ginning turnout percentages are presented in Table 6.11.

**Table 6.11 Mean cotton lint yield (kg/ha) for Gibberellin biosynthesis inhibitor PGR treatments in experiments 8 and 11; notation indicating treatment differences at the P=0.05 level using Tukey's HSD.**

	<i>Exp 8, S1</i>	<i>Exp 8, S2</i>	<i>Exp 11</i>
<b><i>Lint yield (kg/ha)</i></b>			
	<i>(P=0.880)</i>	<i>(P=0.657)</i>	<i>(P=0.016)</i>
Control	1167	1071	702.8 <sup>(b)</sup>
Paclo	1158	1090	667.8 <sup>(ab)</sup>
Unicon	-	-	661.5 <sup>(ab)</sup>
TEP	1159	1020	469.6 <sup>(a)</sup>
PhxCa	1127	1062	584.2 <sup>(ab)</sup>
<b><i>Ginning turnout (%)</i></b>			
	<i>(P&lt;0.001)</i>	<i>(P&lt;0.001)</i>	<i>(P=0.139)</i>
Control	43.87 <sup>(a)</sup>	47.12 <sup>(a)</sup>	48.37
Paclo	46.14 <sup>(b)</sup>	48.11 <sup>(a)</sup>	48.38
Unicon	-	-	48.73
TEP	47.09 <sup>(b)</sup>	50.70 <sup>(b)</sup>	52.65
PhxCa	44.89 <sup>(a)</sup>	48.26 <sup>(a)</sup>	46.31

No treatments differences occurred for lint length (32nds) in experiment 8 (planting 1; P=0.522, planting 2; P=0.689) and experiment 11 (P=0.558). No treatment differences occurred for lint strength (g force per tex) in experiment 8 (planting 1; P=0.701, planting 2; 0.737); all treatments being classed as “strong to very strong”. In experiment 11, treatment 2 (TEP) had a higher lint strength than the control (P=0.007), being classed as “very strong”; no other treatment differences occurred, with all treatments being classed as “intermediate to mean”. Treatment micronaire responses and micronaire quality grades for experiments 8 (planting 1 and 2) and 11 are presented in Table 6.12.

**Table 6.12 Lint fibre micronaire and grading for Gibberellin biosynthesis inhibitor PGR treatments in experiments 8 and 11; notation indicating treatment differences at the P=0.05 level using Tukey’s HSD, quality grades are presented following micronaire value, base grade being G5.**

	<i>Exp 8, S1</i>	<i>Exp 8, S2</i>	<i>Exp 11</i>
<i>Micronaire</i>			
	<i>(P=0.573)</i>	<i>(P=0.018)</i>	<i>(P=0.003)</i>
Control	5.003, G6	5.105 <sup>(b)</sup> , G6	3.33 <sup>(a)</sup> , G4
Paolo	5.208, G7	5.188 <sup>(b)</sup> , G6	3.8 <sup>(ab)</sup> , G5
Unicon	-	-	4.067 <sup>(ab)</sup> , G5
TEP	5.167, G6	4.463 <sup>(a)</sup> , G5	4.667 <sup>(b)</sup> , G5
PhxCa	5.058, G6	4.845 <sup>(ab)</sup> , G5	3.483 <sup>(a)</sup> , G5

## 6.4 Discussion

The growing conditions experienced for all field experiments included within this analysis were significantly hotter and drier than comparable long term means for each site and growing period; being evidenced as an increased number high temperature stress days (days>36 °C), increased day degree (DD) accumulation, a decreased number of cold shock days (temp<11 °C) and decreased rainfall (Appendix 3). Moreover, day of year differences in planting time due to logistics associated with pre-plant irrigation scheduling (ACRI sites, see Chapter 3, Section 3.4.2, Table 3.5) and collaborating-grower farm enterprise activities (specifically, Terry Hie Hie sites), in addition to replanting due to poor establishment (experiment 6) or crop failure (hail, experiment 9), resulted in differences in control treatment growth patterns between experiments. Notwithstanding, lint yields achieved in the 5 field crops examined in this study that were grown to maturity, were at or above the respective mean annual Australian rain grown cotton yields (ABARES, 2019; CA, 2018b).

### 6.4.1 Glasshouse and early field screening; experiments 3, 6 and 7

Limited information exists regarding cotton crop responses to many of the treatments investigated (Appendix 2), notably for early vegetative growth applications. Moreover, most studies reference high-yielding, irrigated cotton crops, a proportion of which being non-transgenic upland cotton or *G. barbadense* varieties. Following initial appraisal of treatment delays in first square, consequent crop and plant level flow-on impacts are evaluated with published literature on a per-treatment basis across experiments 3, 6 and 7.

### ***Delaying crop-level reproductive phase change***

Glasshouse based evaluations in experiment 3 provide preliminary assessments of treatment responses to single PGR applications under controlled environment conditions; both foliar and soil applications of Paclobutrazol (Trt. 4 and 5) and the foliar Uniconazole application (Trt. 6) producing 16-, 15- and 11-day delays in progression to first square, respectively. Data from (field) experiment 7 demonstrated no significant differences in TTFSqu due to an earlier insect-induced square shedding event. In both experiments the TEP treatment grand mean showed an increased time to first square compared to the control (Trt.1), although the delay was not defensible with Tukey's post-hoc HSD test.

### ***Consequent treatment flow-on impacts from Chlormequat chloride***

No Chlormequat chloride (CC) induced differences in leaf area occurred during early- (experiment 3) and mid-flowering (experiments 6 and 7), differing from the decreased mid-flowering leaf area reported by (Dhillon et al., 1980). Differences being potentially attributable to their later (and when comparing to experiment 3, multiple) PGR treatment application timings of first square and first flower, having a longer period of impact into flowering. Decreased total dry matter weights, at early- (experiment 3) and mid-flowering (experiment 7) are analogous with decreased growth rates and mid / late flowering plant heights reported by Dhillon et al. (1980), Echer and Rosolem (2012) and S. S. Rao et al. (2016) for first square onward spray applications.

CC treatments showed some inconsistency in reproductive response, notably between field experiments; being potentially attributable to environment and management interactions obscuring responses (such as plant stand variability in experiment 6), or the timing of early squaring insect pressure impacts in experiment 7. Early flowering sampling in experiment 3 demonstrated decreased proportionate reproductive development (FR:TOT) comparative to the control (Trt.1) via reduced green boll (including flower) number and mean size. Square count and mean size were consistent with control (Trt.1); results could be associated with either reduced early fruit retention or a delay in rate of fruit development, both leading to reduced green boll numbers and could be quantified in future research. However, no differences were evident in mid-flowering fruit load in experiment 6; proportionate reproductive biomass, square, green boll and open boll counts and mean sizes were all consistent with the control (Trt.1). Mid-flowering analysis in experiment 7, which showed increased, possibly compensatory following insect pressure, square numbers to the control (Trt.1) although being consistent in size to the control. No differences occurred in green

or open boll number and green boll size; open boll size was decreased. Thomas (1975) notes the imperative of application timing in determining cotton plant response to CC; results regarding early vegetative stage applications of CC are non-existent, however in contrast to mid-flowering results from experiments 6 and 7, both Thomas (1975) and Marani et al. (1973) noted reduced retention from first flower CC applications of similar concentrations (up to 125 ppm).

No differences occurred in final seed cotton yields, open boll numbers and mean weights from both experiments 6 and 7; at higher concentrations (250-750 ppm), S. S. Rao et al. (2016) and Sawan (2016) both document increased open boll numbers (boll set), increased boll weight and increased seed cotton yields at maturity from later (first to mid flower) CC applications under irrigated field conditions. In experiment 7, no difference occurred from the control (Trt.1) for both lint turnout and lint yield. Comparable to finding of Bhatt and Ramanujam (1971), micronaire was increased in experiment 7, as was lint strength and length. Chloromequat chloride induced 3- and 11-day delays in maturity (progression to 60% open boll) in experiments 6 and 7, respectively; which, when considered with the equal progression to first square, final open boll counts and sizes, may indicate a lengthening of the flowering period (assuming equal fruiting pattern).

#### ***Consequent treatment flow-on impacts from Mepiquat chloride***

No Mepiquat chloride (MC) induced differences in leaf area occurred during early- (experiment 3) and mid-flowering (experiments 6 and 7); differing from mid-flowering results reported by Gonias et al. (2012) and Gausman, Walter, Rittig, Escobar, and Rodriguez (1980), although both utilised later and multiple applications in early flowering of higher (440 ppm) concentrations. Early singular foliar applications of MC reduced total dry matter production in early flowering (experiment 3); comparable to results obtained through Mepiquat chloride seed priming by both Ferrari et al. (2015) and de Almeida and Rosolem (2012) and 3-leaf foliar application by L. Wang et al. (2014). In contrast, no decrease in total dry matter was observed (later) during mid-flowering in experiments 6 or 7, where multiple, early vegetative stage applications occurred.

Differences in reproductive growth from the control (Trt.1) were not evidenced for MC treatments across experiments 3, 6 and 7. No treatment differences occurred in early and mid-flowering for proportionate reproductive biomass accumulation, as well as number and mean size of squares, green bolls and early open bolls. This contrasts with recent seed priming data reported by Fang et al. (2018), that attest fewer floral buds initiated at control

first square in growth chamber grown cotton. Notably, Rosolem, Oosterhuis, and de Souza (2013) detailed the interaction of MC efficacy with temperature; above optimal temperatures, as quantified in experiments 6 and 7, requiring increased concentrations of MC per unit of growth reduction.

Moreover, no treatment differences in time to maturity, final open boll counts, open boll size, lint turn out or lint yield occurred in experiments 6 and 7. Comparative findings of Sawan and Sakr (1990), noting increased seed cotton and lint yields, boll numbers, and boll weights, provide an incongruous contrast owing to later treatment application dates in flowering (80-105 dap) on irrigated *G. barbadense* cotton, aimed at promoting “earliness”. The central proposition from screening experiments in the current study indicates no differences between the specific MC rate, application method, frequency and timing, in invoking either a later transition to first square or subsequent delay in peak resource demand.

#### ***Comparison of consequent treatment flow-on impacts from Paclobutrazol and Uniconazole***

Both soil drench and foliar Paclobutrazol (Paclo) applications were evaluated in experiment 3, 6 and 7; soil drench applications being of interest owing to a lengthy (>3 months) period of residual efficacy in warm climates (Rademacher, 2016). No difference in leaf area occurred across all experiments; however, decreased total dry weight accumulation was evident at early flowering in the glasshouse experiment 3, but not at mid-flowering in field experiment 6 and 7. This contrasted with the findings of Zaghlool and Ibrahim (2005) and Ben-Porath et al. (1988), although both having significantly later (first flower) and multiple PGR treatment application timings that extended treatment efficacy. A decrease in proportional reproductive biomass (FR:TOT) accumulation at early flowering was evident in experiment 3 for the soil drench (but not foliar) application, with no differences in both soil and foliar treatments evident later in flowering in the field experiment 6 and 7. This corresponds with data reported by Zaghlool and Ibrahim (2005), that soil or soil plus early foliar PGR application treatment impacts on growth and development had ceased by early flowering. No differences occurred in final open boll numbers, size, seed cotton, lint percentage (turnout) or lint yield; corroborating with findings of Ben-Porath et al. (1988) that earlier applications have a stronger growth impact with an associated cascade of physiological ramifications, and a lesser impact on late-season fruit development or yield.

Attributable to both being inhibitors of cytochrome P450-dependent mono-oxygenases, which catalyse the same oxidative step in the gibberellin biosynthesis pathway; Uniconazole-P (Unicon) soil and foliar treatment response results principally mirrored those of Paclo. Additionally, Unicon foliar treatment grand means demonstrated small delays in maturity, of 9 and 6 days (experiments 6 and 7, respectively), but were not significant at the  $P=0.05$  level via Tukey's HSD. Contrasting with mid-flowering biomass results from experiment 6, increased green boll numbers at mid flowering in experiment 7 with no difference in size, supported earlier findings Mohamed et al. (2010). No differences occurred in final open boll numbers, size, seed cotton, lint percentage (turnout) or lint yield. Despite a clear treatment delays in first square from both soil and foliar Paclo treatments, as well as foliar Unicon; consequential fruit loads, maturity patterns and yields do not suggest any differences from the control in the subsequent timing of resource demand.

#### ***Comparison of consequent treatment flow-on impacts from Prohexadione calcium and Trinexapac ethyl***

The specific Prohexadione-calcium (PhxCa) treatment rate, application and timing combination evaluated in experiments 3,6 and 7, invoked no differences in leaf area, biomass, reproductive growth and resultant yield outcomes; increases in lint strength and length were observed. Limited comparative analysis exists for PhxCa application in cotton; Inoue, da Costa, Mendes, Mertens, and Goulart (2015) reported no treatment influence on boll maturity, defoliation or seed cotton yields from late season (160 DAP / 85% open boll) applications of higher (275 and 410 ppm) rates.

No differences in leaf area occurred during early- (experiment 3) or mid-flowering (experiments 6 and 7) for Trinexapac ethyl (TEP) treatments. Decreased total dry matter was observed at early- (experiment 3) but not mid-flowering (experiments 6 and 7), from multiple treatment applications. Mid-flowering biomass findings are comparable to those of Correia and Leite (2012), resulting from a smaller (4-fold decrease) concentration and single application at first flower; indicating that the direct growth impacts of both treatments had dissipated by mid-flowering.

Proportionate reproductive biomass accumulation was reduced in early-flowering (experiment 3) but showed no difference from the control at mid-flowering (experiments 6 and 7). No differences in square number or size occurred at either early- (experiment 3) or mid-flowering (experiments 6 and 7). Contrasting results occurred in green boll accumulation; with reduced numbers and size being evidenced in experiment 3 at early

flowering, no difference in experiment 6 at mid-flowering and an increase in number (with no size difference) in experiment 7, also at mid-flowering. No differences occurred in early open boll numbers at mid-flowering in experiments 6 and 7. However open bolls were smaller compared to the control in experiment 7, conceivably commensurate with the increased green boll numbers and potential later development.

TEP produced increased nodes above white flower at control cut-out (4 NAWF) in experiment 7. A similar treatment effect was also suggested in experiment 6, just prior to control cut out (control, Trt.1 at 6 NAWF) ( $P=0.078$ ). Moreover, TEP affected crop progression to maturity (60% open boll); evidenced as a 12-day delay to maturity in both experiments 6 and 7. No treatments impacts were observed regarding open boll number and size at maturity, as well as seed cotton yield for both experiments 6 and 7. Furthermore, lint percentage (turnout) and lint yields showed no differences from the control in experiment 7; all lint quality parameters (strength, micronaire and length) were increased comparative to the control. Delays in progression to physiological cut out and maturity, when considered with the equal progression to first square and final open boll counts and sizes, may indicate a lengthier flowering period (assuming equal fruiting pattern) in TEP treated cotton.

#### **6.4.2 Concept evaluation in field experiments 8, 9 and 11**

Key treatments from preliminary investigations in experiments 3, 6 and 7, were selected for further evaluation across subsequent seasons in experiments 8 (2 plantings), 9 and 11. Both Paclo and TEP treatments being selected on account of more obvious (but not conclusive) mechanisms of both delaying progression to FSqu and resultant flow on impacts on fruit loads and maturity (influencing resource demand). In screening experiments Paclo demonstrated delays in TTFSqu and TEP, whilst demonstrating no significant delay in FSqu, demonstrated an indicative response worth further validation, notably in conjunction with delays in maturity and NAWF. PhxCa was included on account of potential water stress adaptation benefits associated with its ancillary anti-ethylene mode of action. Lastly, both PhxCa and Unicon were included in a proportion of experiments, as a comparative PGR from the same GaBI mode of action sub-group; relating to TEP and Paclo treatments, respectively.

Experiment 8 was within (1<sup>st</sup> planting) and after (2<sup>nd</sup> planting) the standard planting window to determine consistency of results against prior experiments 6 and 7; whilst experiment 9 and 11 were both planted very early as a “proof of concept”, unfortunately with experiment

9 then having to be re-planted (with a smaller number of treatments) outside the optimal window due to hail destruction occurring in late December.

### ***Delaying crop-level reproductive phase change***

Biomass assessments were utilised both as a proxy for time-to-first-square assessments against the control and to provide information regarding the morphological characteristics of any delay in reproductive growth achieved. Where apparent, induced delays in reproductive development evidenced as a decreased square load compared with the control (Trt.1) at early squaring, were consistently demonstrated by a decreased accumulation of total fruiting sites and augmented by a decreased retention of squares on those (reduced number of) sites. Interestingly, mean square size was not impacted by reduced retention.

Across the 4 crops assessed in experiments 8, 9 and 11, PhxCa treatments demonstrated no difference from the control in number of squares, total fruiting sites, retention on those sites or mean size of squares counted, from the control; being similar to findings in earlier experiments 6 and 7. Commensurate with earlier findings in experiment 3, Paclo treatments had reduced square counts at control (Trt.1) early squaring in both experiments 8 (planting 1) and 11, but not with later plantings in experiments 8 (planting 2) and 9. Correspondingly, the total number of sites grown, as well as retention of squares to those sites was also decreased in both experiment 8 (planting 1) and 11, but not with later plantings in experiment 8 (planting 2) and 9. No statistical difference in the number of squares occurred between the control (Trt.1) and Unicon treatments in experiment 11, contrasting with earlier results of Unicon foliar applications in experiment 3. Referencing earlier findings; TEP treatment grand means demonstrated an increased time to first square in experiment 3 and first flower in experiment 7, although neither being cogent by means of Tukey's post-hoc HSD test. Similarly, but of statistical significance, TEP treatments across all experiments (8, both planting dates, 9 and 11) had fewer squares, fewer total sites produced and had a reduced retention of squares to those (lesser quantity of) sites, in comparison to control treatments at early squaring.

### ***Consequent treatment flow-on impacts – were we able to shift peak resource demand?***

Delays in reproductive development were quantified across all TEP treatments investigated and in Paclo treatments in experiments 8 (planting 1) and 11. The cascade of morphological impacts resulting from these delays varied between the two treatments. Comparisons with other published literature utilising PGRs for similar end effect, in cotton or other day-

neutral and indeterminate-flowering crops, is difficult due to a lack of published data. However, comparison with other methods of fruit removal in cotton during early squaring, assists in reckoning the mechanism of flow-on crop responses and resulting resource demand impacts to specific PGR treatments.

Considering Paclo treatments, delays in reproductive growth development coincided with a decrease in leaf area index (LAI) and total dry weight (TOT) in experiment 8 (planting 1) only, with no impact on either LAI or TOT being evident in other experiments (8, planting 2, 9 and 11) at early squaring or at early flowering. No treatment differences occurred in progression to physiological cut out or maturity. Moreover, no Paclo treatment differences were evident in final open boll counts, mean weights or resultant lint yield. Lint percentage (turnout) was increased in experiment 8, planting 1, only and no Paclo treatment differences were evident in lint quality. In summary no conclusive, flow-on impacts of the Paclo induced delay in first square are evidenced in the subsequent flowering period, maturity pattern or yield and associated components. This substantiates earlier findings, that despite a delay in first square, no impact was obtained on the timing of peak resource demand.

Delays in reproductive growth induced by TEP treatments coincided with decreased total dry weights in early squaring across experiments 8 (planting 1 and 2) and 11, with corresponding decreases in LAI, except for the late planted experiment 8, planting 2. These TEP treatment total dry weight decreases were still evident in early flowering across experiments 8 and 9. LAI in early flowering was also decreased in experiments 8 (planting 1) and 11. These findings contrast with Zhai et al. (2018) who documented a compensatory increase in LAI and total dry matter in irrigated Bt cotton following removal of the first 2 – 4 basal fruiting branches at early squaring (imitating insect damage). The timing of LAI and dry matter investigations subsequent to Zhai et al. (2018)'s treatments were not detailed, however differences between can logically be attributed to lag effect of TEP inhibiting growth into flowering and preventing a compensatory vegetative growth response, as seen in Zhai et al. (2018), following removal of (sink) fruiting structures.

Yield and yield component responses to TEP treatments varied across experiments. TEP treatments induced delays in both progression to physiological cut out (4 NAWF) and maturity of mean 15 and 12 days, respectively, across all experiments grown to harvest. This is an increased delay compared with Wilson, Sadras, Heimoana, and Gibb (2003), who documented a 7-day delay in maturity following removal of the first 4 fruiting

branches at first square. Manual fruit removal at first square either had no impact on lint yield (Wilson et al., 2003) or increased lint yields obtained in non-resource constrained irrigated cotton (Dong et al., 2008; Saleem et al., 2016). Documented increases being attributed to increased retention of (mid to late season) bolls on the upper fruiting branches (Hake et al., 1992; G. H. Wang, Gutierrez, Asiimwe, & Zarnstorff, 2011), evidencing a change in fruiting pattern and possibly affecting both intensity (scale) and timing of peak resource demand.

TEP treatment in experiment 8, planting 1, resulted in a decreased final open boll count compared with the control (Trt.1). No differences from the control occurred in final open boll numbers, mean size or resultant seed cotton yield in experiment 8, planting 2. No differences in resultant lint yield occurred for either planting dates in experiment 8. Both experiments also had an increased lint percentage (turnout).

Unseasonably hot and dry late season conditions resulted in an earlier than anticipated season finish in experiment 11 (Tomlinson & Maunder, 2019). On account of the surrounding commercial crop, harvest preparation management of the experiment was based around the control, which combined with the later progression to maturity associated with the TEP treatment, which resulted in residual green bolls and a decreased open boll count in the TEP treatment compared with the control (Trt.1). However, the green boll count did not make up the difference between the control and TEP treatment open boll counts, resulting in a decreased lint yield, with no difference in lint percentage (turnout).

No variation in fibre length from the control existed across all three experiments (8, planting 1 and 2, 11). No difference in micronaire occurred in experiment 8, planting 1; decreased micronaire in planting 2 being possibly attributable to high boll loads relative to canopy, or due to immature fibre associated with managing for defoliation and harvest from planting 1. In contrast, high micronaire in experiment 11 is potentially associated with either increased relative photoassimilate partitioning to a reduced number of bolls on a commensurate sized canopy (as no decrease in total dry weight was evidenced in early flowering) or, possibly to changed (delayed) fruiting patterns caused by TEP treatments, shifting the peak boll filling period into (in this case) less than ideal later season hot/dry conditions, affecting the lint sample mean. Additionally, TEP treatment resulted in increased fibre strength in experiment 11. Fibre strength being associated with the degree of secondary cell wall thickening, with differences in strength depending on the chemical structure of the cellulose laid down in that secondary wall over the 40-day thickening period

per boll (Bange et al., 2017) and as with micronaire, being most likely attributable to a decreased overall boll load. Increased strength and micronaire outcomes are supported by Dong et al. (2008) and Saleem et al. (2016), both in irrigated Bt cotton, after the manual removal of the basal two fruiting branches or squares from basal two branches, respectively, at squaring to simulate early insect damage. This suggests that on the premise of increased retention of later bolls, a changed fruiting pattern (and consequential demand for resources) has occurred.

## 6.5 Conclusions

This study investigated; the impacts of specific Gibberellin biosynthesis inhibiting PGR treatment scenarios on time to FSqu and the consequential effects thereafter on the timing of peak resource demand, as explained by yield, lint quality, biomass and LA accumulation, as well as fruit development, retention and maturity outcomes. The data rejects the null hypothesis that Gibberellin biosynthesis inhibiting PGR treatments induced no plant phenological responses in the timing of crop-level reproductive phase change; consequent changes in peak resource demand were also evidenced but require further quantification as to the magnitude of resource demand shift.

Quantification of time to FSqu in early screening experiments provided no evidence of MC, CC or PhxCa PGR treatment capacity to delay crop-level physiological phase change to reproductive growth. CC treatments delayed crop maturity, but with no significant differences from the control (Trt.1) evident in both early season phase change and end of season yield components; extension of flowering period is a plausible explanation; having water use efficiency implications. Both MC and PhxCa treatments demonstrated no resultant differences from the control (Trt.1) in peak resource demand, as determined by yield, lint quality, biomass and LA accumulation, as well as fruit development, retention and maturity outcomes.

Both early screening and concept evaluation experiments demonstrated Paclo PGR treatment efficacy in delaying crop progression to FSqu, however no flow-on impact on the timing of peak resource demand was demonstrated. Concept evaluation experiments established no conclusive, flow-on impacts in the subsequent flowering period, maturity pattern or yield and associated components. Unicon PGR treatments were not able to delay early reproductive growth under field conditions or delay peak flowering and consequent resource demand. Treatments delayed progression to FSqu under controlled environment conditions, however efficacy in delaying crop-level phase change under early planted field

conditions was not substantiated. Consequential fruit loads, maturity patterns and yields achieved from both screening and concept evaluation experiments do not suggest any utility of Unicon in promoting a time-delay in the occurrence of peak resource demand.

TEP applications were able to delay early crop level phase-transition into reproductive growth. However decreased boll numbers and resultant lint yield compared with the control in a proportion of experiments, confuses the notion of a similar boll load, just moved across time (temporally). Moreover, lint quality changes in micronaire and strength indicate possible influences of increased assimilate provision to the boll load; whether this as a result of a smaller comparative boll load or compensatory growth, and / or increased retention of later fruit, resulting in changed climatic conditions (from the control) through boll filling, is uncertain and requires confirmation. We can infer that that on the basis of a temporal deferral of boll set, as evidenced by delays in TTF<sub>Squ</sub>, early proportionate reproductive biomass accumulation, NAWF and maturity; that the potential for a delay in timing of peak resource (water) use in the TEP treatments occurred. Temporarily delaying or shifting the yield-critical early flowering to cut out period to a later climatic period of lower mean temperatures and vapour pressure deficits (VPD), can improve or stabilise annual yield outcomes and improve downside risk associated with crop establishment outside the optimum window.

Cotton plant response to PGR treatments and the subsequent timing and magnitude of crop plant demand for water across the flowering and boll filling period is affected by many factors, several remaining unquantified in this body of work and requires further investigation and explanation. Higher resolution quantification of the time and space distribution of fruit development and retention across the flowering period, per total crop water input (considering in crop rainfall, soil water changes and runoff) may enhance characterisation of resource demand patterns in response to PGR treatments. Moreover, quantification of the role of plant architecture in achieving PGR induced delays in peak resource demand, notably via lateral branch fruit loads, may provide insights regarding lint quality outcomes. Lastly, accurate quantification of plant stress levels via canopy temperature, in response to both chronic water deficit stress throughout flowering and acute water deficit (and heat) stress events during mid flowering, may provide further understanding around annualised and locality based differences in cotton plant responses to identical PGR treatment scenarios.

This body of work begins to explain the utility of specific Gibberellin biosynthesis inhibiting PGRs to slow early vegetative growth, manipulate the phase-transition to reproduction and temporally shift the subsequent peak resource demand. The resulting data contributes to the understanding of specific cotton plant phenological responses to Mepiquat chloride, Chlormequat chloride, Paclobutrazol, Uniconazole, Trinexapac ethyl and Prohexadione calcium treatment scenarios, under resource constrained rain grown production settings.

## **Chapter 7 “Value of the Opportunity”: Quantifying the System Yield, Input Use Efficiency and Economic Values of PGR Responses**

### **7.1 Introduction**

Chapters 4, 5 and 6 of this thesis presented research regarding capacity of specific PGR treatment scenarios to strategically manipulate cotton plant growth and development, under resource limited rain grown production contexts on Vertic soils and skip row planting configurations. Evaluated Adenine-type synthetic Cytokinin, 6BA, as well as the bioactive Gibberellin A<sub>3</sub> and A<sub>4,7</sub> PGRs, demonstrated no consistent treatment responses indicative of respective targeted physiological mechanisms (Chapter 2, Section 5.4, Table 2.3). Consequently, under rain grown production contexts tested here, the synthetic Adenine-type Cytokinin and bioactive gibberellin treatment scenarios evaluated, hold limited potential in the creation of novel adaptive management strategies to manage climatic variability and promote system resilience.

Foliar applications of Gibberellin biosynthesis inhibiting PGR, Trinexapac ethyl (TEP) demonstrated utility in the targeted physiological mechanisms of slowing growth and delaying crop-level reproductive phase change (as emergence of FSqu). Furthermore, flow-on treatment impacts on fruit development and maturity outcomes demonstrated a temporal shift in peak flowering and consequent resource demand of 12 to 15 calendar days. No other Gibberellin biosynthesis inhibiting PGR treatment scenarios evaluated produced physiological responses to delay peak resource demand. Consequently, only the potential long-term system yield, water use efficiency and economic benefits of the Trinexapac ethyl PGR-mediated opportunity will be explained further.

This chapter details crop response and economic simulations conducted to quantify the systems-value of the TEP PGR delay response. This analysis aimed to:

- Quantify long term lint yield and water use efficiency benefits of TEP induced delays in crop peak resource demand;
- Compare annualised profit outcomes of TEP PGR treatment scenarios;
- Determine the long-term impact on profit outcomes associated with TEP induced delays in peak resource demand; and

- Assess the (economic) functionality of a “proportionate application” (i.e. partial application to a section of the total crop area planted) in Australian rain grown cotton systems.

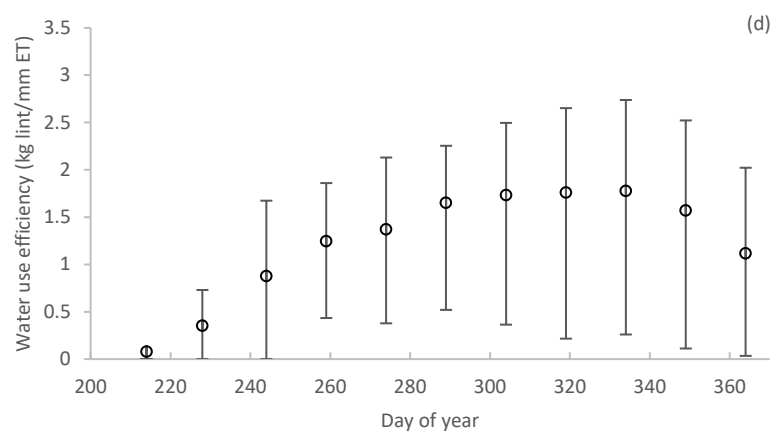
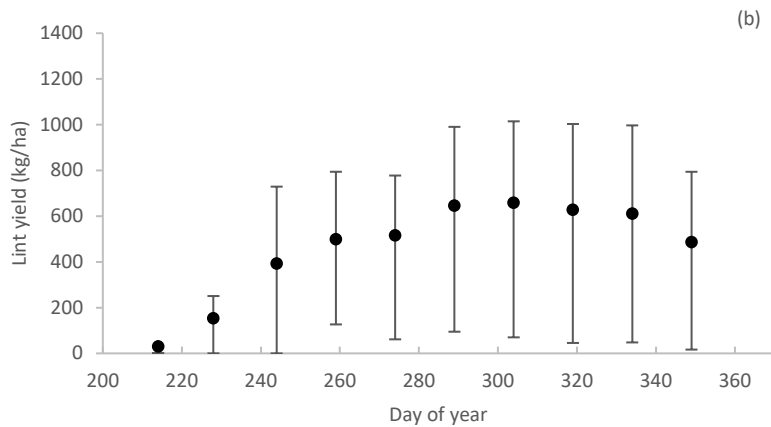
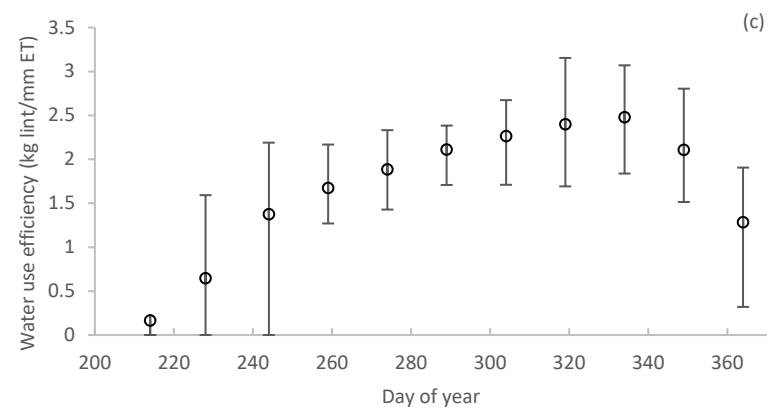
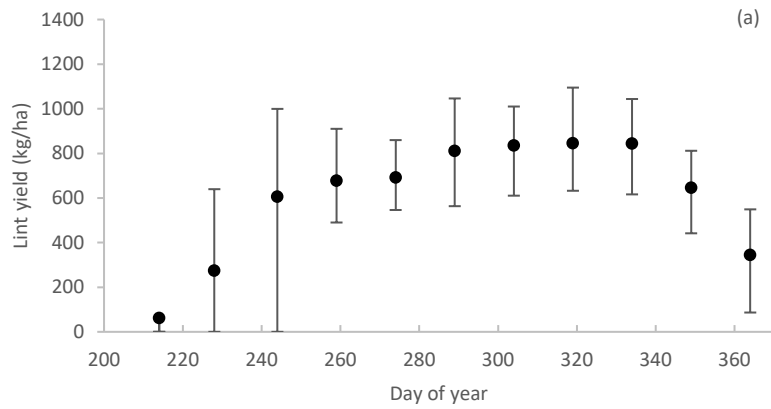
Analysis being presented step-wise with respect to these specific aims; respective methodologies, reporting and discussion of results presented within individual sections.

## **7.2 Quantifying lint yield and water use efficiency benefits of Trinexapac ethyl induced delays in crop peak resource demand**

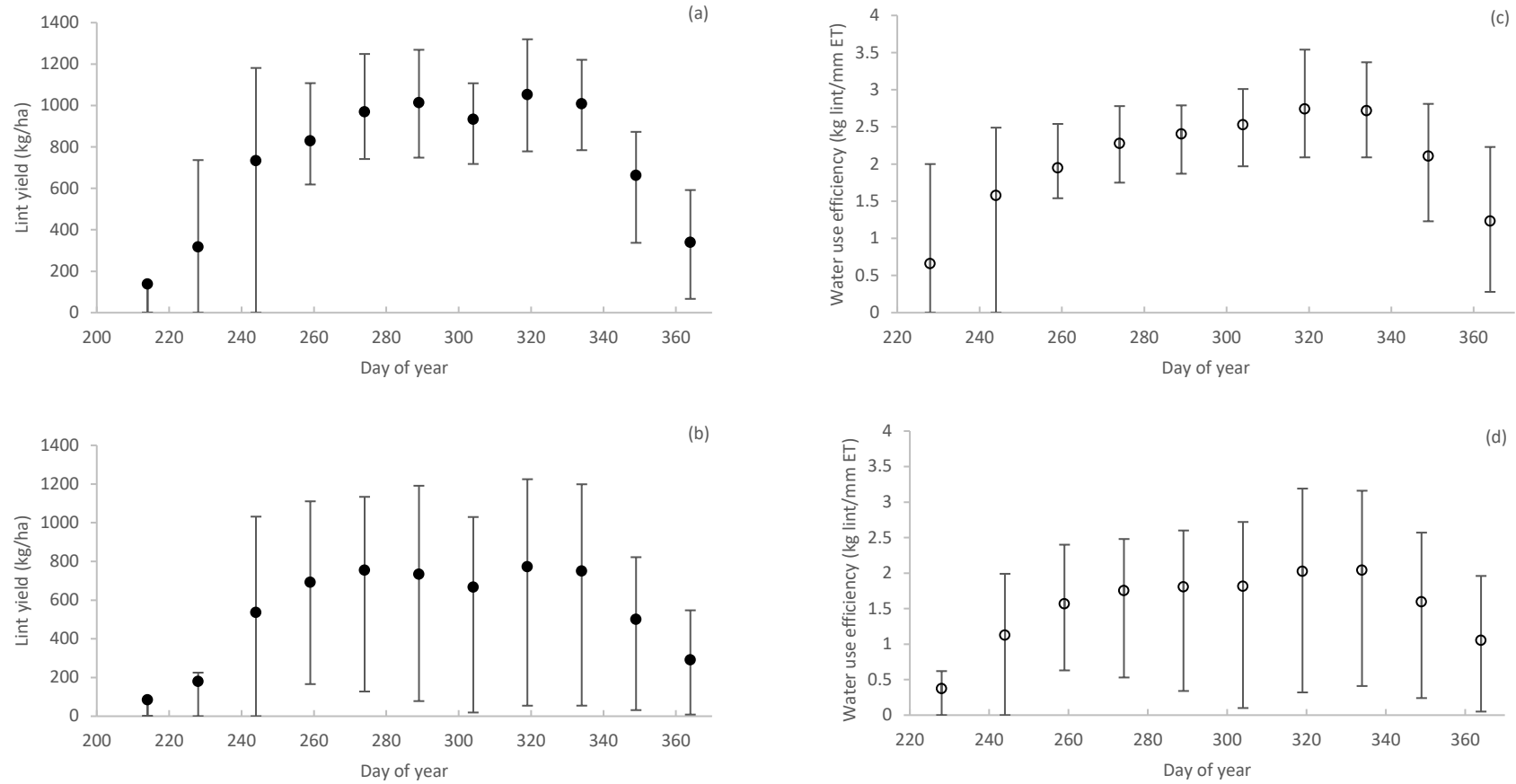
Comparative to and as an extension of, physiological crop response simulations presented in Chapter 6, that characterised mean lint yield and WUE outcomes, as influenced by time of planting, (Chapter 3, section 3.7), Early-stage model adjustments were made to OzCot (Hearn, 1994; Milroy et al., 2004) as specified in Chapter 3, section 3.7. The aim being to provide an initial indication of the lint yield and WUE changes associated with achieving delay in crop level reproductive phase change and consequent peak resource demand, with Trinexapac ethyl PGR treatments (Figures 7.2, 7.3, 7.4).

TEP treatment induced delay in TFSq was calculated from results of glasshouse experiments as an additional 50 DD (Appendix 6). Simulation of delay (“Delayed”) in crop progression to 50% population first square was achieved through manipulating the “DDISQ” function (day degrees to 50% population first square) in the variety input (.inp) file. The standard 74BRvariety.inp file was utilised across all analysis, reaching population 50% FSqu at 426 Day Degrees from planting, with the consequent “delayed” adjustment at 476 Day Degrees from planting.

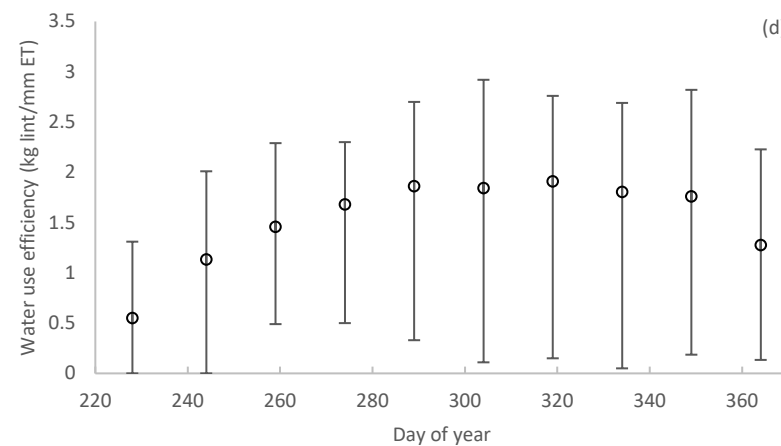
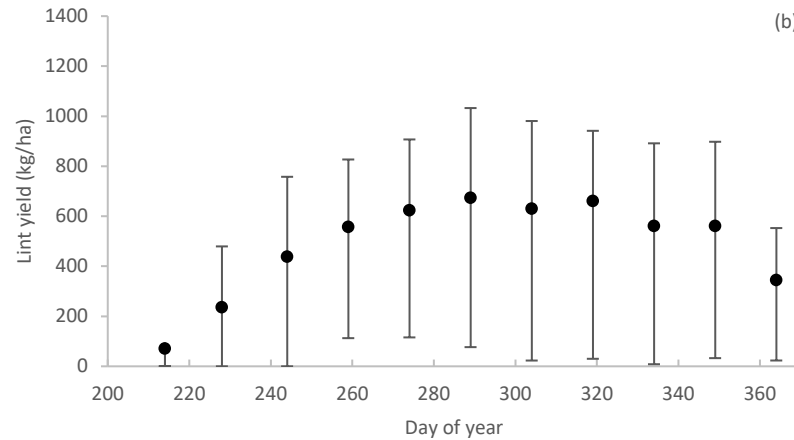
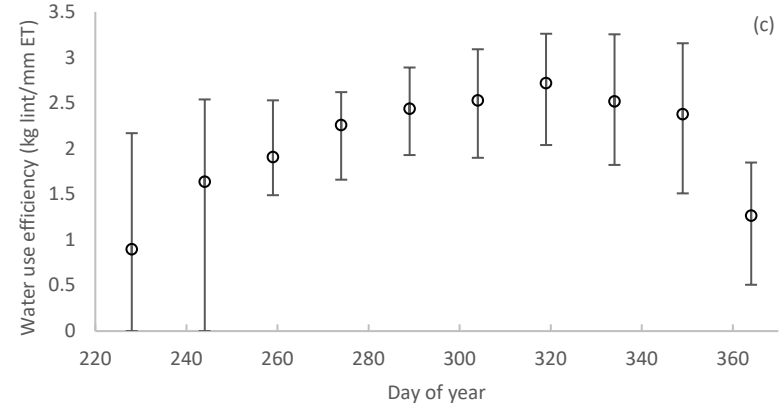
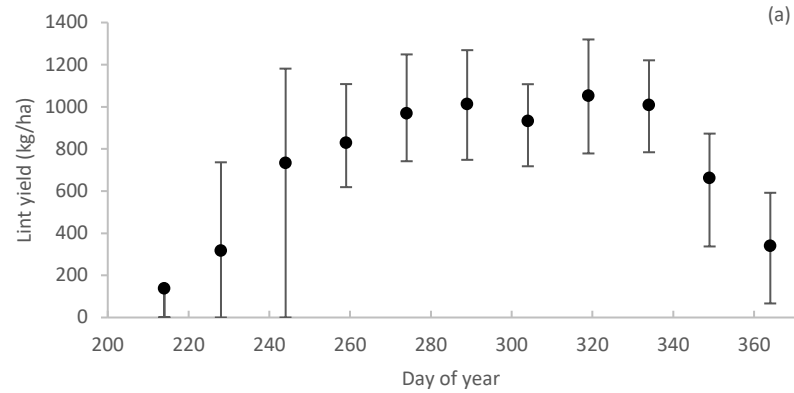
Across the three field experiment sites modelled (Figure 7.2, 7.3, 7.4), delaying progression to FSq in the model resulted in lower mean lint yields and increased downside yield risk (being the potential to incur a decline in yield associated with changing environmental conditions), across all planting dates. Similarly, WUE was also decreased across all experiment sites and planting dates simulated (Figure 7.2, 7.3, 7.4).



**Figure 7.2** Fifty year historical mean lint yields ( $\text{kg}\cdot\text{ha}^{-1}$ ) and water use efficiency (WUE) ( $\text{kg lint} / \text{mm ET}$ ) obtained from physiological crop response simulations using OzCOT (Hearn, 1994), per 15 day establishment intervals (1-Aug to 29-Dec) at Narrabri (ACRI) field site; range is 80% (lower) and 20% (upper) probability of exceedance, a) mean lint yield ( $\text{kg}\cdot\text{ha}^{-1}$ ) from Standard crop growth, b) mean lint yield ( $\text{kg}\cdot\text{ha}^{-1}$ ) from Delayed progression to first square, c) mean WUE ( $\text{kg lint} / \text{mm ET}$ ) from Standard crop growth, d) mean WUE ( $\text{kg lint} / \text{mm ET}$ ) from Delayed progression to first square.



**Figure 7.3** Fifty year historical mean lint yields ( $\text{kg}\cdot\text{ha}^{-1}$ ) and water use efficiency (WUE) ( $\text{kg lint} / \text{mm ET}$ ) obtained from physiological crop response simulations using OzCOT (Hearn, 1994), per 15 day establishment intervals (1-Aug to 29-Dec) at Bellata (BTA) field site; range is 80% (lower) and 20% (upper) probability of exceedance, a) mean lint yield ( $\text{kg}\cdot\text{ha}^{-1}$ ) from Standard crop growth, b) mean lint yield ( $\text{kg}\cdot\text{ha}^{-1}$ ) from Delayed progression to first square, c) mean WUE ( $\text{kg lint} / \text{mm ET}$ ) from Standard crop growth, d) mean WUE ( $\text{kg lint} / \text{mm ET}$ ) from Delayed progression to first square.



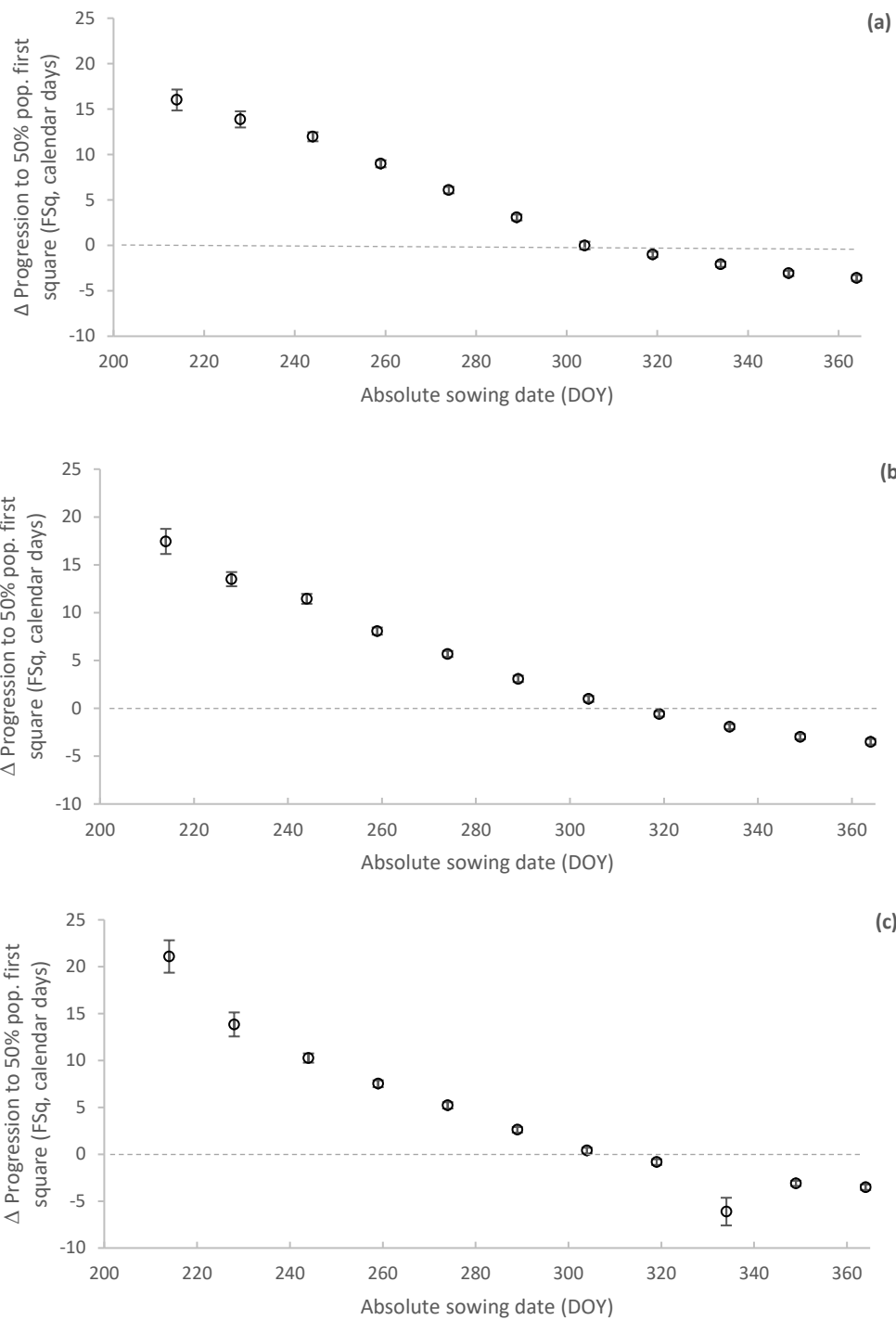
**Figure 7.4 Fifty year historical mean lint yields ( $\text{kg}\cdot\text{ha}^{-1}$ ) and water use efficiency (WUE) ( $\text{kg lint} / \text{mm ET}$ ) obtained from physiological crop response simulations using OzCOT (Hearn, 1994), per 15 day establishment intervals (1-Aug to 29-Dec) at Terry Hie Hie (THH) field site; range is 80% (lower) and 20% (upper) probability of exceedance, a) mean lint yield ( $\text{kg}\cdot\text{ha}^{-1}$ ) from Standard crop growth, b) mean lint yield ( $\text{kg}\cdot\text{ha}^{-1}$ ) from Delayed progression to first square, c) mean WUE ( $\text{kg lint} / \text{mm ET}$ ) from Standard crop growth, d) mean WUE ( $\text{kg lint} / \text{mm ET}$ ) from Delayed progression to first square.**

Unforeseen and substantial differences occurred between what could be feasibly modelled, and the morphological treatment responses demonstrated in three years of field experiments. Syntax interactions occurred within the model and despite partially delaying progression to crop first square, a key difference was that the model adjustment did not hold the cotton plant growth and canopy development (LAI) in stasis. Additionally, any plant internal modifications to biochemistry affecting growth and development would not be accounted by the model.

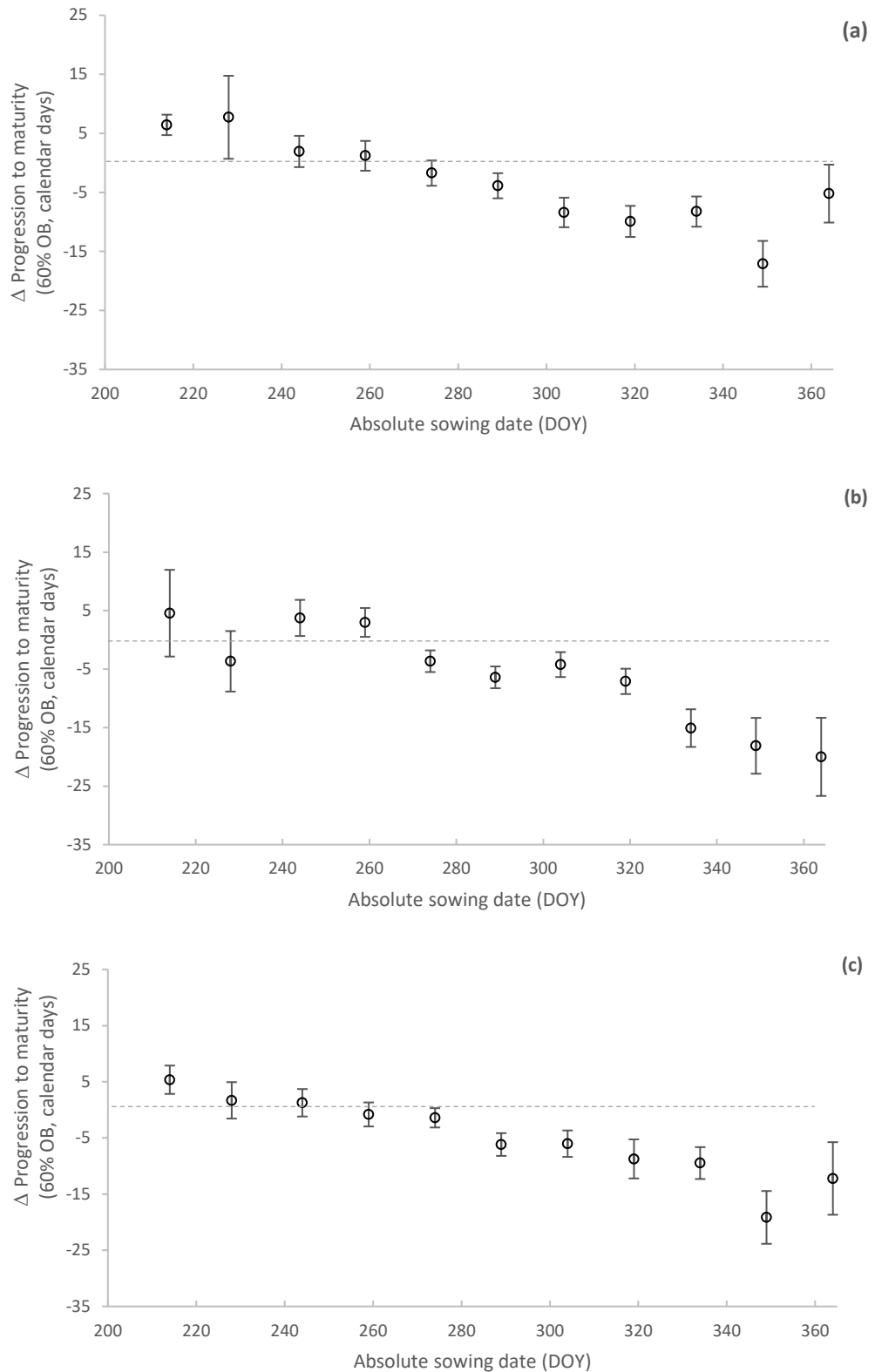
Crop progression to first square (FSq) was consistent with field data for early planting dates; however, from October planting dates (DOY: 300) onwards, modelled outputs across all sites showed an earlier progression to FSq by the delayed types (Figure 7.5), being inconsistent with field data. Importantly with reference to yield outcomes, evaluation of modelled OzCOT crop maturity outcomes for planting date (and site), between standard and delayed type (Figure 7.6), showed a maximum increase of five (calendar) days extension in maturity for very early planted cotton, being shorter than the 12 days delay in progression to both physiological cut out and maturity demonstrated in field experiments. Moreover, and in contrast to field experiment results, model adjustments significantly shortened (up to 20 days) production seasons comparative to the standard, onwards from the 15<sup>th</sup> Sept planting date (Figure 7.6).

In contrast to field experiment data, model adjustments (to invoke delay) also impacted final yield components; reducing both open boll size (as grams of seed cotton per boll) and number of open bolls (per m<sup>2</sup>) at maturity, compared to standard simulation outcomes across all field experiment sites and planting dates modelled; gravity of response increasing as planting date proceeds (Figure 7.7).

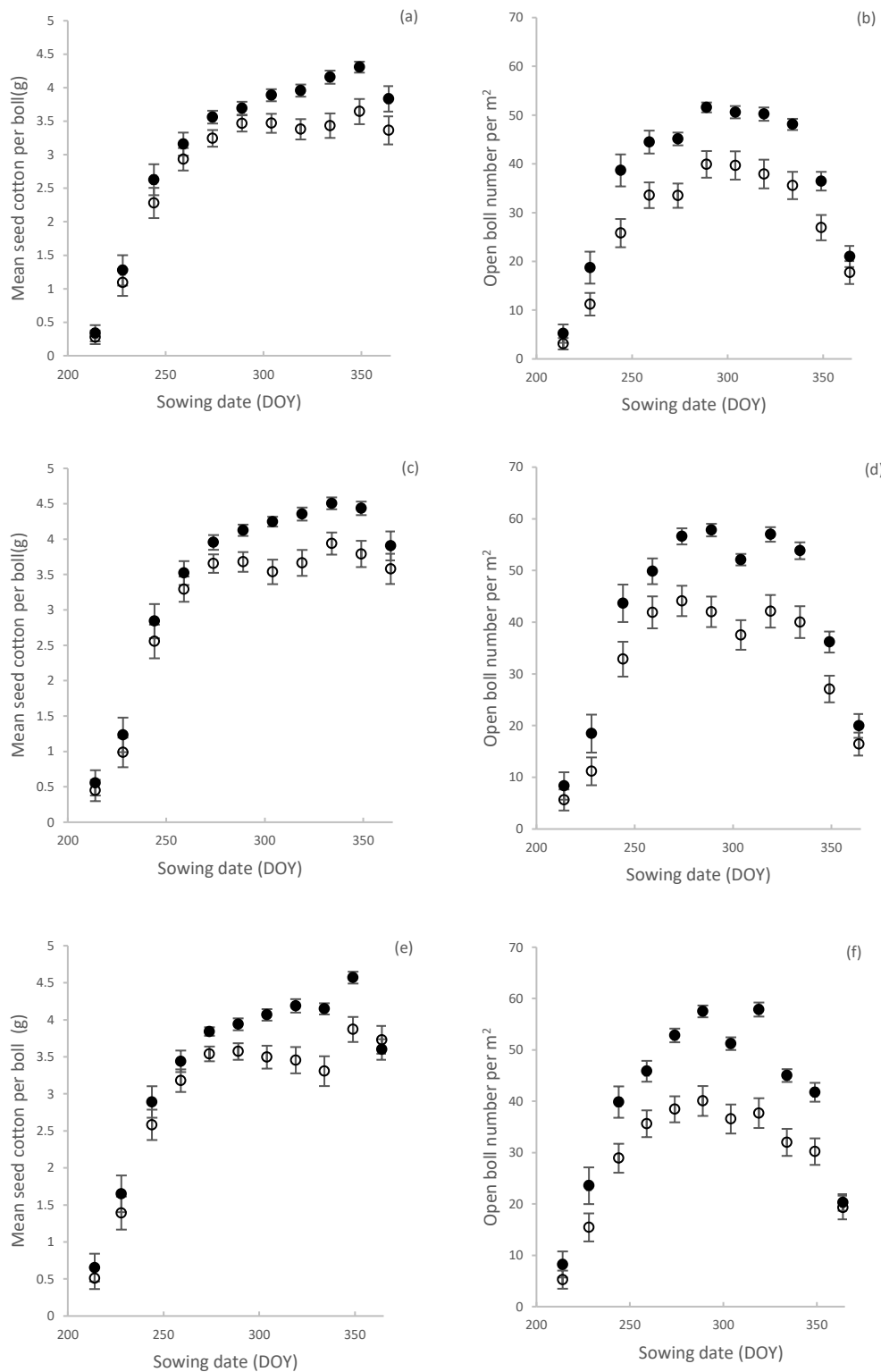
Consequently, model manipulations to invoke a delay in early growth and development were inadequate for the intended evaluations. Validation of yield and input use efficiency values of the TEP PGR adaptive management opportunity was not possible without further significant model adjustment to more closely reflect the associated and flow-on phenological changes observed in experiments.



**Figure 7.5** Difference in fifty year historical mean days to 50% population first square (FSq) of delayed vs standard obtained from crop response simulations using OzCOT (Hearn, 1994), per 15 day establishment intervals (1-Aug to 29-Dec) at a) Narrabri (ACRI), b) Bellata (BTA) and c) Terry Hie Hie (THH) field sites; error bars are  $\pm 1$  standard error of the mean.



**Figure 7.6** Difference in fifty year historical mean days to maturity (as 60% open boll [OB]) of delayed vs standard ideotype obtained from physiological crop response simulations using OzCOT (Hearn, 1994), per 15 day establishment intervals (1-Aug to 29-Dec) at a) Narrabri (ACRI), b) Bellata (BTA) and c) Terry Hie Hie (THH) field sites; error bars are +/- 1 standard error of the mean.



**Figure 7.7** Fifty year historical mean seed cotton per boll (g, [SCBO]) and open bolls (per m<sup>2</sup>, [BOLL]) of delayed vs standard ideotypes obtained from physiological crop response simulations using OzCOT (Hearn, 1994), per 15 day establishment intervals (1-Aug to 29-Dec); error bars are +/- 1 standard error of the mean, (●) standard, (○) delay, a) SCBO Narrabri (ACRI), b) BOLL ACRI, c) SCBO Bellata (BTA) d) BOLL BTA, e) SCBO Terry Hie Hie (THH), f) BOLL THH.

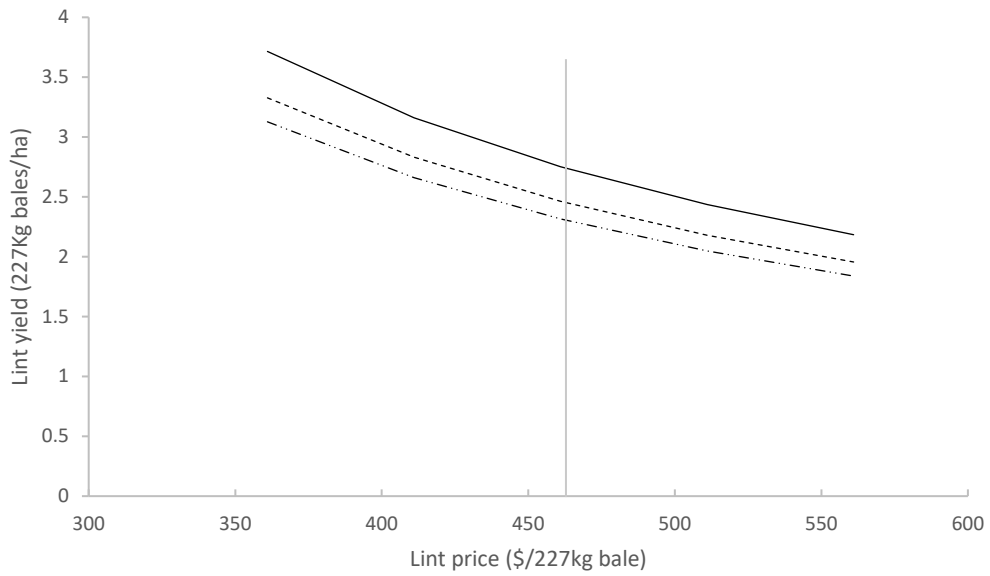
### **7.3 Comparative annualised profit outcomes of Trinexapac ethyl application**

Comparative annualised return (profit) was modelled from Powell and Welsh (2019)'s rain grown cotton gross margin analysis, with an amended PGR gross margin based on the foliar application criteria evaluated in field experiments (Appendix 7). Pricing calculations for commercial TEP product Moddus Evo (Syngenta Australia Pty Ltd) are detailed in Appendix 8, with all assumptions as per Powell and Welsh (2019). Moreover, compatibility and interactions of PGR chemistry with other herbicides utilised have not yet been verified; consequently, combined applications (as indicated) may not be possible, resulting in differing analysis outcomes.

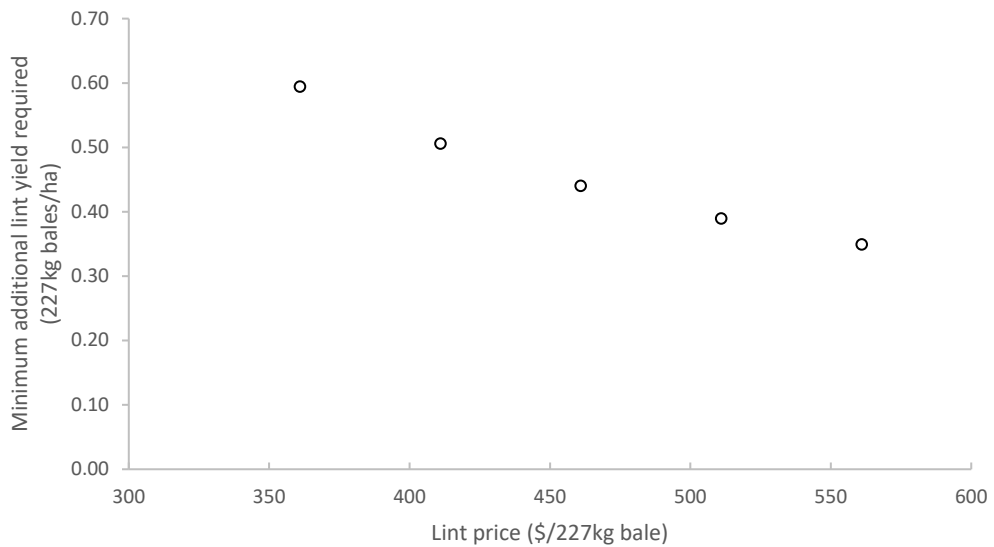
The Australian cotton industry operates in a sophisticated, deregulated market with numerous ginners, merchants and producers. The majority of Australian cotton lint produced is sold using forward contracts (of up to five years and predominately in irrigated production); a complex market exists for hedging cotton lint including using futures, options, bank SWAPS and on-call contracts. Ninety per cent of Australian cotton produced is exported and local prices are highly volatile and heavily influenced by both the international commodity exchange (ICE) No 2 cotton futures contract (New York stock exchange, US Securities and Exchange Commission) and movements in the Australian dollar and basis (Imray, Greenwood, & Dolley, 2019). Consequently, initial gross margin lint commodity pricing is based on the 5-year (2013-2018) nominal mean of \$521/227kg lint bale (ABARES, 2018); all other input pricing being current 12-month industry means (Powell & Welsh, 2019).

Sensitivity analysis (SA) regards the investigation of potential changes or errors of parameter values and /or assumptions, of an (economic) model; being utilised in this case to quantify or otherwise increase understanding of the system (Pannell, 1997). Sensitivity analysis was conducted to determine changes in profit per ½ bale yield increments at five price points centred around the 20-year mean lint price of AU\$461/bale. Trend lines were fitted and solved to obtain the break-even yield points (to engage TEP technology) as influenced by price, which varied with row configuration (Figure 7.8); higher lint prices decreasing the minimum yields required to break even when utilising TEP technology. Comparison with replicate sensitivity analysis conducted on the standard production gross margin, showed the minimum additional yield required (per price point) from the standard, to break even and consequently justify application of TEP technology equating to a

requirement for an extra 0.44 of a bale (100kg lint/ha) at \$461/bale (Figure 7.9); being equal across all row configurations and decreasing as lint prices increased.



**Figure 7.8 Break-even yields required per lint price point when utilising TEP PGR management technology; (—) solid row configuration, (- - -) single skip row configuration, (- · - · - ·), vertical line is 20-year mean lint price of \$461/bale.**



**Figure 7.9 Minimum additional yield required (from the standard) to break-even and justify application of TEP PGR management, per price point, across all row configurations.**

#### 7.4 Long-term economic outcomes from delaying crop peak resource demand

Previously described response simulation limitations prevented absolute quantification of yield, yield variability and downside risk effects associated with delaying peak resource demand. However, by utilising standard OzCOT yield response outcomes per absolute planting dates (Figure 6.1, Chapter 6), three proxy “delay” scenarios (as three absolute planting dates; 31 Aug, 30 Sept and 14 Nov) were analysed to evaluate yield, yield variability and downside risk impacts of delayed peak resource demand, on cumulative 20-year profits outcomes. 20-year annualised lint yield ( $\text{kg}\cdot\text{ha}^{-1}$ ) outcomes were obtained via physiological response simulations using OzCOT (Hearn, 1994; Milroy et al., 2004) per three absolute planting dates (31 Aug, 30 Sept and 14 Nov), for the three field experiment locations. Subsequent cumulative returns (1998-2017) were calculated using the rain grown cotton gross margin analysis (Powell & Welsh, 2019) and applying a consistent 20-year mean price of \$461/bale (Imray et al., 2019) (Figure 7.10).

Cumulative 20-year profits obtained increased with later planting dates (and subsequent later season peak resource demands). Cumulative 20-year profit outcomes across the three field experiment locations increased as absolute planting dates progressed through the season; later planting dates (and consequent dates of crop peak resource demand) demonstrating higher cumulative profits consistently across all three field experiment sites.

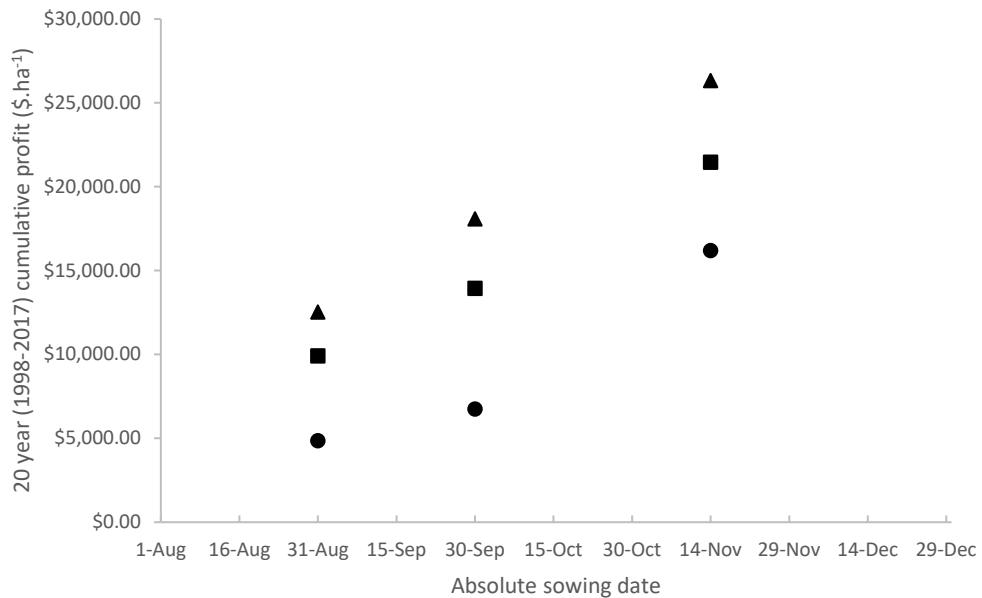
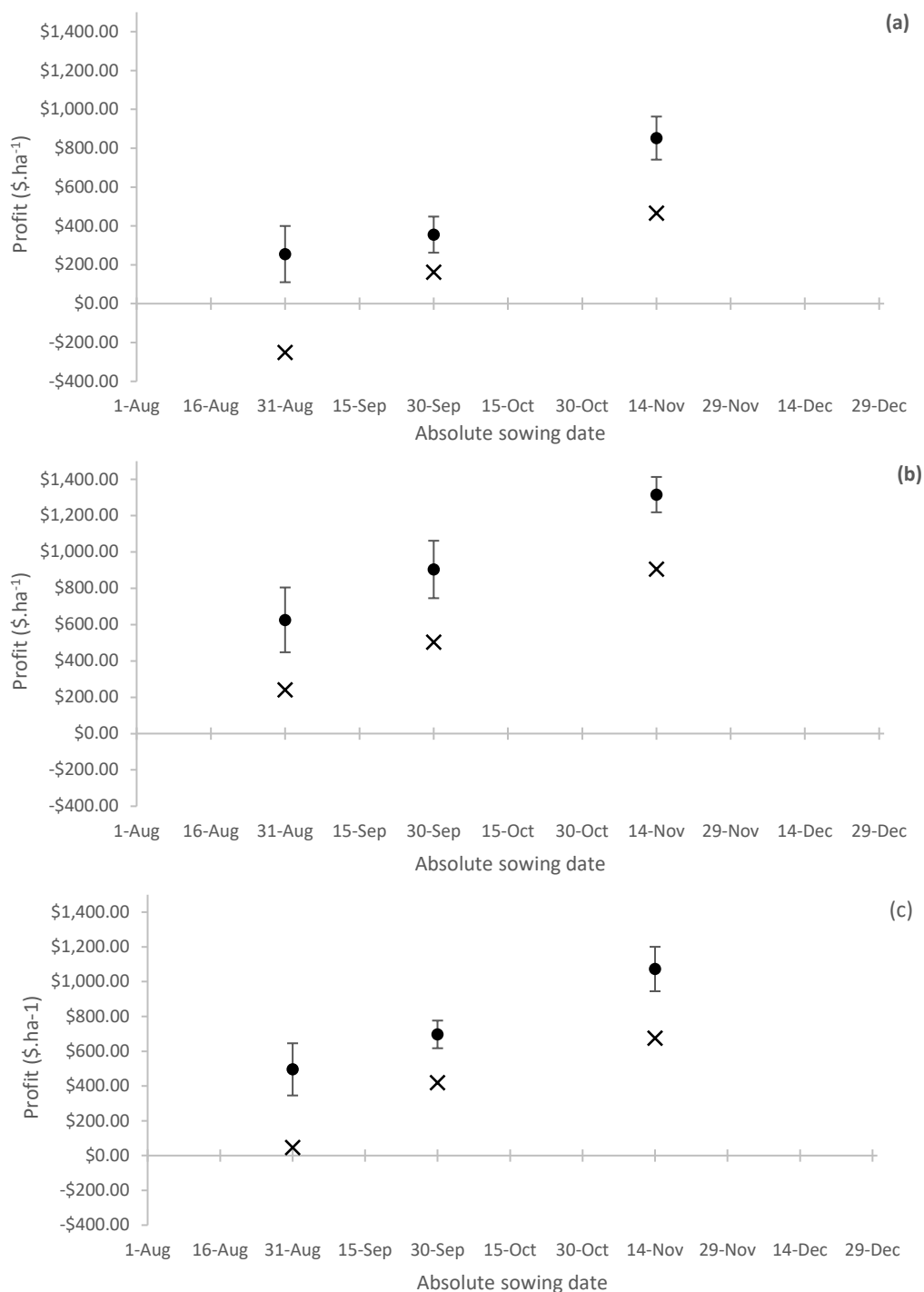


Figure 7.10 20-year cumulative profit per three absolute planting dates (1 Sept, 1 Oct, 15 Nov) and respective experiment locations; (●) ACRI, (■) THH, (▲) BTA.

Subsequently, 20-year mean profit and downside economic risk profiles (as 80<sup>th</sup> percentile values) were calculated per planting date and field experiment site (Figure 7.11). Emulating a delay in peak resource demand, later plantings also demonstrated increased mean returns over the 20-year analysis period, with less variability and reduced downside risk.



**Figure 7.11 20-year (1998-2017) mean annual profit (●) and downside risk (X) per absolute planting date; error bars are +/- 1 standard error of the mean, downside risk is 80% probability of exceedance, (a) ACRI experiment site, (b) BTA experiment site, (c) THH experiment site.**

## 7.5 Economic functionality of a “proportionate application” of Trinexapac ethyl in Australian rain grown cotton systems

The underlying premise of systems applications of PGR adaptive management technologies in Australian rain grown cotton systems is to increase management diversity via in-season responsive management of system limitations and challenges, to manage climate variability. Moreover, such technologies should reduce expenses associated with, as well as increase opportunities for, management implementation. Staggered planting times are currently utilised as a conventional adaptation strategy to hedge production risk against climatic variability; a proportion of the crop being planted at the first opportunity with the remainder planted on the next planting rains (often 2-4 weeks later) (Figure 7.12, b). Consequently, the timing of crop peak resource demand is differentiated between the two plantings; with later planting equating to a later timing of crop peak resource demand. However, due to the uncertainty associated with rain grown production, risk exists that a second planting opportunity may not occur.

### *“Proportionate application” of Trinexapac ethyl as an alternative to successive planting operations*

This thesis proposed the use of foliar TEP application scenarios to be leveraged as an alternate adaptive management strategy to multiple, successive planting times. The entire rain grown cotton crop being planted at the earliest feasibility, with a “proportionate application” of TEP being applied to a percentage (e.g. 1/3) of the crop, to invoke a physiological stasis and result in a delay in crop peak resource demand commensurate with a second planting (Figure 7.12).

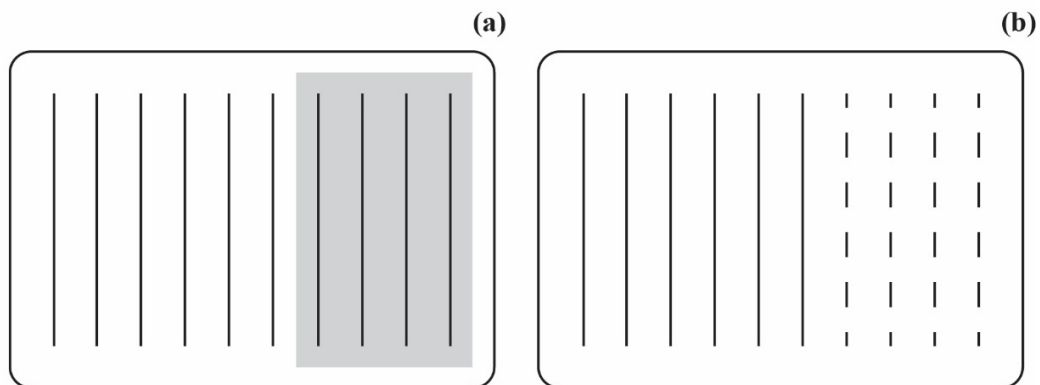
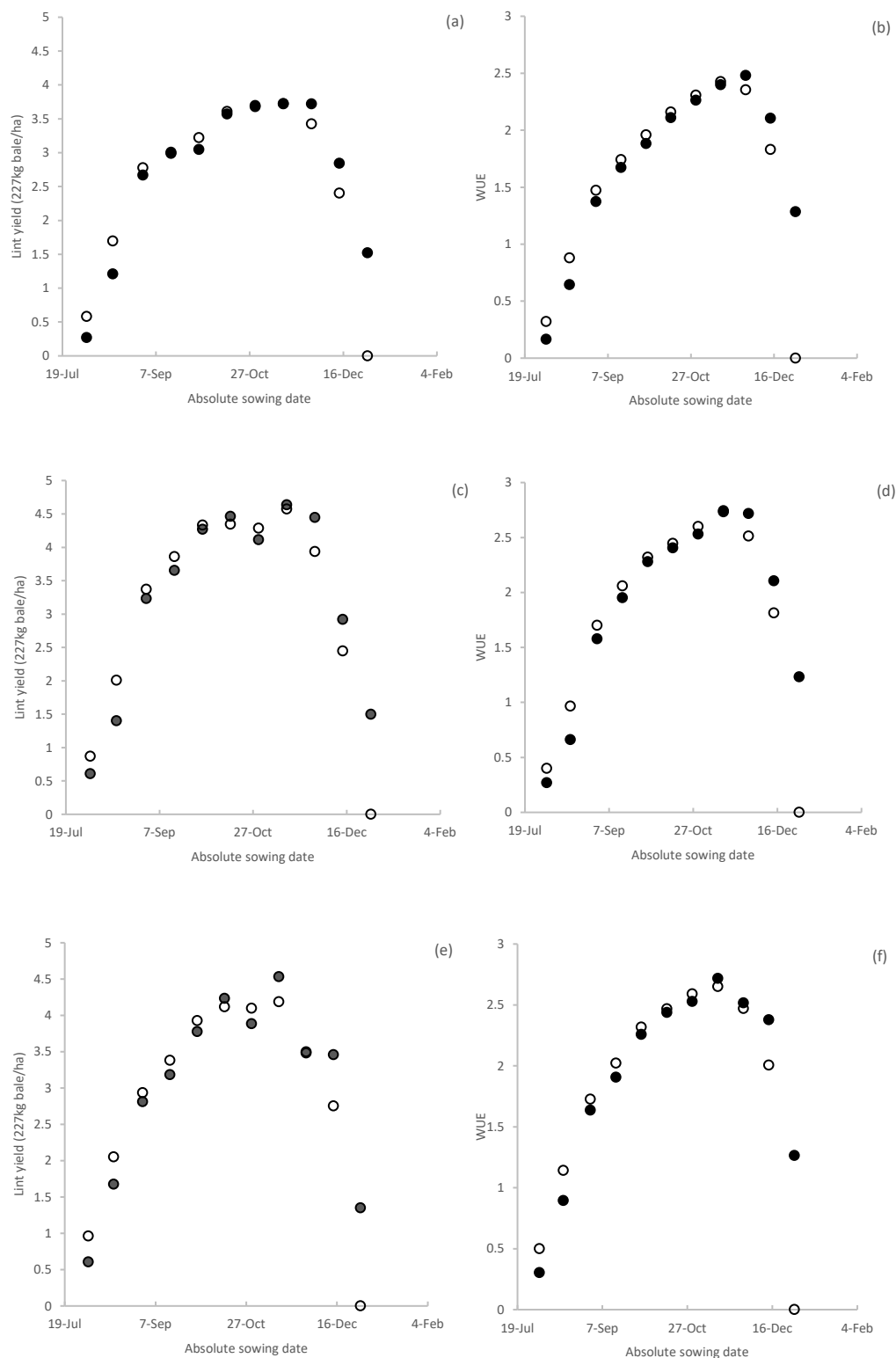


Figure 7.12 Indicative representation of (a) a “proportionate application” of TEP to rain grown cotton crops, as an alternative adaptive management solution to (b) multiple planting operations; (—) planting one, (- - - -) planting two, (■) TEP application.

***Mean lint yields and WUE outcomes of a 1/3 proportionate delay in crop peak resource demand***

As an initial exploratory assessment, analysis to quantify yield and WUE benefits associated with a 1/3 proportionate delay in crop peak resource demand, was conducted utilising standard OzCOT (Hearn, 1994), yield and WUE response outcomes per absolute planting date and field experiment site (Figure 6.1, Chapter 6). Subsequently, a 1/3 proportionate delay in crop peak resource demand was simulated through the sum of; an apportioned 2/3 weighting of the current planting date lint yield and WUE means, with a 1/3 weighting of the successive (15 day) planting date lint yield and WUE means.

60-year modelled mean water use efficiency and lint yield outcomes demonstrated that notably at early planting dates, yield and input use efficiency benefits existed in proportionately delaying crop peak resource demand (Figure 7.13). However, application of a “proportionate delay” strategy (either via a second planting event or application of TEP) was counterproductive in later season planting dates (being past the optimal planting window), ostensibly due to climatic impacts on season length.



**Figure 7.13** Fifty year historical mean lint yields ( $\text{kg}\cdot\text{ha}^{-1}$ ) and water use efficiency (WUE) outcomes obtained from physiological crop response simulations using OzCOT (Hearn, 1994), per 15 day establishment intervals (1-Aug to 29-Dec) for (●) “standard” and (○) “standard + 1/3 delay”; (a) lint yield ACRI, (b) WUE ACRI, (c) lint yield BTA, (d) WUE BTA, (e) lint yield THH, (f) WUE THH.

### ***20-year cumulative profit impacts of a 1/3 proportionate delay in crop peak resource demand***

A preliminary comparative assessment of long-term cumulative profit implications associated with a 1/3 proportionate delay in crop peak resource demand was completed. The proportionate delay in crop peak resource demand being realised via, either a second planting operation, or a commensurate “proportionate application” of Trinexapac ethyl.

20-year annualised profit returns were calculated using Powell and Welsh (2019) rain grown gross margin and the amended TEP PGR gross margin (Appendix 7), utilising the 20-year mean price of \$461/bale (Imray et al., 2019) and assumptions as detailed prior (Section 7.3). Profit outcomes for six specific planting dates were calculated to enable creation of a 15 day “delay” response for three targeted planting dates; 31 August (delay to 15 September), 30 September (delay to 15 October) and 14 November (delay to 29 November). Calculations for a comparative “standard” (i.e. no delay) 20-year profit response estimate was utilised from earlier analysis (Figure 7.10) for the 31 Aug, 30 September and 14 November planting dates.

Subsequent calculation of 20-year cumulative profit responses for a conventional “1/3 delay as a second planting” were obtained by tallying weighted rain grown gross margin (Powell & Welsh, 2019) cumulative profit outcomes; 2/3<sup>rd</sup> from the targeted planting date, with a subsequent 1/3<sup>rd</sup> from the planting date 15 days later (Figure 7.14). Determination of 20-year cumulative profits for “1/3 delay as TEP application” was achieved by, again, tallying weighted gross margin cumulative profit outcomes; the initial 2/3<sup>rd</sup> from the targeted planting date utilising the rain grown gross margin (Powell & Welsh, 2019) with the delayed 1/3<sup>rd</sup> utilising the adjusted TEP PGR gross margin (Appendix 7) from the succeeding 15 day planting date (Figure 7.14). Additionally, at current commercial pricing and with all other inputs and applications being equal, significant difference exists between the costs associated with conducting a second planting operation versus the additional cost (the proportionate area to be treated being already planted) associated with triplicate foliar applications of TEP PGRs during early vegetative growth (Table 7.2).

In conclusion, on a 20-year cumulative basis and under current pricing structures, application of the TEP adaptive management strategies to Australian rain grown cotton systems found no economic systems benefit in comparison to the conventional second planting approach (Table 7.3). Early planting dates close to the optimum late October planting window do establish economic opportunity for TEP applications, although still

being of less benefit than a second planting operation. Conversely, the benefit of TEP applications in avoiding loss due to a lack of second planting opportunity (as in conventional management strategies) cannot be overlooked; however, the timing of initial planting needs to be considered when appraising potential economic advantage.

## 7.6 Conclusions

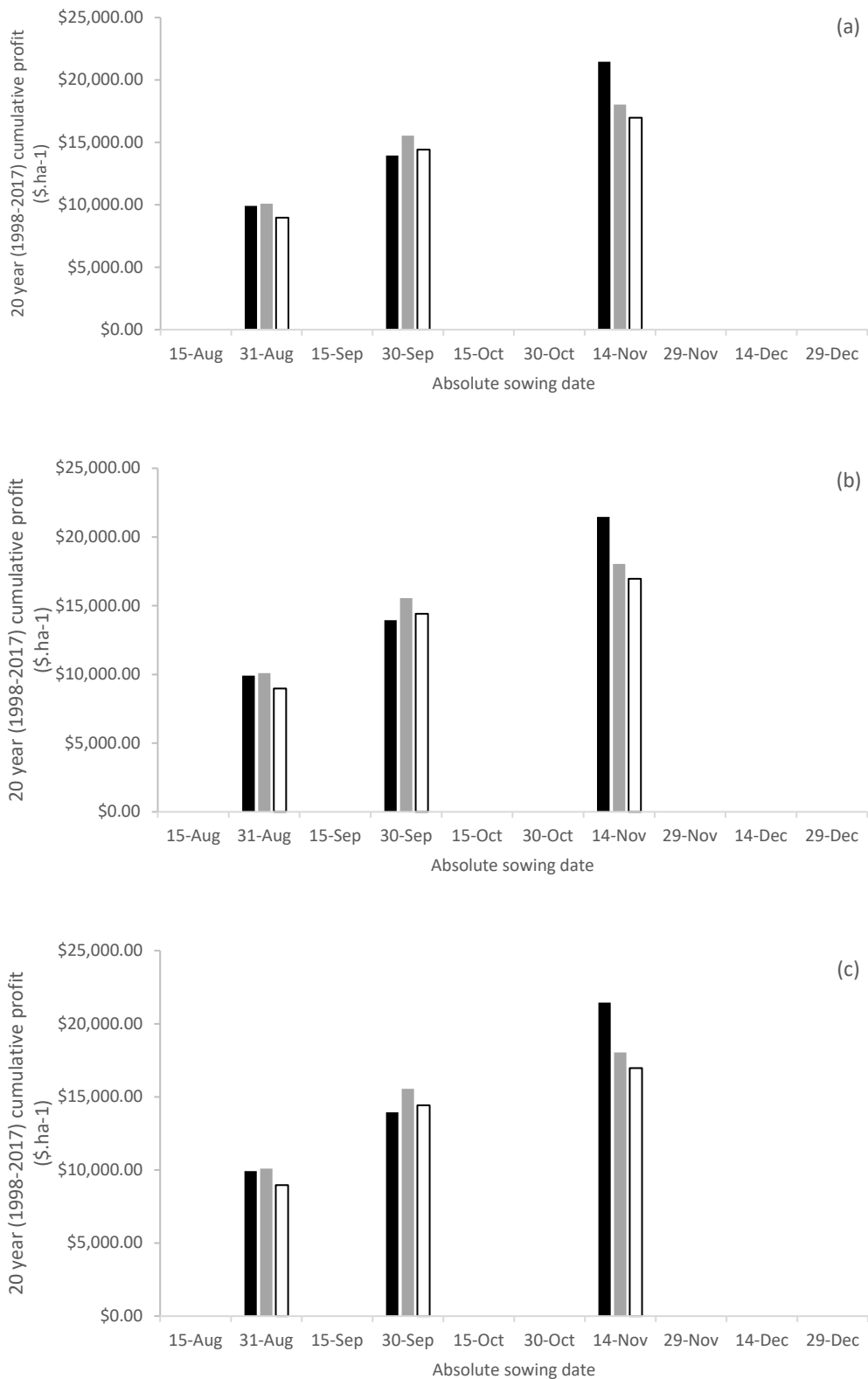
Analysis of the system value of the Trinexapac ethyl PGR treatment scenario was conducted on the premise of demonstrated PGR utility in both slowing growth and delaying crop level reproductive phase change; resulting in a temporal shift in peak flowering and consequent peak resource demand of 12 to 15 calendar days.

Early stage model adjustments were made to provide an initial indication of lint yield and water use efficiency changes associated with achieving a delay in crop level phase change and consequent peak resource demand. Differences occurred between what could feasibly be modelled, and the morphological treatment responses demonstrated in the three years of field experiments; despite delaying progression to 50% population first square, the model adjustments did not hold the cotton plant growth and canopy development (LAI) in stasis. Resultantly, response simulation limitations prevented absolute quantification of longer-term lint yield and WUE, yield variability and downside risk effects associated with TEP induced delays in peak resource demand.

Moreover, sensitivity analysis (lint price and yield) of comparative annualised profit outcomes from both standard and PGR treatment production systems, established that the minimum additional lint yield required to break even and justify a TEP treatment scenario was  $100 \text{ kg}\cdot\text{ha}^{-1}$  (at \$461 per 227kg lint bale); being equal across all row configurations. By utilising 3 differing absolute planting dates, an indicative yield response to a temporal shift in peak resource demand was achieved. Consequently, cumulative 20-year profits increased with later planting dates (and subsequent peak resource demands), across all three field experiment sites. Later planting dates also demonstrated increased 20-year mean profits, with decreased variability and reduced downside economic risk (as 80<sup>th</sup> percentile values).

Comparative analysis of a 1/3 proportionate delay, either as a TEP application or conventional second planting, demonstrated a significant additional cost ( $> \$150\cdot\text{ha}^{-1}$ ) associated with a triplicate foliar application of TEP. Resultantly, on a 20-year cumulative profit basis and under current pricing structures, applications of TEP to Australian rain

grown cotton production systems demonstrated no direct economic benefit in comparison to a conventional second planting. However, the benefit of TEP in avoiding losses associated with a failed second planting opportunity (i.e. no planting rainfall event) cannot be overlooked. Consequently, the timing of the initial planting operation needs to be considered when evaluating any economic advantage; with further sensitivity analysis of both lint and PGR pricing required to determine predictive frameworks for PGR application under water limited rain grown production settings.



**Figure 7.14 20-year cumulative returns per three absolute planting dates (1 Sept, 1 Oct, 15 Nov) and (a) ACRI, (b) BTA and (c) THH experiment locations; [solid black] bar representing standard production, [grey] bar representing 1/3 delay as second planting, [white] bar representing 1/3 delay with TEP PGR foliar application.**

**Table 7.2 Current commercial pricing differential between a second planting opportunity and appliance of triplicate TEP foliar applications in early growth.**

Variable costs		Machinery	\$.ha <sup>-1</sup>	Inputs			Configuration			
				Rate <sup>1</sup>	L.ha <sup>-1</sup>	Band (%)	Cost (\$unit <sup>-1</sup> )	Cost solid (\$ha <sup>-1</sup> )	Solid (\$ha <sup>-1</sup> )	Single (\$ha <sup>-1</sup> )
<i>2nd planting operation</i>										
Oct	Planting: precision planter	295 eng/HP	\$11.50					\$11.50	\$11.50	\$11.50
<i>Triplicate TEP foliar treatment scenario</i>										
Nov	TEP (250g.L <sup>-1</sup> )	with RR spray		0.8	100	\$70.00	\$56.00	\$56.00	\$56.00	\$56.00
Nov	TEP (250g.L <sup>-1</sup> )	Self-propelled	\$1.50	0.8	100	\$70.00	\$56.00	\$57.50	\$57.50	\$57.50
Dec	TEP (250g.L <sup>-1</sup> )	with RR spray		0.8	100	\$70.00	\$56.00	\$56.00	\$56.00	\$56.00

**Table 7.3 Per cent change in 20-year cumulative profit (from standard) obtained from incurring a 15 day delay in peak resource demand via a second planting operation (2<sup>nd</sup> plant) or application of TEP treatment scenario (PGR) across 3 absolute planting dates and 3 field experiment locations.**

Location	31 Aug 2 <sup>nd</sup> plant		30 Sept 2 <sup>nd</sup> plant		14 Nov 2 <sup>nd</sup> plant	
		PGR		PGR		PGR
ACRI	↑ 11.88	↓ -10.26	↑ 18.2	↑ 1.457	↑ 0.88	↓ -6.09
BTA	↑ 0.549	↓ -8.47	↑ 7.13	↑ 0.879	↓ -3.58	↓ -7.87
THH	↑ 1.82	↓ -9.57	↑ 11.53	↑ 3.42	↓ -15.92	↓ -20.93

## **Chapter 8 General Discussion**

Rain grown cotton production is characterised by significant climate variability, notably extremes of temperature and rainfall. Consequentially, water limitation is a focal constraint; with other abiotic stressors directly impacting and/or compounding impacts, on both production quantity and lint quality outcomes. Such elevated system exposure to climate risk results in increased variability of annualised lint production, quality and profit outcomes, as well as increased annualised and industry-proportionate production areas.

Management of climate risk across geographically diverse Australian rain grown cotton production areas is currently centred around the tactical manipulation of multiple agronomic management and genetic solutions simultaneously, so as to moderate existing system limitations and challenges and reach the water limited yield potential of the system. These existing strategies deliver capacity to introduce management diversity to the farming system, but lack application agility, have increased costs associated with implementation and critically, the rainfed-nature of the system often precludes opportunities for implementation. However, leveraging principles underpinning these existing strategies can inform development of novel adaptive management approaches utilising plant growth regulators (PGRs) that augment management diversity, to enable in-season responsive (agile) management of system challenges and limitations under variable climatic conditions; offsetting climatic variability and improving system resilience.

This study was an initial step towards appraisal of bespoke applications of PGRs to create novel adaptive management strategies aimed at improving resilience of rain grown cotton systems. Although PGRs have been evaluated for utility in the strategic manipulation of cotton crop growth and development under irrigated field and controlled environment conditions, there has been minimal extension of this knowledge (particularly around exogenous Gibberellin, Cytokinin and Gibberellin biosynthesis inhibiting PGR treatment scenarios) to resource limited rain grown field conditions, notably in early vegetative growth applications or in skip row planting configuration. Furthermore, a scarcity of detailed information exists as to the systems value of specific PGR adaptive management strategies tailored to Australian rain grown cotton systems. Understanding such responses can enable future development of treatment frameworks for strategic, predictive applications within rain grown cotton systems, that successfully manage climate risk and promote system resilience.

This thesis investigated the utility of exogenously applied PGRs to promote system resilience by tactically modulating cotton growth and development under rain grown production contexts, to augment crop water availability. Obtaining consistency of cotton plant response to PGR treatment scenarios is customarily problematic and dependent upon a multiplicity of temporal and spatial appraisals, to facilitate accurate characterisation. By drawing on preliminary glasshouse and comprehensive field experiments over three production seasons (2016-19) at three locations in New South Wales, provision of a broad-scale indication of specific treatment efficacy was presented. Treatments were positioned around achieving vegetative stage growth responses, negating endogenous hormonal influences associated with reproductive growth. Moreover, to progress research, initial selection of specific PGR chemistry exploited published data from horticulture and tree crops, irrigated cotton and international rain grown production contexts.

Physiological mechanisms underpinning existing agronomic adaptation strategies were identified and resultantly, unique adaptive management opportunities utilising PGRs were proposed for Australian rain grown cotton systems (Chapter 2). Utilising a top-down approach with a systems, crop and plant level focus; promotion of system resilience to climatic variability was examined via three identified response strategies, all aiming to increase crop water availability. Glasshouse and field experiments increased understanding around the capabilities; of synthetic Adenine-type Cytokinin PGRs to promote increased root biomass accumulation and plant root growth area (Chapter 4), of naturally occurring bioactive Gibberellin PGRs to increase early plant structural and canopy biomass (Chapter 5) and of Gibberellin biosynthesis inhibiting PGRs to slow early growth or induce physiological stasis, to delay crop-level reproductive phase change (Chapter 6). Conclusively where applicable, the effectiveness of these approaches was evaluated to establish resource use efficiency benefits and / or economic values of the treatment propositions, to Australian rain grown production systems (Chapter 7) (Table 8.1).

**Table 8.1 Relative thesis chapter contributions toward scientific understanding of PGR utility to promote resilience in rain grown cotton systems.**

<b>Ch.</b>	<b>Objectives</b>	<b>Key findings</b>	<b>Outcome</b>	<b>Future directions</b>
2	Understand the capacity of adaptive management strategies to promote agroecosystem resilience in rain grown cotton production systems.	Identification of key physiological rationales underpinning current adaption strategies.	Unique adaptive management opportunities utilising PGRs devised for Australian rain grown cotton systems.	Quantification of specific PGR capacity to elucidate required growth and development manipulations in rain grown cotton production systems.
4	Determine capability of synthetic Adenine-type Cytokinin PGRs to increase access to skip row soil water reserves.	<ul style="list-style-type: none"> <li>No direct PGR impacts on root growth or utilisation of soil water resources.</li> <li>No flow-on impacts on biomass, LAI, yield and quality outcomes.</li> <li>Treatment induced early square abscission in triplicate, high concentration treatments, delayed crop maturity.</li> </ul>	Increased understanding of cotton plant phenological response to specific 6BA treatment scenarios, under resource-constrained rain grown production setting.	Time series exploration of plant PGR pool resource size to quantify interaction of treatment efficacy and environmental context: early square soluble CHO ( $P_n$ response), subtending leaf ethylene content (abscission response), direct plant water deficit stress measurements, growth rate monitoring.
5	Determine capability of naturally occurring bioactive Gibberellin PGRs to augment early canopy capacity and access to soil water reserves.	<ul style="list-style-type: none"> <li>No evidence of increased root biomass or canopy (LAI) accumulation.</li> <li>Increased plant height, nodes and fruiting sites, with no net benefit in square numbers, leading to yield penalties.</li> <li>Resources (water) required to support promoted growth, for yield benefits.</li> </ul>	Increased understanding of cotton plant phenological response to specific GA <sub>3</sub> and GA <sub>4,7</sub> treatment scenarios, under resource-constrained rain grown production setting.	Rate evaluation of both GA <sub>3</sub> and GA <sub>4,7</sub> treatments under optimal growth (glasshouse) conditions. Quantification of interaction of water resource availability and plant growth rates with treatment responses; continues direct plant water deficit stress measurements, growth rate monitoring, canopy architecture mapping.
6	Determine capability of Gibberellin biosynthesis inhibiting PGRs to shift subsequent peak resource demand to alternative climatic conditions.	<ul style="list-style-type: none"> <li>MC, CC and PhxCa: no delay in phase change or resource demand.</li> <li>Unicon and Pacl: delay phase change, no delay in peak resource demand.</li> <li>TEP: delay phase change, delay in peak resource demand with seasonally dependant yield impacts.</li> </ul>	Increased understanding of cotton plant phenological response to specific GaBI PGR treatment scenarios, under resource-constrained rain grown production setting.	Quantification of magnitude of resource demand shift: distribution of fruit development/retention over time, Role of canopy architecture and lateral branch fruit load in demand shift.
7	Quantifying the system-value (yield, use efficiency, economic) of the adaptive management opportunities.	<ul style="list-style-type: none"> <li>OzCot model incapable of simulating delayed physiology aligned to PGR effects and requires further adjustment.</li> </ul>	Increased understanding of the systems-value of PGR-driven adaptive management strategies.	Investigation of accurate ways to achieve simulated PGR delay utilising Ozcot model Sensitivity analysis of PGR input pricing

### **8.1 Application of synthetic Adenine-type Cytokinin, 6-Benzylaminopurine, to increase root biomass accumulation and promote plant access to soil water reserves**

Adenine-type cytokinin, 6-benzylaminopurine (6BA), was evaluated for the capacity to promote cotton root biomass accumulation, with the objective of improving crop access to skip row soil water reserves (Chapter 4). Enhanced utilisation of stored soil water reserves, and any associated crop growth and yield benefits, are closely related to both the root space available and critically, the penetration and proliferation of the plant root system within that space (Luo et al., 2015). Limitations have been demonstrated in skip row water extraction patterns by cotton plants in Australian (Vertosol) production soils (Goynes, 2005). Therefore, manipulating root system architecture toward a distribution of roots in the soil that optimises water access and uptake (e.g. facilitates absorption in deep soil layers), can increase the total amount of water available to the plant and reduce the length of time where crops are in water deficit stressed conditions, notably post anthesis (Dorlodot et al., 2007).

Plant phytohormones are significant endogenous regulators of cotton root system architecture and can be manipulated through exogenous PGR applications that modulate hormone homeostasis or signalling (Osmont et al., 2007). Exogenous applications of synthetic Adenine-type Cytokinin 6-Benzylaminopurine recently demonstrated utility in promoting adventitious root system architecture under a water limited, rain grown international production setting (Burke, 2011, 2013). However, limited knowledge exists regarding cotton plant responses under rain grown production characterised by skip row planting configurations and Vertic soil contexts.

Treatments were applied as seed priming and seed coatings in preliminary glasshouse evaluations, and as singular and triplicate (at 10 day intervals) foliar applications from four leaf stage, in both preliminary glasshouse and field experiments over the 2016/7, 2017/18 and 2018/19 production seasons at the Narrabri and Bellata experiment sites. These experiments indicated that the 6-Benzylaminopurine treatments evaluated, were not effective in increasing root biomass accumulation, subsequent access to soil water reserves or follow-on lint yield and quality outcomes.

No 6-Benzylaminopurine induced changes to root biomass accumulation or associated plant utilisation of soil water reserves occurred across multiple treatment scenarios under both glasshouse and field contexts. In the field, depth-stratified sampling under the plant line and adjacent skip row demonstrated no foliar treatment differences in the presence of

live cotton roots, or plant utilisation of shallow and deep soil water reserves at mid flowering or maturity; being supported by calibrated electromagnetic conductance surveys to both 0.75 and 1.5m during both early and mid-flowering. Moreover, no consequential treatment impacts on yield components, lint yield or quality were evidenced in both glasshouse and field experiments.

These findings contrast with those of Burke (2011, 2013) in cotton; disparity between the results in rain grown cotton systems can be accounted for in part by differences in both existing environmental contexts and the methodology utilise to quantify plant root growth and associated water uptake. Heavier soil bulk density and unseasonably cold early season conditions (and consequent lower comparative day degree accumulation) in the Australian experiments, may have impacted early plant root growth responses to PGR treatments. Assumption is made that geographical, climatic, genetic and procedural differences may have been contributing aspects to the favourable outcomes demonstrated internationally.

## **8.2 Application of naturally occurring bioactive Gibberellin PGRs to promote canopy capacity and increase access to soil water reserves**

Two bioactive gibberellins were evaluated for capacity to promote early plant canopy and structural biomass development, to intercept radiation and drive above and below ground growth and increase access to soil water reserves (Chapter 5). Following a sigmoidal curve pattern, early season above ground vegetative growth in cotton is comparatively slower than other commercial fibre and oilseed crops; evidencing an initial lag phase prior to early squaring (Robertson et al., 2007). This lag phase results from reduced light interception by a limited leaf area on a small plant (Guthrie et al., 1995; Hansen et al., 1996), with further growth reductions caused by impacts of abiotic stressors on leaf area accumulation of young plants (Robertson et al., 2007); amalgamations of these individually sub-lethal stressors having capability to compromise crop viability (Robertson et al., 2007). Consequently, in early vegetative growth, only a small proportion of available radiation is being harvested and utilised by the plant for assimilate production, to increase the plant vegetative framework (Guthrie et al., 1995).

Vigorous early development maximises cotton crop potential for improved yield and quality outcomes; seed cotton yields being proportionate to expanding plant size in early season cotton plants (Ashley et al., 1965). Promotion of early vegetative growth and canopy development to increase the plant “carrying capacity” and promote increased resilience to episodic water deficits (Biles & Cothren, 2001), is consistent with the nutritional hypothesis of fruit survival in cotton (Ehlig & LeMert, 1973; Turner, 1986).

Opportunity exists to influence this stage effectively because developing squares are not yet dominant sinks for carbohydrates, minerals and water (Guthrie et al., 1995). Moreover, evidenced limitations in seasonal skip row soil water extraction patterns (Goynes, 2005) are compounded by the metabolic costs associated with increased root (vegetative) growth. Reductions in root elongation and sloughing of older roots post physiological cut-out, are associated with assimilate diversion toward boll-loading and result in reductions in soil space exploration and a net-decrease in total root absorption potential and consequent water and nutrient uptake (Robertson et al., 2007). Resultantly, the foremost opportunity to augment root system architecture and facilitate exploration of a larger soil-space for water (and nutrient) capital, is during early vegetative growth due to the genetic predominance of early root biomass accumulation (Robertson et al., 2007). Consequently, exogenous application of plant growth regulators can be utilised to influence endogenous hormone concentrations, transport and / or associated receptor sites (Li et al., 2016); plant responses being dependant on varietal, management and environmental contexts (Livingstone & Parker, 1994; Rademacher, 2000).

Promotion of early root growth utilising Gibberellins as a limited water adaption strategy, has been demonstrated in other crops including maize (Silverman et al., 2017; Yuan et al., 2014), okra (Jasmine & Merina, 2012), tomato (Choudhury et al., 2013) and in cotton as a proportionate component of a proprietary preparation PGRIV (Zhao & Oosterhuis, 1995); Gibberellins have also been utilised to promote root elongation in horticultural aquatic plant, *Lemna minor* (Inada & Shimmen, 2000). Early vegetative stage foliar applications of Gibberellin A<sub>3</sub>, has demonstrated utility in promoting increased LAI under irrigated international production environs (Hansen et al., 1996; Larson et al., 1997; Walhoad, 1958). However, a scarcity of detailed information exists regarding cotton crop and plant (notably RSA) response to Gibberellin A<sub>3</sub> and especially Gibberellin A<sub>4,7</sub> applications, under rain grown production contexts (Appendix 2); location, proximity and method of exogenous Gibberellin application having implication for plant phenological response (Oosterhuis & Zhao, 1994).

Gibberellins A<sub>3</sub> and A<sub>4,7</sub> were foliar applied from 4 leaf stage, both as single and triplicate applications at 10-day intervals. Preliminary screening and concept evaluation assessments were completed through early glasshouse and field experiments over the 2016/7, 2017/18 and 2018/19 production seasons at the Narrabri and Terry Hie Hie experiment sites. Bioactive Gibberellin A<sub>3</sub> and A<sub>47</sub> demonstrated similar treatment responses and were not

effective in promoting early canopy or structural biomass development, or the accession of additional soil water reserves.

Synopsis of findings for both total dry weight accumulation and corresponding differences in leaf area index (LAI) claim no consistent contributory impact of the specific GA<sub>3</sub> and GA<sub>4,7</sub> treatment scenarios evaluated. Across the five field experiments, no GA<sub>3</sub> or GA<sub>4,7</sub> foliar treatment differences in total dry weight accumulation were evidenced at early squaring, early or mid-flowering. Additionally, no GA<sub>3</sub> treatment impacts on canopy capacity as described by leaf area index (LAI), were verified at early squaring or early flowering. Correspondingly, GA<sub>4,7</sub> treatments evidenced no effects in LAI accumulation at early squaring, early- and mid-flowering.

Moreover, weight of evidence from quantitative analysis of both glasshouse and field experiments indicated no positive treatment impacts of either GA<sub>3</sub> or GA<sub>4,7</sub> treatment applications on root biomass accumulation or associated plant utilisation of soil water reserves, across multiple treatment scenarios. Preliminary glasshouse screening of foliar and seed priming GA<sub>3</sub> treatments verified no treatment impacts on root length and root biomass. In the field, core break sampling of GA<sub>4,7</sub> treatments at mid flowering confirmed no difference from the control in the depth-stratified presence of live cotton roots or plant utilisation of shallow and deep soil water reserves, both under the plant line and adjacent skip row. These findings were substantiated by calibrated electromagnetic conductance surveys; no GA<sub>3</sub> or GA<sub>4,7</sub> treatment differences were evidenced in cumulative volumetric soil moisture to both 0.75 and 1.5 m during early and mid-flowering.

The evidenced lack of root growth and canopy capacity differences as well as associated increases in height and the production of fruiting sites, had flow on implications for early fruit retention and subsequent maturity and yield components. GA<sub>3</sub> and GA<sub>4,7</sub> impacts on reproductive growth across all field experiments showed increased development of total fruiting sites at early squaring, with a contrasting decrease in fruit retention and subsequent total square counts. No flow-on GA<sub>3</sub> or GA<sub>4,7</sub> treatment differences in proportionate reproductive biomass, square number and size, green boll number and size were evident at early flowering. A delay in maturity due to decreased early fruit retention was evident at mid flowering as decreased green boll weights, for both treatments. Delayed maturity was evidenced as smaller open boll sizes, however no corresponding differences in lint yield were noted.

### **8.3 Application and systems value of synthetic Gibberellin biosynthesis inhibiting PGRs to slow growth or induce physiological stasis**

Six anti-gibberellin PGRs were evaluated for the capacity to slow vegetative growth or induce physiological stasis in early vegetative stage cotton plants; with the objective of delaying reproductive phase change and subsequent crop peak resource demand (Chapter 6). The rain dependant nature of the production system delineates a priority to plant at the earliest opportunity, often earlier than the optimum window. Ensuring crop development results in the critical early flowering to physiological cut out period coinciding with peak seasonal evaporative demand and the probable occurrence of extreme heat events. Temporarily delaying crop maximum water requirements into a later climatic period of lower mean temperatures and vapour pressure deficits can improve or stabilise annualised yield outcomes and improve the downside risk associated with crop establishment outside the optimum window. Gibberellin biosynthesis inhibiting PGRs reduce the bioactive Gibberellin content of treated plants by disrupting specific stages of the Gibberellin biosynthesis pathway, particular to the individual chemical (Rademacher, 2000). At a plant level, this resulted in reduced shoot elongation and a decreased number of main-stem nodes, being key to cotton plant reproductive development (V. A. da Costa & Cothren, 2012). Significant variation exists between GaBI PGR chemicals in cotton plant phenotypic response to treatment, many of which are unquantified in early vegetative growth stage cotton or rain grown production contexts.

Widely utilised Mepiquat chloride, in addition to cotton-atypical PGRs, Chlormequat chloride, Paclobutrazol, Uniconazole, Trinexapac ethyl and Prohexadione-calcium, were soil-drench (glasshouse only) and foliar applied from 4 leaf stage, both as single and triplicate applications at 7-day intervals. Preliminary screening and concept evaluation assessments were completed through early glasshouse and subsequent detailed field experiments during the 2016/17, 2017/18 and 2018/19 production seasons at the Narrabri and Terry Hie Hie sites.

Preliminary glasshouse and field screening evaluations showed contrasting outcomes in PGR capacity to delay physiological phase change to reproductive growth/ progression to 50% population first square. In preliminary glasshouse-based evaluations of single treatment applications, both foliar and soil applications of Paclobutrazol and the foliar Uniconazole application produced 16-, 15- and 11-day delays in progression to 50% population first square, respectively. Early square shedding events caused by cold shock and the impact of sucking insect pests meant no substantiating differences in progression

to population 50% first flower was demonstrated in the two subsequent preliminary field experiments.

Accordingly, observed flow-on treatment impacts observed in preliminary glasshouse and field experiments were also partially divergent, notably during early flowering measurements; although, Trinexapac ethyl treatments did demonstrate utility in delaying reproductive development. Preliminary glasshouse and field evaluations of foliar Chloromequat- and Mepiquat chloride treatments evidenced decreases in total dry mass accumulation at early flowering; however, no consistent treatment differences in canopy development, mid-flowering fruit load and subsequent lint yield, turnout and yield components were demonstrated in field assessments. Concomitant evaluations of Paclobutrazol and Uniconazole soil and foliar applications demonstrated similar findings; decreases in proportionate reproductive biomass evidenced in the glasshouse, being associated with a delay reproductive phase-change, were not translated to field evaluations. Consequential fruit loads, maturity patterns and yield outcomes suggested no treatment differences in the subsequent timing of resource demand. Prohexadione-calcium treatments invoked no differences in LAI, biomass, reproductive growth and resultant yield outcomes in both glasshouse and field experiments. Preliminary glasshouse and field evaluations of Trinexapac ethyl demonstrated no LAI differences at early- or mid flowering. Both total and proportionate reproductive biomass, as well as green boll numbers and size, were decreased in early flowering with no consistent impacts evident at mid-flowering. Furthermore, early open bolls were comparatively smaller at mid-flowering, being commensurate with delayed development. Treatment induced delays in progression to physiological cut out and maturity were evidenced in both preliminary field experiments. No treatment impacts were observed on yield components at maturity, on ginning turnout or lint yield in both preliminary field experiments.

Foliar Paclobutrazol and Trinexapac ethyl treatments were further evaluated during subsequent field experiments during the 2017/18 and 2018/19 production seasons, at both the Narrabri and Terry Hie Hie experiment sites; Prohexadione calcium also being included on account of its anti-ethylene mode of action. Detailed biomass assessments were utilised both as a proxy for reproductive phase change measurements and to characterise delays in reproductive growth achieved. Where apparent, induced delays in reproductive development (evidenced as a reduced comparative square load), were due to firstly a decreased accumulation of total fruiting sites, and secondly by a decreased retention of squares on those sites. The mean square size was not impacted by this reduced retention. During early squaring, Prohexadione calcium treatments demonstrated no difference from

the control across all experiments in number of squares, total fruiting sites, retention on those sites or mean square size. Time of planting impacted Paclobutrazol treatment responses; earlier plantings (only) having decreased square counts from both a reduced total number of sites grown and reduced retention at early squaring. Trinexapac ethyl treatments across all experiments had comparatively fewer squares, fewer total sites and a reduced retention of squares to those sites at early squaring.

Physiological impacts resulting from delays in reproductive development varied between the Paclobutrazol and Trinexapac ethyl treatments. Paclobutrazol demonstrated inconsistent treatment responses in LAI and total biomass accumulation at both early squaring and flowering. No differences in progression to physiological cut out or maturity were evidenced, with no consequent differences in final open boll counts, mean weights or resultant lint yield. No conclusive flow-on impacts of the Paclobutrazol delay in first square are evidenced in the subsequent flowering period, maturity pattern or yield and associated components; substantiating earlier findings that despite a delay in first square, no impact was obtained on the timing of peak resource demand. Delays in reproductive growth induced by Trinexapac ethyl treatments coincided with decreased total dry weights and LAI at early squaring across three out of four experiments, the effect being still evident at early flowering. Trinexapac ethyl treatments induced mean delays in progression to physiological cut-out and maturity of 15 and 12 days, respectively. Yield and component responses varied across experiments, owing to hot and dry late season conditions experienced.

Foliar Trinexapac ethyl treatments were able to delay early reproductive growth with evident impacts on later reproductive development, being evidenced as; a reduced square load at control first square and differences in progression to physiological cut out and maturity under field conditions. However, decreased boll numbers and resultant lint yield compared with the control in a proportion of experiments, confounds the notion of a similar boll load, just moved temporally. Moreover, lint quality changes in micronaire and strength indicate possible influences of increased assimilate provision to the boll load; whether this as a result of a smaller boll load or compensatory growth, and / or increased retention of later fruit, resulting in changed climatic conditions (from the control) through boll filling, is uncertain and requires confirmation.

Analysis of the system value of the Trinexapac ethyl PGR treatment scenario established that the minimum additional lint yield required to break even and justify a TEP treatment scenario was 100 kg.ha<sup>-1</sup> (at \$461 per 227kg lint bale); being equal across all row

configurations. Being indicative of a delay in peak resource demand, later absolute planting dates demonstrated increased cumulative and mean 20-year profit outcomes, with decreased variability and reduced downside economic risk (as 80<sup>th</sup> percentile values); across all three field experiment sites.

Comparative analysis of a 1/3 proportionate delay in crop peak resource demand, achieved through either as TEP application or conventional second planting, demonstrated a significant additional cost ( $> \$150.\text{ha}^{-1}$ ) associated with a triplicate foliar application of TEP. Resultantly, on a 20-year cumulative profit basis and under current pricing structures, applications of TEP to Australian rain grown cotton production systems demonstrated no economic benefit in comparison to a conventional second planting, but provide benefit in avoiding losses associated with a failed second planting opportunity (i.e. no planting rainfall event). Further sensitivity analysis of both lint and PGR pricing is required to determine frameworks for PGR application under water limited rain grown production settings.

#### **8.4 Future research opportunities**

This study considered the utility of PGRs to strategically manipulate cotton crop growth and development, under water limited rain grown production contexts; for the promotion of crop water availability through three identified physiological response strategies.

Consequently, identified outcomes require further clarification around:

- Increased control of field evaluation scenarios, akin to Mahan and Payton (2018) rain-fed matrix, to enable quantification of plant response to treatment scenarios in line with a quantified stratification of seasonal conditions. Furthermore, such research can be combined with improved application (viz. higher resolution monitoring) of plant sensing technologies; to facilitate development of treatment criteria framework to promote system resilience through improved decision support.
- Improve understanding of OzCOT model mechanistic response to manipulations caused by PGRs increasing the day degree requirements to reach 50% population first square, including of particular note the influence on LAI.
- Analysis of value of residual profile water post TEP treatment scenarios, for following season crop (cereal, legume) production; incorporation into systems value proposition analysis.
- Risk sensitivity analysis of TEP “proportionate application” regarding relative production area implementation within a wider farming enterprise.

- Full economic analysis of TEP proposition including sensitivity analysis of PGR and lint pricing, sensitivity analysis of the proportion of crop total area treated, resulting impacts on profit outcomes (including accounting for the real cost of money); overlaid with analysis of climatic scenarios and the use of seasonal forecasting models; to inform application scenarios to drive adoption.

### **8.5 Concluding remarks**

Petitioning a novel “systems” phase of research; this thesis informs the discourse regarding system and crop level adaptive management opportunities to offset climate variability and promote system resilience, under resource-constrained Australian rain grown cotton production systems. Under capricious climatic conditions, in-season responsive (agile) management of system challenges and limitations introduces management diversity, reduces management implementation expenses and increases opportunity for management implementation. The resultant outcome being potentially increased farm-gate profits, through the provision of increased crop resource use efficiencies and improved yield and lint quality outcomes, with less annualised variability.

Unique Australian rain grown cotton production system-level adaptive management opportunities are identified and the utility of specific PGRs to accomplish, through the strategic and reliable manipulation of cotton plant growth and development, is described. The data contributes to the understanding of cotton plant phenological responses to specific exogenous PGR treatment scenarios, under resource-constrained rain grown production settings and where applicable provides insight to the systems-value of such PGR-driven adaptive management strategies.

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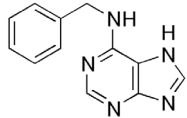
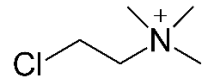
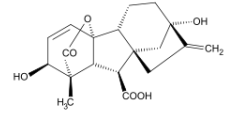
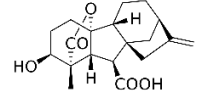
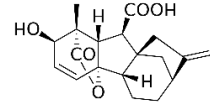
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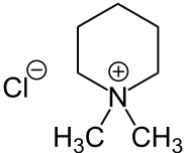
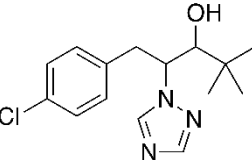
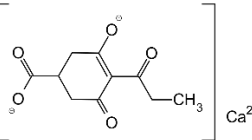
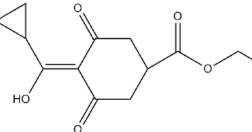
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## Appendices

## Appendix 1 Nomenclature and structural description of plant growth regulator active ingredients

ISO name (abbreviation) and proprietary source	Systematic IUPAC and other names	CAS N <sup>o</sup>	Structure and formula	Molecular mass
<b>6-Benzylaminopurine (6BA)</b> MaxCel, 19 g.L <sup>-1</sup> , Valent BioSciences LLC, Libertyville, Illinois, USA	<i>N</i> -(Phenylmethyl)-7 <i>H</i> -purin-6-amine Benzyl adenine 6-Benzyladenine 6BAP	1214-39-7	C <sub>12</sub> H <sub>11</sub> N <sub>5</sub> 	225.225g.mol <sup>-1</sup>
<b>Chlormequat,</b> <b>as Chlormequat chloride (CC)</b> Cycocel 750A, 582 g.L <sup>-1</sup> , BASF Australia Ltd, Southbank, VIC, Aust.	2-Chloro- <i>N,N,N</i> -trimethylethanaminium Chlorocholine Chlorcholine	7003-89-6 999-81-5 (chloride salt)	C <sub>5</sub> H <sub>13</sub> CIN 	122.62g.mol <sup>-1</sup>
Gibberellin A <sub>3</sub> (GA <sub>3</sub> ) ProGibb SG, 400 g.Kg <sup>-1</sup> , Valent BioSciences LLC, Libertyville, Illinois, USA	(3 <i>S</i> ,3 <i>aS</i> ,4 <i>S</i> ,4 <i>aS</i> ,7 <i>S</i> ,9 <i>aR</i> ,9 <i>bR</i> ,12 <i>S</i> )-7,12-Dihydroxy-3-methyl-6-methylene-2-oxoperhydro-4 <i>a</i> ,7-methano-9 <i>b</i> ,3-propenoazuleno[1,2- <i>b</i> ]furan-4-carboxylic acid Gibberellic Acid GA <sub>3</sub>	77-06-5	C <sub>19</sub> H <sub>24</sub> O <sub>6</sub> 	346.379g.mol <sup>-1</sup>
<b>Gibberellin A<sub>4,7</sub> (GA<sub>4,7</sub>)</b> <b>as Gibberellin A<sub>4</sub></b> <b>and Gibberellin A<sub>7</sub></b> ProVide, 113 g.Kg <sup>-1</sup> , Valent BioSciences LLC, Libertyville, Illinois, USA	(GA <sub>4</sub> ) (1 <i>R</i> ,2 <i>R</i> ,5 <i>R</i> ,8 <i>R</i> ,9 <i>S</i> ,10 <i>R</i> ,11 <i>S</i> ,12 <i>S</i> )-12-hydroxy-11-methyl-6-methylidene-16-oxo-15-oxapentacyclo[9.3.2.15,8.01,10.02,8]heptadecane-9-carboxylic acid (GA <sub>7</sub> ) (1 <i>R</i> ,2 <i>R</i> ,5 <i>R</i> ,8 <i>R</i> ,9 <i>S</i> ,10 <i>R</i> ,11 <i>S</i> ,12 <i>S</i> )-12-hydroxy-11-methyl-6-methylidene-16-oxo-15-oxapentacyclo[9.3.2.15,8.01,10.02,8]heptadec-13-ene-9-carboxylic acid	468-44-0 (GA <sub>4</sub> ) 510-75-8 (GA <sub>7</sub> )	C <sub>19</sub> H <sub>24</sub> O <sub>5</sub> (GA <sub>4</sub> )  C <sub>19</sub> H <sub>22</sub> O <sub>5</sub> (GA <sub>7</sub> ) 	332.39g.mol <sup>-1</sup> (GA <sub>4</sub> ) 330.39g.mol <sup>-1</sup> (GA <sub>7</sub> )

<p><b>Mepiquat,</b> <i>as Mepiquat chloride (MC)</i> Reward, 38 g.L<sup>-1</sup>, Adama Australia Pty Ltd, St Leonards, NSW, Aust</p>	1,1-dimethylpiperidin-1-ium;chloride	15302-91-7 24307-26-4 (chloride salt)	C <sub>7</sub> H <sub>16</sub> ClN	149.66g.mol <sup>-1</sup>	
<p><b>Paclobutrazol (Paclo)</b> Condense, 4 g.L<sup>-1</sup>, CropCare Australia Pty Ltd, Pinkenba, QLD, Aust.</p>	(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol	76738-62-0	C <sub>15</sub> H <sub>20</sub> ClN <sub>3</sub> O	293.80g.mol <sup>-1</sup>	
<p><b>Prohexadione calcium (PhxCa)</b> RegalisPlus, 100 g.Kg<sup>-1</sup>, BASF Australia Ltd, Southbank, VIC, Aust.</p>	calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate	127277-53-6	C <sub>10</sub> H <sub>10</sub> O <sub>5</sub> Ca	250.26g.mol <sup>-1</sup>	
<p><b>Trinexapac ethyl (TEP)</b> Moddus Evo, 250 g.L<sup>-1</sup>, Syngenta Australia Pty Ltd, Macquarie Park, NSW, Aust.</p>	ethyl (RS)-4-cyclopropyl(hydroxy)methylene-3,5-dioxocyclohexanecarboxylate	95266-40-3	C <sub>13</sub> H <sub>16</sub> O <sub>5</sub>	252.30g.mol <sup>-1</sup>	

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**Uniconazole (Unicon)**

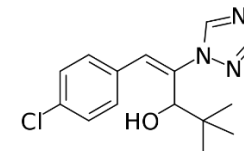
Sunny, 50 g.L<sup>-1</sup>, Sumitomo Chemical  
Australia Pty Ltd, Epping, NSW  
Aust.

(E)-1-(4-Chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-  
1-yl)pent-1-en-3-ol

83657-22-1

C<sub>15</sub>H<sub>18</sub>ClN<sub>3</sub>

275.78g.mol<sup>-1</sup>



**Appendix 2 Cotton plant (*G. hirsutum*) response to true plant growth regulator compounds, adapted from Cothren and Oosterhuis (2010)**

PGR Group	Compound	Application	Environment	Affected trait, effect, response	Reference
Aux	1-naphthylacetic (NAA)	acid 20 ppm, foliar, 960 L.ha <sup>-1</sup> total spray volume, applied twice at 90 and 120 days after planting	Field, irrigated	Increased boll number, boll weight and lint yield.	Sawan and Sakr, 1998
Aux	1-naphthylacetic (NAA)	acid		Low concentrations increases fibre fineness; high concentrations decreases fibre fineness	Bhatt <i>et al.</i> , 1972
Aux	1-naphthylacetic (NAA)	acid		Decreased boll shedding, increased yields	Murty <i>et al.</i> , 1976
Aux	1-naphthylacetic (NAA)	acid		Increased boll numbers, 8-10% yield increase	Negi and Singh, 1956
Aux	1-naphthylacetic (NAA)	acid		Increased fibre length	Patel, 1992
Aux	Indol-3-ylbutyric (IBA)	acid		Increases NADH oxidase activity in roots, indicating an increase in root respiration.	Urwiler and Oosterhuis, 1986
Aux	Indole-3-acetic (IAA)	acid 0.1 ppm, applied as 200 uL solution directly to developing ovary, once at 0 or 6 days post anthesis	Glasshouse	Regulation of all (MADS-box members) AG-subfamily gene expression associated with ovule and ovary development.	deMoura <i>et al.</i> , 2017
Aux	Indole-3-acetic (IAA)	acid 10 uM, 2 hour soak prior to ethylene exposure, on primary leaf explant of 2 leaf stage plant.	InVitro	Suppression of increased cellulase and polygalacturonase activity in abscission zone associated with increased ethylene levels.	Mishra <i>et al.</i> , 2008
Aux	Indole-3-acetic (IAA)	acid		Improved length and fineness of fibre	Bhatt <i>et al.</i> , 1972
Aux	Indole-3-acetic (IAA)	acid		Overcome trifluralin damage	Hassawy and Hamilton, 1971
AuxTI	2,3,5 triiodobenzoic acid (TIBA)			Yield increase, increased boll size and boll number per plant, lowered position of first fruiting branch	Freytag and Coleman, 1973
AuxTI	2,3,5 triiodobenzoic acid (TIBA)			Reduced plant size and dry weight; reduced seed cotton yield	Thomas, 1967
AuxTI	2,3,5 triiodobenzoic acid (TIBA)			Increased boll set, earlier progression to cut out, no significant yield effect	Thomas, 1967
Ck	6-Benzylaminopurine (6BA)	25 umol.mol <sup>-1</sup> , foliar, no described total spray volume, one application at 2-4 leaf stage.	Glasshouse	Phytotoxic leaf lesions, increased hypocotyl diameter, reduction in apical dominance, increased root length and increased mean boll weight, reduced water deficit stress (via bioassay), reduced transpiration.	Burke, 2011.

Ck	6-Benzylaminopurine (6BA)	25 $\mu\text{mol}\cdot\text{mol}^{-1}$ , foliar, no described total spray volume, one application at 2-4 leaf stage.	Field, pre-plant then rain grown.	irrigated,	increased hypocotyl diameter, increased lateral root growth, reduced apical dominance, increased lint yield, reduced water deficit stress (via bioassay)	Burke, 2013.
Ck	6-Benzylaminopurine (6BA)	24.75 ppm, seed soaking for 12 h, air dry, planted immediately	Growth hydroponic	chamber,	Increased number of floral buds initiated at control first square (via increased sucrose accumulation in apical buds, upregulation of floral initiation genes)	Fang <i>et al.</i> , 2018
Ck	6-Benzylaminopurine (6BA)	24.75 ppm, seed soaking for 12 h, air dry, planted over 2 years	Field, irrigated		Increased Photosynthesis rate (leaf carbohydrate content, translocation rate and sucrose, starch content in floral buds, reduced abscission rate), increased retention and boll numbers boll numbers, lint yield (12.1-13.3%)	Fang <i>et al.</i> , 2019
Ck	6-Benzylaminopurine (6BA)	10 $\text{mg}\cdot\text{L}^{-1}$ , foliar at 300 $\text{L}\cdot\text{ha}^{-1}$ , two applications one day apart, at squaring	Field, irrigated		Increased Photosynthesis rate (leaf carbohydrate content, translocation rate and sucrose, starch content in floral buds, reduced abscission rate), increased retention and boll numbers boll numbers, lint yield (7.7-8.5%)	Fang <i>et al.</i> , 2019
Ck	6-Benzylaminopurine (6BA)	20 $\text{mg}\cdot\text{L}^{-1}$ , foliar at 300 $\text{L}\cdot\text{ha}^{-1}$ , two applications one day apart, at squaring	Field, irrigated		Increased Photosynthesis rate (leaf carbohydrate content, translocation rate and sucrose, starch content in floral buds, reduced abscission rate), increased retention and boll numbers boll numbers, lint yield (8.2-11%)	Fang <i>et al.</i> , 2019
Ck	6-Benzylaminopurine (6BA)	100 $\mu\text{mol}\cdot\text{L}^{-1}$ , foliar, 2.5 $\text{mL}/\text{pot}$ , one application at 5-leaf stage.	Growth chamber		Increased leaf abscission in young plants at high concentrations due to Ck induced ethylene production.	Grossmann, 1991.
Ck	6-Benzylaminopurine (6BA)	50 $\text{mg}\cdot\text{L}^{-1}$ , foliar at flowering (60DAP),	Pot, field		Counteracted water deficit stress impacts on net photosynthesis rate, transpiration rate and stomatal conductance.	Kumar <i>et al.</i> , 2001
Ck	6-Benzylaminopurine (6BA)	5 $\text{mg}\cdot\text{L}^{-1}$ , foliar at flowering (60DAP),	Pot		Effect of drought stress imposed during flowering, on RuBPCO, PEPC and CA was alleviated	Pandey <i>et al.</i> , 2000
Ck	6-Benzylaminopurine (6BA)	5 $\mu\text{M}$ , foliar, sprayed to drip at first-flower (55DAP)	Glasshouse		Decreased impact of water deficit stress on enzyme activation (carbonic anhydrase, RuBPCo) and improved seed cotton yield outcomes in 6BA treated cotton plants. Increased flower retention and OB no. but decreased seed cotton yield in unstressed, 6BA treated plants.	Pandey <i>et al.</i> , 2003
Ck	6-Benzylaminopurine (6BA)	Foliar, applied once at flowering, 5 $\text{mg}\cdot\text{L}^{-1}$	Field, irrigated		Increased OB mean weight and fibre length under both optimal and low temperature contexts. Increased boll sucrose content and associated sucrose synthase and sucrose phosphate synthase activity.	Wang <i>et al.</i> , 2011
Ck	CPPU	250 $\mu\text{mol}\cdot\text{mol}^{-1}$ , seed soaking for 10 sec., air dry, planted at 6 days post treatment.	Field, rain grown.		Increased lateral root production, increased root length, increased yield, reduced water deficit stress levels (via stress test bioassay)	Burke and Sanchez, 2018
Ck	CPPU	5 $\mu\text{mol}\cdot\text{mol}^{-1}$ , foliar, no defined total spray volume, one application at cotyledon.	Field, rain grown.		Increased lateral root production, increased root length, increased yield, reduced water deficit stress levels (via stress test bioassay)	Burke and Sanchez, 2018

Ck	CPPU	250 $\mu\text{mol}\cdot\text{mol}^{-1}$ , seed soaking for 10 sec., air dry, planted at 6 days post treatment.	Glasshouse	Increased lateral root production.	Burke and Sanchez, 2018
Ck	CPPU	5 $\mu\text{mol}\cdot\text{mol}^{-1}$ , foliar, no defined total spray volume, one application at cotyledon.	Glasshouse	Increased lateral root production.	Burke and Sanchez, 2018
Ck	CPPU	25 ppm, foliar, no defined total spray volume, one application at cotyledon / 2-leaf stage	Glasshouse	Increased root:shoot ratio, increased lateral root growth (length)	deBenedetto, 2015
Ck	Kinetin (KIN)	5 $\text{mg}\cdot\text{L}^{-1}$ , foliar at first square in 203L.ha spray solution.	Field	Decreased (comparative to control) multi (6) year trend in lint yield, turnout and boll size.	Hedin and McCarty, 1994a
Ck	Kinetin (KIN)	0.56 $\text{L}\cdot\text{ha}^{-1}$ Burst®, foliar, first square x 2, at 203 $\text{L}\cdot\text{ha}^{-1}$ spray solution.	Field	No treatment difference in OB mean weight, decreased lint ginning turnout and lint yield across 2 year mean.	Hedin and McCarty, 1994b
Ck	Kinetin (KIN)	Foliar	Field	Increased GB avg weight, increased OB no, increased ginning turnout	Mayeux et al, 1987
Ck	Kinetin (KIN), with $\text{GA}_3$	65.7 $\text{mg}\cdot\text{L}^{-1}$ , foliar, 4 leaf stage,	Growth chamber	Analysed for 14d post application; no change from control in net photosynthesis, dark respiration and daily carbon gain, no differences detected in leaf area or shoot dry weight.	Bednarz and van Iersel, 1998
Ck	Thidiazuron	$10^{-100}$ $\mu\text{mol}\cdot\text{mol}^{-1}$ , foliar, no defined total spray volume, one application at 20-30 days post planting.	Growth chamber	Induction of defoliation	Suttle, 1985
Ck	Zeatin glycosides	Foliar, undefined	Field, irrigated and rain grown	Increased seed cotton yields proportionate to water stress; rain grown achieving increased yield outcomes compared to 60% and 100% ET replacement.	Mayeux et al, 2011
EthAI	1-MCP	10g.ai/ha, foliar, no defined total spray volume, one application at first square.	Growth chamber	Under heat stress conditions; delayed senescence, decreased lipid peroxidation, membrane leakage and leaf soluble sugar contents, increased chlorophyll content. Under water deficit stress conditions; decreased lipid peroxidation, membrane leakage and soluble sugar content, increased water use efficiency, leaf water potential and chlorophyll content.	Chen <i>et al.</i> , 2015
EthAI	1-MCP	2.4g.ai/L, as gaseous application.	Glasshouse	Increased water use efficiency under well watered conditions, increased reproductive node number under water deficit stress conditions.	daCosta and Cothren, 2011
EthAI	1-MCP	25/50g.ai/ha, foliar, 93L.ha <sup>-1</sup> total spray volume, one application at 93 days after planting (16 nodes).	Field, irrigated	Mid bloom application to reduce ethephon (abiotic stress) application effect; increased fruit set in upper portion of canopy (increased boll number).	daCosta, Cothren and Bynum, 2011

EthAI	1-MCP	10g.ai/ha, foliar, no defined total spray volume, three applications at first square, first square + 7 days, first square + 14 days.	Field, irrigated	Increased seed cotton yield, lint yield and turnout	deBrito <i>et al.</i> , 2013
EthAI	1-MCP	10g.ai/ha, foliar, 187L.ha <sup>-1</sup> total spray volume, applied once at first square	Growth chamber	Lower level of plant water deficit stress (increased membrane integrity and antioxidant enzyme activity)	Kawakami <i>et al.</i> , 2010
EthAI	1-MCP	25g.ai/ha, foliar, 103L.ha <sup>-1</sup> total spray volume, canopy or ambient temperature triggered applications from first square.	Field, irrigated and rain grown	Increased fruit retention under irrigated and rain grown conditions and under irrigated conditions, decreased mean daily canopy temperature and associated changes in net photosynthesis, transpiration, PSII quantum yield.	Maeda <i>et al.</i> , 2018
EthAI	1-MCP	10uL.L <sup>-1</sup> , gas exposure prior to ethylene treatment, on primary leaf explant of 2 leaf stage plant.	InVitro	Suppression of increased cellulase and polygalacturonase activity in abscission zone associated with increased ethylene levels.	Mishra <i>et al.</i> , 2008
EthAI	1-MCP	250nL.L <sup>-1</sup> gas exposure prior to ethylene application	Growth chamber, hydroponic	Maintenance of shoot elongation and ethylene-responsive gene (GhACS6, GhACO5, GhEIN4, GhLTL1) expression in shoot apices, in presence of ethylene; duration of protection did not exceed 48 h.	Su and Finlayson, 2012
EthBI	AVG	30g.ai/ha, foliar, no defined total spray volume, two applications at first square, first square + 7 days.	Field, irrigated	Increased seed cotton yield, lint yield and turnout	deBrito <i>et al.</i> , 2013
EthBI	AVG	30g.ai/ha, foliar, no defined total spray volume, two applications at first flower, first flower + 7 days.	Field, irrigated	Increased seed cotton yield, lint yield and turnout	deBrito <i>et al.</i> , 2013
EthBI	AVG	125g.ai/ha (830 ppm), foliar, no defined total spray volume, applied once at early squaring (9-10 node, 52 days after planting)	Glasshouse	Under waterlogging conditions; prevented ethylene accumulation in leaf tissue resulting in increased boll numbers, leaf growth, nitrogen acquisition and photosynthesis.	Najeeb <i>et al.</i> , 2015
EthBI	AVG	125g.ai/ha (830 ppm), foliar, no defined total spray volume, applied once at early squaring (9-10 node, 52 days after planting)	Field, irrigated	Under waterlogged conditions; increased lint yield, boll number, boll weight and fruit retention	Najeeb <i>et al.</i> , 2016
EthBI	AVG	125g.ai/ha (830 ppm), foliar, no defined total spray volume, applied once at early squaring (9-10 node, 52 days after planting)	Glasshouse	Under increased [CO <sub>2</sub> ] and waterlogged conditions; increased fruit retention	Najeeb <i>et al.</i> , 2018
GaBI	Chlormequat chloride (and Mepiquat chloride)	Seed priming, 3 h, air-dried, 1000 ppm	Field,	Seed treatment reduced chlorosis and necrosis of cotton treated with fluometuron, no untreated seed control to determine effect of CCC.	Corben and Frans, 1991

GaBI	Chlormequat (CC)	chloride			At low concentrations, coarser fibre; higher concentrations, increased length and fineness; decreased strength and yield	Bhatt <i>et al.</i> , 1972
GaBI	Chlormequat (CC)	chloride	31.2 ppm / 62.4 ppm, foliar, once at first flower, mid/peak or late flowering, 224 L.ha <sup>-1</sup> total spray volume.	Field, low density	Reduced retention at first flower application, increased suppression of late boll set (boll number) and also size with subsequent / later applications, higher yielding crops more sensitive to yield reduction.	Thomas, 1975; (DeSilva, 1971; Kittock <i>et al.</i> 1975)
GaBI	Chlormequat (CC)	chloride	80 ppm, foliar, two applications at 55 and 75 dap.	Field, irrigated	Earlier application (only) lowered seed cotton yield via reducing vegetative growth earlier and having a larger impact on leaf area and subsequent photosynthetic capacity. Later application (only) increased seed cotton yield relative to control via optimising photosynthate partitioning	Dhillon <i>et al.</i> , 1980
GaBI	Chlormequat (CC)	chloride	100 ppm, foliar, 30 days after planting (first square)	Greenhouse	Response to rain post application investigated, unfortunately no PGR untreated control included.	Echer and Rosolem, 2012
GaBI	Chlormequat (CC)	chloride	125 ppm, foliar, first flower, once, 400 L.ha <sup>-1</sup> total spray volume	Field, irrigated	Decreased growth rate, decreased height at harvest, reduction in boll retention	Marani, 1973
GaBI	Chlormequat (CC)	chloride			Significant yield increase	Rao <i>et al.</i> , 1980
GaBI	Chlormequat (CC)	chloride	500 ppm, foliar, 960 L.ha <sup>-1</sup> total spray volume, three applications at 60-80 days after planting	Field, drip irrigated.	Increased boll number, boll weight, seed cotton weight and water use efficiency	Rao <i>et al.</i> , 2016
GaBI	Chlormequat (CC)	chloride	250/500/750 ppm, foliar, 960 L.ha <sup>-1</sup> total spray volume, one application at 105 days after planting.	Field, irrigated	Increased boll number, boll weight and lint yield.	Sawan, 2016
GaBI	Chlormequat (CC)	chloride			Increased yields	Singh, 1970
GaBI	Chlormequat (CC)	chloride			Promoted drought resistance	Singh, 1975
GaBI	Chlormequat (CC)	chloride			Reduced plant height and yield	Thomas, 1964
GaBI	Mepiquat chloride (MC)				Erratic yield response by cultivar	Briggs, 1981
GaBI	Mepiquat chloride (MC)				Reduced rate of root density growth	Cappy and Cothren, 1980
GaBI	Mepiquat chloride (MC)		no defined concentration, seed soaking for 12 h soaking, air dry, planted immediately	Growth chamber, hydroponic	Increased number, length area and volume of lateral roots and lateral root primordia, reduced aerial biomass, increased root:shoot ratio in 20 day old seedlings	Chen, <i>et al.</i> , 2018
GaBI	Mepiquat chloride (MC)				Seed treatment reduced chlorosis and necrosis of cotton treated with fluometuron	Corben and Frans, 1991

GaBI	Mepiquat chloride (MC)	12g.ai/kg seed, spray application to seed, air dry, planted post treatment.	Glasshouse		Decreased shoot length, no impact on root growth at 21 days after planting	deAlmeida and Rosolem, 2012
GaBI	Mepiquat chloride (MC)					Echer and Rosolem, 2012
GaBI	Mepiquat chloride (MC)				Mitigates symptom expression of Verticillium wilt	Erwin <i>et al.</i> , 1979a, b
GaBI	Mepiquat chloride (MC)	150 ppm, seed soaking for 12 h, air dry, planted immediately	Growth chamber, hydroponic		Decreased number of floral buds initiated at control first square	Fang <i>et al.</i> , 2018
GaBI	Mepiquat chloride (MC)				Smaller bolls, increased flower production	Feaster <i>et al.</i> , 1980
GaBI	Mepiquat chloride (MC)				promoted maintenance of leaf turgor potential in water-stressed plants	Fernandez and Cothren, 1990
GaBI	Mepiquat chloride (MC)				Lack of effect on carbon use efficiency in well-watered and water-deficient plants	Fernandez <i>et al.</i> , 1992
GaBI	Mepiquat chloride (MC)				Increase in fine root hairs	Fernandez <i>et al.</i> , 1991
GaBI	Mepiquat chloride (MC)	3.4g.ai/kg seed, seed soak.	Glasshouse		Decreased early vegetative growth stage height and stem diameter, increased yield.	Ferrari <i>et al.</i> , 2015
GaBI	Mepiquat chloride (MC)				Darker green leaves	Gausman <i>et al.</i> 1978; Gausman <i>et al.</i> 1980a; Walter <i>et al.</i> , 1980
GaBI	Mepiquat chloride (MC)				Thicker leaves with reduced surface area	Gausman <i>et al.</i> 1980a
GaBI	Mepiquat chloride (MC)				Stimulation of CO <sub>2</sub> uptake	Gausman <i>et al.</i> 1980b
GaBI	Mepiquat chloride (MC)				Reduced wind blown damage to cotton	Gausman <i>et al.</i> , 1981
GaBI	Mepiquat chloride (MC)	41.94g.ai/ha, foliar, 94 L.ha <sup>-1</sup> , three applications at first square (FS), FS + 10 days, first flower.	Field, irrigated		Reduced plant height and leaf area, changed canopy structure evidenced via increased canopy extinction coefficient, increased radiation use efficiency (less light intercepted by MC treatment for same biomass production) due to changes in photosynthetic capacity of leaves and light distribution through canopy.	Gonias <i>et al.</i> , 2012
GaBI	Mepiquat chloride (MC)				Height reduction under luxuriant growth conditions	Heilman, 1981
GaBI	Mepiquat chloride (MC)				Increased gross canopy photosynthesis	Hodges <i>et al.</i> , 1991
GaBI	Mepiquat chloride (MC)	18/45/60g.ai/ha, foliar, no defined total spray volume, three applications at first square (FS), FS + 10 days, first flower.	Field, irrigated		Mepiquat interaction with increasing plant density; increased boll weight and increased amount of harvestable bolls located on lower/middle fruiting positions (optimal distribution).	Mao <i>et al.</i> , 2015
GaBI	Mepiquat chloride (MC)	15/30g.ai/ha, foliar, 150 L.ha total spray volume, one application at first square.	Growth chamber		Mepiquat interaction with temperature; above / below optimum decreased effectiveness of MC on plant height and dry matter accumulation, requiring increased concentration of MC per unit of growth reduction.	Rosolem <i>et al.</i> , 2013

GaBI	Mepiquat chloride (MC)	0.06/0.11/0.17/0.23kg.ai/ha, foliar, 150 L.ha <sup>-1</sup> total spray volume, one application at first flower.	Field, irrigated	MC interaction with insect induced fruit removal during flowering; increasing concentrations of MC required to counteract increased vegetative growth due to fruit removal.	Samples <i>et al.</i> , 2015
GaBI	Mepiquat chloride (MC)			Increased boll weight, seed and lint index, lint yield and earliness	Sawan and Sakr, 1990
GaBI	Mepiquat chloride (MC)			Reduced boll rot	Snow <i>et al.</i> , 1981
GaBI	Mepiquat chloride (MC)			Increased levels of petiole nitrates	Stedman <i>et al.</i> , 1982
GaBI	Mepiquat chloride (MC)			More favourable water status, higher leaf water potential	Stuart <i>et al.</i> , 1980, 19891, 1984
GaBI	Mepiquat chloride (MC)			Reduction in canopy width, reduced internode length, reduced bolls/plant, individual boll weight increase	Walter <i>et al.</i> , 1980
GaBI	Mepiquat chloride (MC)	80 ppm, foliar, no defined total spray volume, one application at 3 leaf.	Growth chamber	Suppression of Gibberellin metabolism and biosynthesis genes (GhEXP and GhTHZ, DELLA), resulting in decreased GA <sub>3</sub> and GA4 levels (and plant height) in elongating internode.	Wang <i>et al.</i> , 2014
GaBI	Mepiquat chloride (MC)			Increased drought resistance of cotton seedlings	Xu and Taylor, 1992
GaBI	Mepiquat chloride (MC)	15/30g.ai/ha, foliar, no defined total spray volume, three applications between first square and early flowering.	Field, irrigated	Increased lint yield, K use and use efficiency.	Yang <i>et al.</i> , 2014
GaBI	Paclobutrazol	125 ppm, 375 ppm, foliar, single application, FF, FF+2 wk, FF+4 wk	Field, drip irrigated.	Reduced plant height, number of main-stem nodes, dry weight of stems and leaves. Early application having stronger growth impact, no impact on flowering, lint quality or yield.	Ben-Porath <i>et al.</i> , 1988
GaBI	Paclobutrazol	soil drench, 0.05 g/m <sup>2</sup> , 0.1 g/m <sup>2</sup> , once after first irrigation,	Field, irrigated	Decreased node of first fruiting branch. No difference in FF, FOB or yield.	Cimen et al, 2003
GaBI	Paclobutrazol	soil drench, 0.05 g/m <sup>2</sup> , 0.1 g/m <sup>2</sup> , once after first irrigation,	Field, irrigated		Cimen et al, 2015
GaBI	Paclobutrazol	soil drench, 0.05 g/m <sup>2</sup> , 0.1 g/m <sup>2</sup> , once after first irrigation,	Field, irrigated	Increased micronaire	Temiz et al, 2009
GaBI	Paclobutrazol	50 ppm, seed priming, priming + foliar at FF.	Field, irrigated ( <i>G. barbadense</i> )	Inhibited early growth (dry weight, stem length, node development, leaf area); effect over by reproductive transition.	Zaghlool et al, 2005
GaBI	Prohexadione-calcium				Inoue et al, 2015
GaBI	Trinexapac ethyl (TEP)	500 ppm, foliar, 200 L.ha <sup>-1</sup> , one application at 12-leaf / first flower.	Pots, field conditions.	No toxicity symptoms, no significant impact on plant height or biomass accumulation at 50 days post application.	Correia and Leite, 2012
GaBI	Uniconazole	500 ppm, foliar, two applications at FF + 14 d	Field, irrigated ( <i>G. barbadense</i> )	Decreased height at maturity, increased fruiting branch number, increased number of green and open bolls, decreased time to first open boll.	Mohamed et al, 2010
Gibb	Gibberellin A3			Increased fibre length	Bhatt and Ramanujam, 1971

Gibb	Gibberellin A3	0.0001 ppm, applied directly to abscission zone	14 day old cotton seedling explants (excised abscission zones), growth chamber	Increased number of cells in abscission zone, increased starch content in abscising cells	Bornman, Addicott and Spurr, 1966
Gibb	Gibberellin A3	0.0001 ppm, applied directly to abscission zone	14 day old cotton seedling explants (excised abscission zones), growth chamber	Increased abscission, adaxial commencement of abscission process	Bornman, Spurr and Addicott, 1967
Gibb	Gibberellin A3	Seed soak, 10-3 M, 5 mins + chill treated at 5°C, 24 hrs	Growth chamber	Reduced chilling injury in germinating cotton; increased speed and percentage germination	Cole and Wheeler, 1974
Gibb	Gibberellin A3	0.06 ppm, foliar x3, at 20, 40 and 60 DAP	Field, irrigated	Increased photosynthetic pigments (Chlorophyll a and b, carotenoids) at 90 days post treatment.	daCosta et al, 2017
Gibb	Gibberellin A3	1 ppm, applied as 200 uL solution directly to developing ovary, once at 0 or 6 days post anthesis	Glasshouse	No regulation of (MADS-box members) AG-subfamily gene expression associated with ovule and ovary development.	deMoura <i>et al.</i> , 2017
Gibb	Gibberellin A3	0.1 ppm, Foliar at one leaf / 15 DAP, applied in 2 day intervals for 20 days	Glasshouse	Induced more rapid emergence and taller seedlings; no impact on node development, increased dry weight of stems and petioles, no impact on leaf or root dry weight	Ergle, 1958
Gibb	Gibberellin A3	4.5 ppm, seed soaking for 12 h soaking, air dry, planted immediately	Growth chamber, hydroponic	Increased number of floral buds initiated at control first square (via increased sucrose accumulation in apical buds, upregulation of floral initiation genes)	Fang <i>et al.</i> , 2018
Gibb	Gibberellin A3	4.5 ppm, seed soaking for 12 h soaking, air dry, planted over 2 years	Field, irrigated	Increased Photosynthesis rate (leaf carbohydrate content, translocation rate and sucrose, starch content in floral buds, reduced abscission rate), increased retention and boll numbers, lint yield (6.3-7.5%)	Fang <i>et al.</i> , 2019
Gibb	Gibberellin A3	35.7 ppm, foliar, once at 7 leaf.	Glasshouse	Increased leaf area, height, net photosynthesis rate at 2 weeks post treatment	Hansen et al, 1996
Gibb	Gibberellin A3			Taller plants, less yield	Lane, 1958
Gibb	Gibberellin A3	47.6 ppm, 3-5 L, single banded foliar application.	growth chamber and field	At 2 week post treatment; increases in height, leaf area, leaf fresh weight. Increased net photosynthesis due to greater leaf area. Increase lint yield	Larson et al, 1997
Gibb	Gibberellin A3	0.1 ppm, applied directly to abscission zone	Explants (excised abscission zones), growth chamber	Increased abscission, evidenced translocatability of Gibberellin to opposite untreated petiole (abscised), A3>A4>A7 in efficacy	Lyon and Smith, 1966
Gibb	Gibberellin A3	150 and 200 ppm, foliar at 5 weeks post planting	Glasshouse	Increased number of flowers, increased shedding and increased retention; overall net increase in flower / boll number	Mathur and Mittal, 1964
Gibb	Gibberellin A3	0.9 mM, foliar, weekly from 6 weeks post planting	Field, irrigated	Induced fasciation at flowering	Nadjimov et al, 1999

Gibb	Gibberellin A3	0.04 ppm, foliar x2, at 36 and 67 days post transplant, transplant at 7 days after germination.	Hydroponics	Increased root absorption area and root volume at 90 days post transplant (25 days post treatment)	Onanuga <i>et al.</i> , 2012
Gibb	Gibberellin A3			Flower bud and boll abscission reduced, no yield impact	Subbiah and Mariakulandia, 1972
Gibb	Gibberellin A3	foliar	Field, irrigated	Increased vegetative growth, decreased boll size (at maturity) and abscission, later maturation.	Walhood, 1958
Other	Adenosine-3'-5'cyclic (AMP, Monophosphate)			Reduced chilling injury in germinating cotton	Cole and Wheeler, 1974
Other	Antitranspirants			Prevented chilling injury to seedling cotton	Christiansen and Ashworth, 1978
Other	Epibrassinolide	0.048 ppm, applied as 200 uL solution directly to developing ovary, once at 0 or 6 days post anthesis	Glasshouse	Regulation of 3 (MADS-box members) AG-subfamily genes; GhMADS3, 4, 7; expression being associated with ovule and ovary development.	deMoura <i>et al.</i> , 2017
Other	Putrescine (PUT)	10 mM, foliar (brush), applied to drip to floral buds 24 h prior to flowering	Growth chamber	Increased seed set under high temperature stress conditions.	Bibi <i>et al.</i> , 2010

**Appendix 3 Terry Hie Hie (THH), Bellata (BTA) and Narrabri (ACRI) field experiment in-crop rainfall and temperature metrics ([BOM], 2019); total in-crop day degree accumulation (DDAcum), number of in-crop days occurrence of heat stress (daily max > 35°C) and cold chill (daily min <11°C)**

Experiment	Cropping period		In-crop rainfall (mm)	Temperature		Cold chill
	Planted	Maturity		DDAcum	Heat stress	
4. 16.17 ACRI ERCF	20/10/16	20/4/17	243.6	2412.45	70	24
5. 16.17 BTA ERF	3/11/16	4/4/17	209.65	2305.2	73	5
6. 16.17 ACRI GABI	21/11/16	25/5/17	231.8	2304.9	68	31
7. 16.17 THH GABI	21/10/16	3/4/17	307.6	2400.9	73	1
8. 17.18 ACRI Resil_S1	19/10/17	24/4/18	233.8	2457.8	67	1
8. 17.18 ACRI Resil_S2	7/12/17	24/4/18	104.4	2000.4	66	1
9. 18.19 ACRI Resil_2b	12/1/19	19/3/19 <sup>(a)</sup>	48.8	1043.6	42	0
10. 18.19 BTA Resil	30/11/18	4/2/19 <sup>(b)</sup>	39.6	1149.55	46	0
11. 18.19 THH Resil	19/9/18	22/2/19	186.6	2211.75	67	12

<sup>(a)</sup> Not grown to maturity; crop replanted following hailstorm and was terminated at 50% plant population first flower

<sup>(b)</sup> Not grown to maturity; due to severe drought conditions crop was terminated at 50% plant population first flower

**Appendix 4 Cotton plant agronomic response to synthetic Adenine-type Cytokinin PGR, 6-Benzylaminopurine**

**Table 1 Summary of 6BA PGR treatment impacts on root biomass accumulation and access to soil water reserves across experiments 1, 2, 4, 5, 8, 9 and 10.**

Trt. description	Early vegetative	Early flowering	Mid flowering	Post harvest
Seed prime, 0.01mg.L <sup>-1</sup> , 20hr	<b>Root length, root dry weight and Root:Shoot ratio;</b> no treatment differences (13 DAP, exp. 1).			
Seed prime, 850mg.L <sup>-1</sup> , 10s				
Seed coat, 9.97mg cwt				
Seed coat, 49.99mg cwt				
Foliar, 150mg.L <sup>-1</sup> 4L once	<b>Root length;</b> no treatment differences (24 DAP, exp. 2).			
Foliar, 67mg.L <sup>-1</sup> 4L once				
Foliar, 33mg.L <sup>-1</sup> 4L once				
Foliar, 25mg.L <sup>-1</sup> 4L once	<b>Root dry weight;</b> all treatments decreased (24 DAP, exp. 2).			
	<b>Root:Shoot ratio;</b> decreased in 25 and 33mg.L <sup>-1</sup> treatments (24 DAP in exp. 2).			
Foliar, 25mg.L <sup>-1</sup> 4L 3x10 d		<b>Volumetric soil moisture, cumulative to 0.75m and 1.5m at plant line and adj. skip row;</b> no treatment differences (78 DAP, exp. 8).	<b>Volumetric soil moisture, cumulative to 0.75m and 1.5m at plant line and adj. skip row;</b> no treatment differences (110 DAP, exp. 8). <b>Presence of roots, to 1m under plant line and adj. skip row;</b> no treatment differences (108 DAP, exp. 9). <b>Gravimetric soil moisture, stratified to 1m in plant line and adj. skip row;</b> no treatment differences (108 DAP, exp. 9).	<b>Presence of roots, to 1m under plant line and adj. skip row;</b> no treatment differences (237 DAP, exp. 4). <b>Gravimetric soil moisture, stratified to 1m in plant line and adj. skip row;</b> no treatment differences (237 DAP, exp. 4).
Foliar, 100mg.L <sup>-1</sup> 4L 3x10 d		<b>Volumetric soil moisture, cumulative to 0.75m and 1.5m at plant line and adj. skip row;</b> no treatment differences (78 DAP, exp. 8).	<b>Volumetric soil moisture, cumulative to 0.75m and 1.5m at plant line and adj. skip row;</b> no treatment differences (110 DAP, exp. 8).	

**Table 2 Summary of 6BA PGR treatment impacts on early above ground across experiments 1, 2, 4, 5, 8, 9 and 10.**

Trt. description	Early vegetative	Early squaring	Early flowering	Mid flowering
Seed prime, 0.01mg.L <sup>-1</sup> , 20hr	<b>Height, total dry weight;</b> no treatment differences (13 DAP, exp. 1).			
Seed prime, 850mg.L <sup>-1</sup> , 10s				
Seed coat, 9.97mg cwt				
Seed coat, 49.99mg cwt				
Foliar, 150mg.L <sup>-1</sup> 4L once	<b>Total dry weight;</b> decreased in 150, 67 and 25mg.L <sup>-1</sup> treatments (24 DAP in exp. 2).	<b>Total dry weight, LAI, Fr:Tot, Total sites, retention, Squ no, avg. Squ wt;</b> no treatment differences (44 DAP, exp. 9).	<b>Total dry weight, LAI, Fr:Tot, Squ, GB, OB no. and avg. weight;</b> no treatment differences (66 DAP, exp. 9).	<b>Total dry weight, LAI, Fr:Tot, Squ, GB, OB no. and avg. weight;</b> no treatment differences (124 DAP, exp. 4; 113 DAP, exp. 5).
Foliar, 67mg.L <sup>-1</sup> 4L once				
Foliar, 33mg.L <sup>-1</sup> 4L once				
Foliar, 25mg.L <sup>-1</sup> 4L once				
Foliar, 25mg.L <sup>-1</sup> 4L 3x10 d		<b>Total dry weight, LAI, Fr:Tot;</b> no treatment differences (55 DAP, exp. 8; 44 DAP, exp. 9). <b>Total sites, avg. Squ wt;</b> no treatment differences (55 DAP, exp. 8; 44 DAP, exp. 9).	<b>Total dry weight, LAI;</b> no treatment differences (81 DAP, exp. 8; 66 DAP, exp 9, 10). <b>Fr:Tot, Squ, GB no. and avg. wt;</b> no treatment differences at 81 DAP in exp. 8, 66 DAP in exp 9 and 10.	<b>Total dry weight, LAI;</b> no treatment differences (124 DAP, exp. 4; 113 DAP, exp. 5; 116 DAP, exp 8). <b>Fr:Tot, Squ, GB, OB no. and avg. wt;</b> no treatment differences (124 DAP, exp. 4; 113 DAP, exp. 5; 116 DAP, exp 8).
Foliar, 100mg.L <sup>-1</sup> 4L 3x10 d		<b>Squ no., retention;</b> decreased (55 DAP, exp 8), no difference (44 DAP, exp 9).	<b>Fr:Tot, Squ no.;</b> less than the control (81 DAP, exp 8). <b>GB no., Squ and GB avg. wt;</b> no treatment differences (81 DAP, exp 8).	<b>Total dry weight, LAI;</b> no treatment differences (116 DAP, exp 8). <b>Fr:Tot, GB avg. wt;</b> less than the control (116 DAP, exp 8). <b>Squ no., GB no, Ob no, Squ and OB avg. wt;</b> no treatment differences (116 DAP, exp 8).

**Table 3 Summary of 6BA PGR treatment impacts on maturity, yield components, lint yield and quality across experiments 4, 5 and 8.**

<b>Trt. description</b>	<b>Maturity</b>	<b>Yield components</b>	<b>Lint yield and turnout</b>	<b>Quality outcomes</b>
Foliar, 25mg.L <sup>-1</sup> 4L once		<b>Final open boll no. and OB avg. weight;</b> no treatment differences (124 DAP, exp. 4; 113 DAP, exp. 5).	<b>Lint yield and seed cotton turnout;</b> no treatment differences (124 DAP, exp. 4; 113 DAP, exp. 5).	<b>Lint length, strength, micronaire;</b> no treatment differences (124 DAP, exp. 4; 113 DAP, exp. 5).
Foliar, 25mg.L <sup>-1</sup> 4L 3x10d	<b>Progression to 60% OB;</b> no difference from control (exp 8).	<b>Final open boll no. and OB avg. weight;</b> no treatment differences (124 DAP, exp. 4; 113 DAP, exp. 5, exp 8).	<b>Lint yield and seed cotton turnout;</b> no treatment differences (124 DAP, exp. 4; 113 DAP, exp. 5, exp 8).	<b>Lint length, strength, micronaire;</b> no treatment differences (124 DAP, exp. 4; 113 DAP, exp. 5; exp 8).
Foliar, 100mg.L <sup>-1</sup> 4L 3x10d	<b>Progression to 60% OB;</b> delayed compared to control (exp 8).	<b>Final open boll no. and OB avg. weight;</b> no treatment differences (exp. 8)	<b>Lint yield</b> no treatment differences from the control (exp 8). <b>Seed cotton turnout;</b> higher than the control (exp. 8).	<b>Lint length, strength, micronaire;</b> no treatment differences from the control (exp. 8).

## Appendix 5 Cotton plant agronomic response to bioactive Gibberellin PGRs

**Table 1 GA<sub>3</sub> impacts in root biomass accumulation and access to soil water reserves across experiments 1, 2, 4, 5, 8, 9, 10 and 11; asterix (\*) indicating treatment differences at the P<0.05 level, n.s. indicating no significant treatment difference at the P<0.05 level .**

Trt. description	Early vegetative	Early flowering	Mid flowering	Post defoliation / harvest
Seed prime, 200mg.L <sup>-1</sup> , 20hr	<i>Exp. 1</i> Root length: ↑ 5.2% n.s. Root dry weight: ↓ 46.3% n.s. Rt:Sht ratio: ↓ 52.58% *			
Seed prime, 500mg.L <sup>-1</sup> , 10sec	<i>Exp. 1</i> Root length: ↓ 30.3% n.s. Root dry weight: ↓ 58.9% * Rt:Sht ratio: ↓ 50.8% *			
Foliar, 80 mg.L <sup>-1</sup> , 2L, once	<i>Exp. 2</i> Root length: ↑ 4.7% n.s. Root dry weight: ↑ 8.7% n.s. Rt:Sht ratio: ↑ 7.5% n.s.			
Foliar, 160 mg.L <sup>-1</sup> , 2L, once	<i>Exp. 2</i> Root length: ↑ 0.6% n.s. Root dry weight: ↑ 9.43% n.s. Rt:Sht ratio: ↓ 12.7% n.s.			
Foliar, 80 mg.L <sup>-1</sup> , 2L, 3x 10d		<i>Exp. 8</i> Vol. soil moisture, cumulative. 0.75m PL: ↑ 5.7% n.s. 0.75m SK: ↑ 5.6% n.s. 1.5m PL: ↑ 0.7% n.s. 1.5m SK: ↑ 4.7% n.s.	<i>Exp. 8</i> Vol. soil moisture, cumulative. 0.75m PL: ↑ 6.9% n.s. 0.75m SK: ↑ 3.2% n.s. 1.5m PL: ↑ 2.4% n.s. 1.5m SK: ↑ 3.0% n.s.	<i>Exp. 9</i> EC <sub>t</sub> (1.5m) PL: ↓ 1.6% n.s. EC <sub>t</sub> (1.5m) SK: ↑ 2.1% n.s.

**Table 2 GA<sub>3</sub> impacts on early above ground biomass accumulation in exp. 1, 2, 4, 5, 8, 9, 10 and 11; asterix (\*) indicating treatment differences at the P<0.05 level, n.s. indicating no significant treatment difference at the P<0.05 level.**

Trt. descr.	Early vegetative	Early squaring	Early flowering	Mid flowering				
Seed prime, 200mg.L <sup>-1</sup> , 20hr	<i>Exp. 1</i> <b>Height:</b> ↑ 83.9% * <b>Tot dry wt:</b> ↓ 16.8% n.s.							
Seed prime, 500mg.L <sup>-1</sup> , 10sec	<i>Exp. 1</i> <b>Height:</b> ↑ 38.4% n.s. <b>Tot dry wt:</b> ↓ 38.8% n.s.							
Foliar, 80 mg.L <sup>-1</sup> , 2L, once	<i>Exp. 2</i> <b>Height:</b> ↑ 40.8% * <b>Tot dry wt:</b> ↑ 5.8% n.s.							
Foliar, 160 mg.L <sup>-1</sup> , 2L, once	<i>Exp. 2</i> <b>Height:</b> ↑ 53.0% * <b>Tot dry wt:</b> ↑ 2.2% n.s.							
Foliar, 80 mg.L <sup>-1</sup> , 2L, 3x 10d		<i>Exp. 8</i> <b>Tot dry wt:</b> ↑ 3.1% n.s. <b>LAI:</b> ↓ 1.1% n.s. <b>Fr:Tot:</b> ↓ 48.3% * <b>Squ No:</b> ↓ 64.8% * <b>Avg squ wt:</b> ↑ 42.0% n.s. <b>Total sites:</b> ↑ 93.1% * <b>Retention:</b> ↓ 82.21% *	<b>Tot dry wt:</b> <b>LAI:</b> <b>Fr:Tot:</b> <b>Squ No:</b> <b>Avg squ wt:</b> <b>GB No:</b> <b>Avg GB wt:</b>	<i>Exp. 8</i> ↓ 13.4% n.s. ↓ 13.2% n.s. ↓ 31.2% n.s. ↑ 21.6% n.s. ↓ 27.8% n.s. ↓ 12.3% n.s. ↓ 51.6% n.s.	<i>Exp. 9</i> - 0% n.s. ↓ 7.5% n.s. ↓ 28.4% n.s. ↓ 21.2% n.s. ↓ 52.2% * ↑ 26.9% n.s. ↓ 25.4% n.s.	<b>Tot dry wt:</b> <b>LAI:</b> <b>Fr:Tot:</b> <b>Squ No:</b> <b>Avg squ wt:</b> <b>GB No:</b> <b>Avg GB wt:</b> <b>OB No:</b> <b>Avg OB wt:</b>	<i>Exp. 4</i> ↑ 11.2% n.s. ↑ 22.2% n.s. ↓ 7.2% n.s. ↑ 24.2% n.s. ↑ 10.0% n.s. ↓ 27.2% n.s. ↑ 28.9% n.s. - 0% n.s. - 0% n.s.	<i>Exp. 8</i> ↓ 8.0% n.s. ↑ 29.5% * ↓ 50.9% * ↑ 860% * ↓ 24.9% n.s. ↑ 46.1% n.s. ↓ 71.7% * - 0% n.s. - 0% n.s.

**Appendix 6 Calculation methodology of day degrees delay in progression to 50% plant population first square, attributable to Trinexapac ethyl (TEP) PGR foliar treatment scenario in experiment 3 and utilised in OzCot crop simulation modelling to determine any associated lint yield and water use efficiency benefits**

1. 2016 glasshouse experiment 3, TEP treatment showed a 3.1 calendar day delay in progression to FSq.
2. Glasshouse set points at 28°C / 20°C (max / min) across 59 days of experiment (30/9/16 to 28/11/16).
3. Day degree accumulation at squaring dates: 25.5 DAP to 28.6 DAP  
(25<sup>th</sup> Oct, 26<sup>th</sup> Oct, 27<sup>th</sup> Oct, 28<sup>th</sup> Oct, 29<sup>th</sup> Oct)
4. Calculation of accumulated day degrees difference (Table 1).

**Table 1 daily recorded max and min temperatures and difference in day degree accumulation between Trt. 1 (Control) FSqu (25/10/16) and Trt. 8 (TEP) FSqu (28/10/16), in Glasshouse 3 (ACRI, Narrabri NSW) during squaring in experiment 3.**

	Max	Min	DDAccum.	
25/10	34.15	21.1	7.8125	(50%)
26/10	34.27	21.1	15.685	
27/10	34.06	21.42	15.74	
28/10	34.15	21.1	9.375	(60%)
			<b>48.6125DD</b>	<b>(50DD)</b>

**Appendix 7 Rain grown cotton gross margins; Table 1 being standard (Powell and Welsh, 2019), Table 2 being amended to include triplicate foliar application of Trinexapac ethyl at 7-day intervals**

**Table 1 Standard Australian rain grown cotton gross margin (Powell and Welsh, 2019).**

Dryland cotton, Bollgard 3 RRF, 2019-20										Standard (Powell and Welsh, 2019)								
Variable costs by operation	Machinery	Inputs				Configuration												
		\$ ha <sup>-1</sup>	Rate ha <sup>-1</sup>	Unit	Banding (%)	Cost (\$ unit <sup>-1</sup> )	Cost solid plant (\$ ha <sup>-1</sup> )	Cost single skip (\$ ha <sup>-1</sup> )	Cost double skip (\$ ha <sup>-1</sup> )	Solid plant (\$ ha <sup>-1</sup> )	Single skip (\$ ha <sup>-1</sup> )	Double skip (\$ ha <sup>-1</sup> )						
<b>Prior fallow management</b>																		
Nov Fallow spray: Glyphosate (450g L-1)	Self propelled	\$1.50	2	L	100	\$5.00	\$10.00			\$11.50	\$11.50	\$11.50						
Nov Fallow spray: 2,4-D amine (625g L-1)	with above		0.4	L	100	\$6.00	\$2.40			\$2.40	\$2.40	\$2.40						
Jan Fallow spray: Glyphosate (450g L-1)	Self propelled	\$1.50	2	L	100	\$5.00	\$10.00			\$11.50	\$11.50	\$11.50						
Jan Fallow spray: Fluoxypyr (333g L-1)	with above		0.4	L	100	\$29.00	\$11.60			\$11.60	\$11.60	\$11.60						
Mar Fallow spray: Glyphosate (450g L-1)	Self propelled	\$1.50	2	L	100	\$5.00	\$10.00			\$11.50	\$11.50	\$11.50						
Mar Fallow spray: Pendimethalin (440g L-1)	with above		2.25	L	100	\$11.00	\$24.75			\$24.75	\$24.75	\$24.75						
<b>Nutrition</b>																		
Aug Fertiliser: Urea (32kg N/ha, drilled, once, pre-plant adj. to plant line)	295 eng/HP	\$20.00	70	kg	#	\$0.62	\$43.40	\$28.64	\$21.70	\$63.40	\$48.64	\$41.70						
<b>Planting and in-crop farming</b>																		
Oct Planting: precision planter	295 eng/HP	\$11.50								\$11.50	\$11.50	\$11.50						
Oct Seed: Roundup Ready Flex® Bollgard3®	with above		9	kg	#	\$8.96	\$80.64	\$53.22	\$40.32	\$80.64	\$53.22	\$40.32						
<b>Crop protection, application and licence fee</b>																		
Dec Insecticide: Sulfoxalox (500g kg-1), target: mites	Self propelled	\$1.50	0.14	kg	30	\$303.00	\$12.73	\$8.40	\$6.36	\$14.23	\$9.90	\$7.86						
Feb Insecticide: Diafenthiuron (500g L-1), target: silver leaf whitefly, aphids, mites	Self propelled	\$1.50	0.6	L	100	\$63.00	\$37.80			\$39.30	\$39.30	\$39.30						
Oct Herbicide: Pendimethalin (45g L-1)	Self propelled	\$1.50	2.2	kg	100	\$14.00	\$30.80			\$32.30	\$32.30	\$32.30						
Nov Herbicide: Roundup Ready® Plantshield® (690g kg-1 Glyphosate)	Self propelled	\$1.50	1.2	kg	100	\$8.00	\$9.60			\$11.10	\$11.10	\$11.10						
Dec Herbicide: S-metolachlor (960g L-1)	Self propelled	\$1.50	1	L	100	\$11.00	\$11.00			\$12.50	\$12.50	\$12.50						
Jan Herbicide: Roundup Ready® Plantshield® (690g kg-1 Glyphosate)	Self propelled	\$1.50	1.2	kg	100	\$8.00	\$9.60			\$11.10	\$11.10	\$11.10						
Mar Licence: Bollgard 3® stacked RRF licence fee, end point novelty			62.5	227kg lint bale	#					\$168.00	\$168.00	\$168.00						
Jun Other: Agronomic consultant	Contractor	\$35.00								\$35.00	\$35.00	\$35.00						
Jun Refuse: conventional cotton 5% <sup>40</sup>										\$28.42	\$20.26	\$14.21						
<b>Defoliation</b>																		
Mar Defoliation: Thidiazuron (500g L-1)	Self propelled	\$3.50	0.15	L	##	\$64.00	\$9.60	\$9.60	\$6.72	\$13.10	\$13.10	\$10.22						
Mar Defoliation: Ethephon (720g L-1)	with above		0.5	L	##	\$7.00	\$3.50	\$3.50	\$2.45	\$3.50	\$3.50	\$2.45						
Mar Defoliation: Crop oil	with above		1	L	##	\$6.00	\$6.00	\$6.00	\$4.20	\$6.00	\$6.00	\$4.20						
Mar Defoliation: Thidiazuron (500g L-1)	Self propelled	\$3.50	0.15	L	##	\$64.00	\$9.60	\$9.60	\$6.72	\$13.10	\$13.10	\$10.22						
Mar Defoliation: Ethephon (720g L-1)	with above		2.5	L	##	\$7.00	\$17.50	\$17.50	\$12.25	\$17.50	\$17.50	\$12.25						
Mar Defoliation: Crop oil	with above		1	L	##	\$6.00	\$6.00	\$6.00	\$4.20	\$6.00	\$6.00	\$4.20						
Apr Defoliation: Paraquat (360g L-1)	Self propelled	\$3.50	2	L	##	\$6.00	\$12.00	\$12.00	\$8.40	\$15.50	\$15.50	\$11.90						
Defoliation: Crop oil	with above		1	L	##	\$6.00	\$6.00	\$6.00	\$4.20	\$6.00	\$6.00	\$4.20						
<b>Picking, cartage and ginning</b>																		
Apr Stripping: contract, round baler incl. end of field stacking	CS690	\$200.00			100					\$200.00	\$200.00	\$200.00						
Apr Stripping: plus fuel			21	L	100	\$0.97	\$20.33	\$20.33	\$20.33	\$20.33	\$20.33	\$20.33						
Apr Stripping: plus wrap			10.35	227kg lint bale						\$33.12	\$33.12	\$33.12						
May Cartage: lift and stack			1.65	227kg lint bale						\$5.28	\$5.28	\$5.28						
May Cartage: freight			12.47	227kg lint bale						\$39.90	\$39.90	\$39.90						
May Ginning: fibre processing			70	227kg lint bale						\$224.00	\$224.00	\$224.00						
June Levies: Research levy and industry lobby group (Cotton Australia) levy			3.75	227kg lint bale						\$12.00	\$12.00	\$12.00						
<b>Farming: post harvest</b>																		
Jun Farming: Crop mulching (refer BT licence pupae busting requirements)	Contractor	\$35.00								\$35.00	\$35.00	\$35.00						
Jun Farming: Chisel plough plant line (refer BT licence pupae busting requirements)	295 eng/HP	\$13.50								\$13.50	\$13.50	\$13.50						
<b>Other</b>																		
Nov Crop insurance										\$128.00	\$72.00	\$64.00						
June Cotton: Lint quality discount (est)			25	227kg lint bale						\$80.00	\$80.00	\$80.00						
										<b>TOTAL VARIABLE COSTS \$/ha:</b>	<b>\$1,452.57</b>	<b>\$1,342.01</b>	<b>\$1,284.92</b>					
<b>Income</b>																		
				Rate ha <sup>-1</sup>	Unit		Income (\$ unit <sup>-1</sup> )				Configuration							
June Cotton: Lint			3.2	227kg lint bale			\$521.00				Solid plant (\$ ha <sup>-1</sup> )	Single skip (\$ ha <sup>-1</sup> )						
June Cotton: Seed			3.2	227kg lint bale			\$100.00				Double skip (\$ ha <sup>-1</sup> )							
										<b>TOTAL GROSS INCOME \$/ha:</b>	<b>\$1,987.20</b>	<b>\$1,987.20</b>	<b>\$1,987.20</b>					
										<b>GROSS MARGIN \$/ha:</b>	<b>\$534.63</b>	<b>\$645.19</b>	<b>\$702.29</b>					
<b>Notes:</b>																		
Two year cycle as per financial year: 1st July - 30th June																		
* refer: Dryland conventional cotton, refugia for Bollgard 3®, 2019-20 gross margin																		
* \$44/round module																		
* \$57/round module, 12 round modules/truck																		
* \$33/round module, 30km to gin																		
* discretionary amount																		
* as gin yard																		
<table border="1"> <tr> <td>100</td> <td>66</td> <td>50</td> </tr> <tr> <td>100</td> <td>100</td> <td>70</td> </tr> </table>													100	66	50	100	100	70
100	66	50																
100	100	70																
yield dependent variable																		

**Table 2 Standard Australian rain grown cotton gross margin (Powell and Welsh, 2019), amended to include triplicate foliar application of Trinexapac ethyl at 7-day intervals; (■) additional PGR applications.**

Dyland cotton, Bollgard 3 RRF, 2019-20										Amended for TEP application scenario from Standard (Powell and Welsh, 2019)		
Variable costs by operation	Machinery	Inputs			Banding (%)	Cost (\$/unit <sup>2</sup> )	Cost solid plant (\$/ha <sup>1</sup> )	Cost single skip (\$/ha <sup>2</sup> )	Cost double skip (\$/ha <sup>2</sup> )	Configuration		
		\$/ha <sup>1</sup>	Rate/ha <sup>1</sup>	Unit						Solid plant (\$/ha <sup>1</sup> )	Single skip (\$/ha <sup>2</sup> )	Double skip (\$/ha <sup>2</sup> )
<b>Prior fallow management</b>												
Nov Fallow spray: Glyphosate (450g/L-1)	Self propelled	\$1.50	2	L	100	\$5.00	\$10.00			\$11.50	\$11.50	\$11.50
Nov Fallow spray: 2,4-D amine (625g/L-1)	with above		0.4	L	100	\$6.00	\$2.40			\$2.40	\$2.40	\$2.40
Jan Fallow spray: Glyphosate (450g/L-1)	Self propelled	\$1.50	2	L	100	\$5.00	\$10.00			\$11.50	\$11.50	\$11.50
Jan Fallow spray: Fluroxypyr (333g/L-1)	with above		0.4	L	100	\$29.00	\$11.60			\$11.60	\$11.60	\$11.60
Mar Fallow spray: Glyphosate (450g/L-1)	Self propelled	\$1.50	2	L	100	\$5.00	\$10.00			\$11.50	\$11.50	\$11.50
Mar Fallow spray: Pendimethalin (440g/L-1)	with above		2.25	L	100	\$11.00	\$24.75			\$24.75	\$24.75	\$24.75
<b>Nutrition</b>												
Aug Fertiliser: Urea (32kg N/ha, drilled, once, pre-plant adj. to plant line)	295 eng/HP	\$20.00	70	kg	#	\$0.62	\$43.40	\$28.64	\$21.70	\$63.40	\$48.64	\$41.70
Oct Planting: precision planter	295 eng/HP	\$11.50								\$11.50	\$11.50	\$11.50
Oct Seed: Roundup Ready Flex® Bollgard3®	with above		9	kg	#	\$8.96	\$80.64	\$53.22	\$40.32	\$80.64	\$53.22	\$40.32
<b>Crop protection, application and licence fee</b>												
Dec Insecticide: Sulfoxaflor (500g/kg-1), target: mirids	Self propelled	\$1.50	0.14	kg	30	\$303.00	\$12.73	\$8.40	\$6.36	\$14.23	\$9.90	\$7.86
Feb Insecticide: Diafenthiuron (500g/L-1), target: silver leaf whitefly, aphids, mites	Self propelled	\$1.50	0.6	L	100	\$63.00	\$37.80			\$39.30	\$39.30	\$39.30
Oct Herbicide: Pendimethalin (45g/L-1)	Self propelled	\$1.50	2.2	kg	100	\$14.00	\$30.80			\$32.30	\$32.30	\$32.30
Nov Herbicide: Roundup Ready® Plantshield® (690g/kg-1 Glyphosate)	Self propelled	\$1.50	1.2	kg	100	\$8.00	\$9.60			\$11.10	\$11.10	\$11.10
Nov PGR: Trinexapac-ethyl (250g/L-1)	with above		0.8	L	100	\$70.00	\$56.00			\$56.00	\$56.00	\$56.00
Nov PGR: Trinexapac-ethyl (250g/L-1)	Self propelled	\$1.50	0.8	L	100	\$70.00	\$56.00			\$57.50	\$57.50	\$57.50
Dec Herbicide: S-metolachlor (960g/L-1)	Self propelled	\$1.50	1	L	100	\$11.00	\$11.00			\$12.50	\$12.50	\$12.50
Dec PGR: Trinexapac-ethyl (250g/L-1)	with above		0.8	L	100	\$70.00	\$56.00			\$56.00	\$56.00	\$56.00
Jan Herbicide: Roundup Ready® Plantshield® (690g/kg-1 Glyphosate)	Self propelled	\$1.50	1.2	kg	100	\$8.00	\$9.60			\$11.10	\$11.10	\$11.10
Mar Licence: Bollgard 3® stacked RRF licence fee, end point royalty			52.5	227kg lint bale	#					\$168.00	\$168.00	\$168.00
Jun Other: Agronomic consultant	Contractor	\$35.00								\$35.00	\$35.00	\$35.00
Jun Refuge: conventional cotton 5% <sup>40</sup>										\$28.42	\$29.36	\$14.21
<b>Defoliation</b>												
Mar Defoliation: Thidiazuron (500g/L-1)	Self propelled	\$3.50	0.15	L	##	\$64.00	\$9.60	\$9.60	\$6.72	\$13.10	\$13.10	\$10.22
Mar Defoliation: Ethephon (720g/L-1)	with above		0.5	L	##	\$7.00	\$3.50	\$3.50	\$2.45	\$3.50	\$3.50	\$2.45
Mar Defoliation: Crop oil	with above		1	L	##	\$6.00	\$6.00	\$6.00	\$4.20	\$6.00	\$6.00	\$4.20
Mar Defoliation: Thidiazuron (500g/L-1)	Self propelled	\$3.50	0.15	L	##	\$64.00	\$9.60	\$9.60	\$6.72	\$13.10	\$13.10	\$10.22
Mar Defoliation: Ethephon (720g/L-1)	with above		2.5	L	##	\$7.00	\$17.50	\$17.50	\$12.25	\$17.50	\$17.50	\$12.25
Mar Defoliation: Crop oil	with above		1	L	##	\$6.00	\$6.00	\$6.00	\$4.20	\$6.00	\$6.00	\$4.20
Apr Defoliation: Paraquat (360g/L-1)	Self propelled	\$3.50	2	L	##	\$6.00	\$12.00	\$12.00	\$8.40	\$15.50	\$15.50	\$11.90
Apr Defoliation: Crop oil	with above		1	L	##	\$6.00	\$6.00	\$6.00	\$4.20	\$6.00	\$6.00	\$4.20
<b>Picking, cartage and ginning</b>												
Apr Stripping: contract, round baler incl. end of field stacking	CS690	\$200.00			100					\$200.00	\$200.00	\$200.00
Apr Stripping: plus fuel			21	L	100	\$0.97	\$20.33	\$20.33	\$20.33	\$20.33	\$20.33	\$20.33
Apr Stripping: plus wrap			10.35	227kg lint bale						\$33.12	\$33.12	\$33.12
May Cartage: lift and stack			1.65	227kg lint bale						\$5.28	\$5.28	\$5.28
May Cartage: freight			12.47	227kg lint bale						\$39.90	\$39.90	\$39.90
May Ginning: fibre processing			70	227kg lint bale						\$224.00	\$224.00	\$224.00
June Levies: Research levy and industry lobby group (Cotton Australia) levy			3.75	227kg lint bale						\$12.00	\$12.00	\$12.00
<b>Farming: post harvest</b>												
Jun Farming: Crop mulching (refer BT licence pupae busting requirements)	Contractor	\$35.00								\$35.00	\$35.00	\$35.00
Jun Farming: Chisel plough plant line (refer BT licence pupae busting requirements)	295 eng/HP	\$13.50								\$13.50	\$13.50	\$13.50
<b>Other</b>												
Nov Crop insurance										\$128.00	\$72.00	\$64.00
June Cotton: Lint quality discount (est)			25	227kg lint bale						\$80.00	\$80.00	\$80.00
										<b>TOTAL VARIABLE COSTS \$/ha: \$1,622.07</b>		
										<b>\$1,511.51</b>		
										<b>\$1,454.42</b>		
<b>Income</b>												
										Configuration		
										Solid plant (\$/ha <sup>1</sup> )	Single skip (\$/ha <sup>2</sup> )	Double skip (\$/ha <sup>2</sup> )
June Cotton: Lint			3.2	227kg lint bale		\$521.00				\$1,667.20	\$1,667.20	\$1,667.20
June Cotton: Seed			3.2	227kg lint bale		\$100.00				\$320.00	\$320.00	\$320.00
										<b>TOTAL GROSS INCOME \$/ha: \$1,987.20</b>		
										<b>\$1,987.20</b>		
										<b>\$1,987.20</b>		
										<b>GROSS MARGIN \$/ha: \$365.13</b>		
										<b>\$475.69</b>		
										<b>\$532.79</b>		
<b>Notes:</b>												
Two year cycle as per financial year: 1st July - 30th June												
*refer: Dryland conventional cotton, refugia for Bollgard 3®, 2019-20 gross margin												
**\$44/round module												
**\$37/round module, 12 round modules/track												
**\$53/round module, 50km to gin												
***discretionary amount												
***at gin yard												
***yield dependent variable												

**Appendix 8 Calculation methodology of Trinexapac ethyl pricing as included in amended PGR gross margin**

<i>Moddus Evo (Syngenta)</i>	\$1,400	20	L	(+GST)	<i>Delta Ag, Narrabri (23/1/2020)</i>	
	\$350	5	L	(+GST)		
	250	g.L <sup>-1</sup>	(active)			
	250	g	1000	mL		
	1	g	4	mL		
	1000	mg	4	mL		
	1	mg	0.004	mL		
<b>Rate:</b>	2000	mg.L <sup>-1</sup>	in	100	L	spray soln.
	8	mL.prod	in	1	L	spray soln.
	800	mL.prod	in	100	L	spray soln.
	800	mL.ha <sup>-1</sup>				
<i>(check:)</i>	200000	mg.100L <sup>-1</sup>				
	2000	l	L			
<b>Cost:</b>	\$1,400	20	L			
	\$70	1	L			
	\$70	1000	mL			
	\$0.07	1	mL			
	\$56.00	ha	(per application)			