FINAL REPORT

CRDC ID: CSP2001

Project Title: Modern Systems Agronomy for Resilient Cotton Production

Confidential or for public release? For Public Release

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Submission checklist.

Please ensure all documentation has been completed and included with this final report:

☐ Final report template (this document)
☐ Final Technical Report
☐ Final Schedule 2: IP register
☐ Final Schedule 3: Acknowledgment
☐ Final financial report
☐ PDF of all journal articles (for CRDC's records)

Signature of Research Provider Representative:

Date submitted: 28/5/2026

Part 2 - Monitoring & Evaluation

This data is for CRDC's internal M&E requirements. Please complete all fields and add additional rows into each table if required.

Achievement against milestones in the Full Research Proposal

Milestone	Achieved/ Partially Achieved/ Not Achieved	Explanation
1.1 Identify novel agronomic practices that require detailed research validation.	*Achieved*	Several industry consultations have occurred to understand and identify opportunities for the use of novel plant growth hormones in dryland and water limited cotton systems. Consultations suggest that there are several novel plant growth hormones that may be useful in cotton production systems, but they are not currently utilised. It was identified that there was an opportunity to further understand how plant growth regulators may be utilised in Australian dryland and water limited cotton systems.
1.2 Understand industry use of early-season mepiquat chloride in relation to current industry recommendations.	*Achieved*	As part of the CSP2001 project, we have been engaging with growers, consultants and CottonInfo representatives throughout cotton growing regions. An online survey and discussions with young agronomists were initiated at the Narrabri CCA meeting in July 2023. Data suggested that consultants were most likely to rely on weekly height and node measurements, and measurements of a specific internode for making early-season mepiquat chloride (MC) decisions. Further discussions with consultants and growers throughout the 2023/24 cotton season highlighted variability in MC strategies across regions and at the farm scale. There was a keen interest for additional research of early-season MC applications across key cotton regions.
2.1 Extend and validate hormone/PGR approaches deemed as potential opportunities from recently completed thesis research to broaden industry application.	*Achieved*	Four field experiments were conducted to investigate if Trinexapac-ethyl (TEP) can delay flowering in dryland cotton systems. Our data showed that multiple applications of TEP could delay flowering by up to 20 days, and delay maturity up

		to 27 days. In comparison, early season application of MC was ineffective as a method of delaying development, whereas pruning of the plant at flowering had the potential to cause longer delays in maturity, up to 23 days later than the TEP treatments. The application of TEP had the potential to decrease yield, particularly if a cold finish to the season resulted in the plants unable to mature fruit. The seasons that TEP was tested were atypical, and varied greatly from the hot, dry seasons that Australian dryland cotton growers often contend with. Therefore, further research should explore a broader range of temperatures and environmental conditions through both experimental research and modelling to better understand decision-support for applying TEP as a risk management strategy in cotton systems, in conjunction with further consultation with growers and consultants to determine viable agronomic practice.
2.2 Develop broader understanding and impact of mechanisms that drive the change between vegetative and reproductive growth and establish whether PGRs can play a role in managing this.	*Achieved*	To develop broader understanding and impact of mechanisms that drive the change between vegetative and reproductive growth and establish whether Plant Growth Regulators (PGRs) can play a role in managing this, we investigated three research questions: (1) Do PGR's either promoting or inhibiting gibberellins affect the switch from vegetative to reproductive growth of cotton; (2) Do gibberellic acids (GA_3 or $GA_{4,7}$) stimulate floral initiation across different planting times in dryland cotton systems; and (3) which TEP application rate and frequency are optimal to manipulate phase transition of cotton. Field and glasshouse experiments were conducted from the 2020-21 to 2022-23 cotton seasons. Data showed significant delays in growth and development of cotton when plants were applied with early applications of gibberellin or gibberellin inhibiting PGRs, suggesting that gibberellic pathways were altered, however, further refinement of timing, rates and other environmental interactions is required. Improving this understanding may enable growers to have additional agronomic strategies to manipulate phase transitions in cotton crops to target optimal environmental conditions in a season.

2.3 Develop an improved understanding of the concept of 'crop determinacy' and assess its role in providing resilience in crop production in stressed environments.	Achieved	To develop an improved understanding of the concept of "crop determinacy" and assess its role in proving resilience in crop production in stressed environments, three field experiments were conducted to investigate how PGRs affect crop determinacy compared with variety. Reliable and consistent manipulation of crop determinacy using Aminoethoxyvinylglycine (AVG), GA_3 or $GA_{4,7}$ was not observed throughout these three field experiments conducted. However, there are some responses that are interesting and have potential application in cotton systems, although further research is required.
2.4 Establish whether there are alternative hormone/PGR approaches that can enhance cotton growth in stressed environments.	Achieved	To establish whether there are alternative hormones/PGR approaches that can enhance cotton growth in stressed environments, glasshouse and field experiments were conducted to (1) to investigate the effect that two rates of Abscisic Acid (ABA) have on the physiology of cotton in well-watered and water deficit conditions; and (2) understand the effects of ABA on growth, water use and yield of cotton in the field. Data showed significant reductions in stomatal conductance of cotton applied with ABA for approximately two to three days, with higher application rates having a marginally longer lasting effect. The short-term effects of ABA on cotton physiology likely contributed to no significant changes in plant water use, fruit retention or yield of plants grown in field conditions. Thus, ABA was not shown to enhance cotton growth in stressed environments.
2.5 Identify potential new hormone/PGR formulations that could assist cotton agronomy.	Achieved	Several potential new hormone/PGR formulations that could potentially assist cotton agronomy were broadly screened for yield improvements across field experiments across multiple years. PGR's tested included Abscisic acid (ABA), Forchlorfenuron (CPPU), Gibberellin $A_{4,7}$ and Benzyladenine ($GA_{4,7}$ + BA) and Glycine Betaine (GB). Our data showed inconsistent yield responses for the respective application timings and rates for products tested. These results may have been partially due to mild environmental conditions and a lack of hot, dry temperatures to fully test yield responses across a range of climatic conditions.

4.2 Develop oversight and leadership on industry related issues pertaining to cotton agronomy in a systems context.	Achieved	The Australian cotton production manual was reviewed in February 2022. The manual is very comprehensive, and no changes were suggested for the 2022 cotton season. As more data becomes available on the implications of early-season MC use across regions with the new proposed project, there may be an opportunity to update the Australian cotton production manual with additional information and case studies.
4.3 Communicate results of research to industry.	Achieved	Results have been presented at grower field days, the CCA seminar, the Australian Cotton Conference, and in the CRDC Spotlight magazine. Outcomes of these experiments will continue to be presented as per the communication plan.
4.5 Engage with growers, consultants and CottonInfo representatives to conduct grower/consultant-led research to understand the effects of various alternative early-season mepiquat chloride application strategies on cotton growth and yield	Achieved	Grower/consultant-led experiments were conducted at Cecil Plains, Aberdeen, Wee Waa and Griffith to better understand the effects of alternative early-season application strategies on cotton growth and yield. Although there were no significant differences in yield, results showed that early-season MC applications may benefit relative yield, however, there may be some losses in relative yield with very high rates of MC. Preliminary results have been presented at four field days during the 2023/24 cotton season, at the CCA seminar in conjunction with consultants across the regions, and at the Australian Cotton Conference, August 2024.
4.6 Engage with growers and consultants to conduct a field demonstration of selected novel PGR approaches (based on CSP2001 data and grower interest) that can enhance cotton growth. This will require consultation with growers and technical providers.	Achieved	A grower and consultant-led field demonstration testing the effects of a novel PGR for cotton, Trinexapac-ethyl (TEP) on the growth and yield of cotton was conducted during the 2023/24 cotton season at Terry Hie Hie. Our results showed that the application of TEP consistently delayed time to first flower, with some delays also seen in time to maturity (60% open boll). Our data also showed that yields of cotton applied with TEP were relatively greater compared with unsprayed control cotton, with a significant increase of more than 1 bale/ha in some treatments. Preliminary results have been presented at a field day during the 2023/24 cotton season and in the Winter 2024 edition of the CRDC Spotlight magazine. Results have also

		been presented at the Australian Cotton Conference 2024.
5.1 Submit milestone/progress reports to CRDC to provide an update on project activities	Achieved	Six-monthly progress reports have been submitted to CRDC to provide an update on project activities and preliminary experimental results.
5.2 Deliver final project report documenting project outcomes	Achieved	A final project report documenting project outcomes has been submitted to CRDC in October 2024.
5.3 Evaluation and reflection report on grower led trials	Achieved	Five grower/consultant led trials (four early season MC and one novel PGR) were conducted during the 2023/24 cotton season. These trials provided an opportunity for a greater understanding of current practice and potential PGR use across key cotton regions in irrigated, semi-irrigated, and dryland systems. Engagement with growers and consultants facilitated industry collaborations, and opportunities for field demonstrations expanded outreach of the research. Overall, there is a growing interest in early-season MC application that will require further research and dissemination to ensure that plant growth, and thus yields, are not limited. Growers and consultants have also expressed an interest in better understanding novel PGRs such as TEP for altering crop development and enabling alternative tools to minimise production risk for dryland systems.

Outputs produced *(Please refer to examples document to assist in completing this section).*

Output	Description
CRDC Progress Reports	Progress reports have been submitted to CRDC throughout the course of the project providing preliminary experimental results and project updates
Presentations	Presentations on PGR research included: CSIRO cotton seminars Australian Cotton Conference 2022, 2024 Australian Cotton Research Conference 2023 Crops Consultants Australia Seminar 2024 Australian Agronomy Conference (October 2024)
Extension resources and services delivered to industry	CRDC Spotlight magazine article (Winter 2024) Australian Cottongrower article (Dec 23-Jan 24 issue) DCRA update (May 2024) <u>Presentations at field days:</u> Coleambally grower meeting (Nov 2023) Coleambally field day (Feb 2024)

	Griffith field walk (Mar 2024) Mullaley field day (April 2024) Bundy field day (April 2024) CCA Seminar (June 2024)
CRDC Final Report	A final project report documenting project outcomes has been submitted to CRDC in October 2024.

Outcomes from project outputs *(Refer to examples document).*

Outcome	Description
Increased knowledge of plant growth regulators (PGRs) for Australian cotton systems	Results from field studies identified that the early application of Trinexapac-ethyl (TEP) consistently delayed the development of dryland cotton. Field trials during the 2023-24 cotton season showed there was no significant effect on yield with mepiquat chloride (MC) application in Cecil Plains, Wee Waa or Aberdeen. However, in Griffith, relative yield losses were observed with high rates of MC, indicating that early-season application rates of MC can lead to growth restrictions that reduce final yield. It would be beneficial to conduct further research to determine what conditions lead to yield reductions across multiple seasons so that grower/consultant perceptions are not reliant on a single year of data. Several potential new hormone/PGR formulations that could potentially assist cotton agronomy were broadly screened for yield improvements across field experiments across multiple years. Novel PGR's tested included Abscisic acid (ABA), Forchlorfenuron (CPPU), $GA_{4,7}$ and 6-Benzyladenine ($GA_{4,7}$ + BA) and Glycine Betaine. Our data showed inconsistent yield responses for the respective application timings and rates for products tested.
Collaboration	Collaborations with industry representatives, growers and consultants have resulted in several on-farm field experiments across the regions, and connections with industry representatives have enabled study of various PGR products to understand effects of these on growth, development and yield across several cotton systems.
Industry capacity building	Knowledge, skills and industry networks of project stakeholders have been increased leading to a better understanding of plant functioning (hormones) and agronomic practices across cotton regions, and grower/consultant and industry stakeholder engagement and collaboration to achieve project outcomes.

Part 3 – Technical Report

Projects may require different approaches to the structure of the Technical report. A detailed technical report should normally include the following items (Note - PhDs only - CRDC will accept your awarded Thesis as the Technical Report, please attach and copy and paste the abstract here):

- *Table of contents (if necessary – depends on the length and complexity of your report)*
- *Executive summary*
- *Introduction*
- *Materials and methods*
- *Results*
- *Discussion*
- *Conclusions*
- *Key word index and*
- *A full list of industry and scientific publications, presentations, extension activities and other outputs.*

Please contact your R&D manager if you would like to adopt a different approach.

Introduction

Australian cotton production is characterised by high yielding, high quality cropping systems, earning in excess of $2.5b in export revenues annually. Crops are grown under an expanding scope of climatic conditions and diverse agronomic management practices, driven by broad industry use of transgenic technologies. Abiotic stressors and their management are key drivers of lint quality and yield. Australian cotton production systems are predominantly driven by water availability and the compounding impact of deficit on other system constraints.

A key industry challenge is to minimise such climate-induced year to year production variability and maintain existing lint quality repute. An industry-led strategic imperative also exists to significantly increase productivity and profitability on cotton farms within 5 years, being partly achieved through both optimising cotton farming systems and protecting them from biotic threats and environmental stresses.

This project aimed to address these strategic requisites by increasing crucial independent research capacity and capabilities for applied cotton systems research. The project aimed to investigate responsive agronomic practices for irrigated, rain-fed and abiotically stressed crops by leveraging plant growth regulator chemistry and applying modern sensing technologies and data analytics. This project continued research into the use of crop growth regulators seeking to modify or support crop growth to assist development of novel agronomic approaches to improve production resilience.

Background

Australian rainfed cotton production occurs in warm regions with intermittent and highly variable rainfall throughout the growing season. Predominate areas for rainfed cotton production are northern New South Wales and Southern Queensland (Bange et al., 2005). Figure 1 shows how in-crop rainfall is skewed where the number of seasons that receive more rainfall are less frequent than seasons that receive very low rainfall, and highlights the variability in when rainfall occurs (Conaty et al., 2018). This environmental variability affects the area of land planted to cotton, and crop yields.

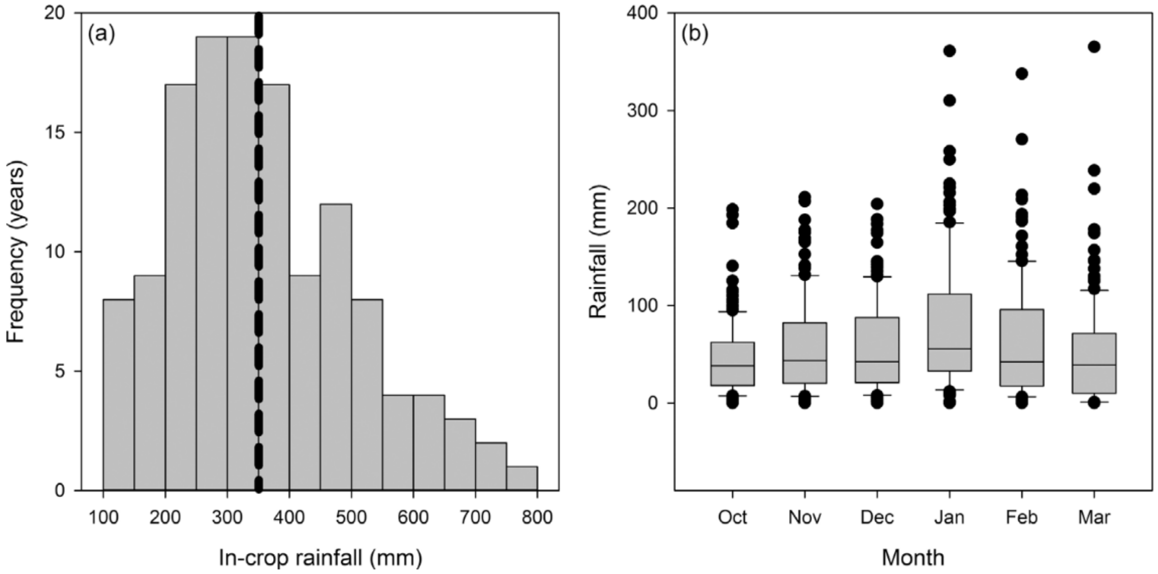


Figure 1: (a) Frequency distribution of in-crop rainfall (October to March), where the dashed line represents the mean rainfall; and (b) box plot highlighting monthly variation in rainfall measured between 1885 and 2015 at Wee Waa, NSW (Conaty et al., 2018).

Recently, rainfed cotton production has expanded, partly due to improved varietal choices and partly in response to high prices and thus increased profitability with cotton cropping compared with alternative summer crops such as sorghum (Godfrey et al., 2023).

Agronomic management practices can reduce the risk associated with such variable in-crop rainfall. Dryland cotton growers can use planting row configuration to manage their growing costs and maintain yield and fibre quality. Skip-row sowing configurations decrease plant population and can increase soil water availability, thereby reducing the downside risk in years with low rainfall. Time of planting is also an important consideration, and sowing dates of rainfed crops are varied according to soil water availability dictating planting opportunities. The length of sowing windows in dryland crops are often longer than for irrigated crops. Therefore, season length in a region will be a major factor in cultivar choice. Cultivars with phenological plasticity have been shown to benefit rainfed environments. In rainfed systems with high climate variability and long season growth, it has been found that cultivars that are less determinate (i.e., more indeterminate) are preferential in delivering higher yields (Stiller et al., 2005), because of their ability to regrow after significant stress (Bange et al., 2016). Identifying cultivars more suited to rainfed systems is one focus of the CSIRO cotton breeding program (Conaty et al., 2022). It has been identified that plant growth hormones (also known as plant growth regulators) may provide additional agronomic management strategies that could be beneficial to Australian dryland cotton growers. However, there are a number of different hormones to consider, with varied functions within the plant.

Plant hormones help regulate the plant by responding to various signals from the plant and environment. Hormones are regulated in different plant tissues during the different developmental stages. Many plants synthesise hormones in all parts of the plant, including in the roots, stems, and leaves. There are five major plant hormones: auxin, cytokinin, gibberellin, abscisic acid, and ethylene (Table 1). Each type of hormone has a specific function, with several secondary functions. Generally, auxins, gibberellins and cytokinins act as growth stimulators, whereas abscisic acid and ethylene act as growth inhibitors.

Table 1: Types of plant hormones and their location and function within the plant. Glycine betaine is an osmoprotectant, rather than a plant hormone.

Hormone	**Locations in the plant**	**Functions**

Auxin	Present in the seed embryo, young leave, and the meristem of apical buds. Acid growth hypothesis suggests that the release of H+ from cells into the walls causes cell-wall loosening. This leads to cell expansion under turgor pressure. Auxin may also regulate gene expression.	• Stimulate cell elongation, cell division in cambium, differentiation of phloem and xylem, root initiation on stem cuttings, lateral root development in tissue culture • Delaying leaf senescence • Suppression of lateral bud growth when supplied from apical buds • Inhibition of promotion of fruit and leaf abscission through ethylene stimulation • Fruit setting and growth induced through auxin in some plants • Auxin can delay fruit ripening • Stimulation of flower parts, femaleness of dioecious flowers, and high concentration of ethylene in flowering plants
Cytokinin	Synthesised in roots and then transported to other parts of the plant. Synthesised in young, rapidly dividing cells of the shoot and root apical meristems and transported to the shoot via the xylem.	• Stimulation of cell division, growth of lateral buds, and apical dominance • Stimulation of shoot initiation and bud formation in tissue culture • Leaf cell enlargement that stimulates leaf expansion • Enhancement of stomatal opening in some plant species • Etioplasts converted into chloroplasts through stimulation of chlorophyll synthesis
Gibberellin	Present in the mainstems of apical buds and roots, young leaves, and embryo	• Stimulates stem elongation • Leads to development of seedless fruits • Delays senescence in leaves and citrus fruits • Ends seed dormancy in plants that require light for induction of germination
Abscisic acid	Mostly found near leaves, stems and unripe fruit. ABA is synthesised in all cells that contain plastids, and is transported in the xylem and phloem	• Stimulates closure of stomata • Inhibits shoot growth • Inducing seeds for synthesising storage of proteins
Ethylene	Present in the tissues of ripening fruits, nodes of stems, senescent leaves and flowers.	• Leads to release of dormancy state • Stimulates shoot and root growth along with differentiation • Leaf and fruit abscission

		- Stimulation of femaleness in dioecious flowers - Stimulates flower opening - Stimulation of flower and leaf senescence - Stimulations of fruit ripening
Osmoprotectant (Glycine betaine)	Synthesised in the chloroplast	- Stabilises the structure of enzymes, complex proteins, and membranes. - Promotes cell division - Protects mitochondrial complex II under salt stress conditions

Adapted from: https://biologywise.com/plant-hormones-their-functions (accessed 16/11/23)

This research project focuses on gibberellins, gibberellin inhibitors, ethylene inhibitors, and the osmoprotectant glycine betaine, so these will be discussed in further detail below.

Gibberellins (GAs) are a group of plant hormones that stimulate germination, the elongation of the stem, and flowering. There are more than 70 gibberellins isolated, with GA_3 being the most widely studied and predominately used commercially. The major uses of gibberellins (predominately GA_3) in viticulture, horticulture and agriculture are to manage fruit crops, malt barley, increase sugar yield in sugarcane, and increase yield of seedless grape varieties. However, a combination of GA_4 and GA_7 are used to control russeting, a defect that appears in the fruit of some varieties of apples and pears, which leads to visual depreciation of quality, and therefore affects commercialisation (Salazar-Cerezo et al., 2018). GA_4 is less persistent than GA_3 and GA_7 and is therefore better suited where too long-lasting effects are unwanted. Due to the close chemical similarity of GA_4 and GA_7, their separation in the manufacturing process is very difficult. As a result, the content of GA_7 in commercial products varies (~insignificant amounts to 40%) (Rademacher and Brahm, 2012). $GA_{4,7}$ can also be combined with the cytokinin 6-benzyladenine, to improve the fruit quality of apples (Rademacher, 2015).

The main hormonal functions of GAs in plants are the promotion of longitudinal growth (e.g., plant height and internode length), the induction of hydrolytic enzymes in germinating seeds, the induction of bolting in long-day plants, and the promotion of fruit setting and development (Rademacher, 2015). The biosynthesis of GAs in plants can be separated into three stages according to the nature of the enzymes involved and their location in the cell (Figure 2); terpene clyclases act in proplastids, monooxygenases are associated with the endoplasmic reticulum, and dioxygenases are located in the cytosol (Gupta and Chakrabarty, 2013; Rademacher, 2017; Rademacher and Brahm, 2012). Inhibitors of GA biosynthesis led to less cell elongation and cell division, thereby making plants more compact. This may have many benefits in crop production. For example, mepiquat chloride (MC) is a quaternary ammonium compound that inhibits cyclases involved in early stages of GA metabolism, which lead to the formation of *ent*-kaurene (Rademacher, 2015; Rademacher, 2017). In comparison, the late stages of GA biosynthesis are catalysed by dioxygenases, which require 2-oxoglutaric acid as a co-substrate. Trinexapac-ethyl (TEP) inhibits these reactions because of its structural relationship to 2-oxoglutaric acid, and through inhibition of flavanone 3-hydroxylase which is involved in the biosynthesis of flavonoids (Rademacher, 2015). Therefore, there are many different biochemical pathways that may be promoted or inhibited with the application of exogenous hormones.

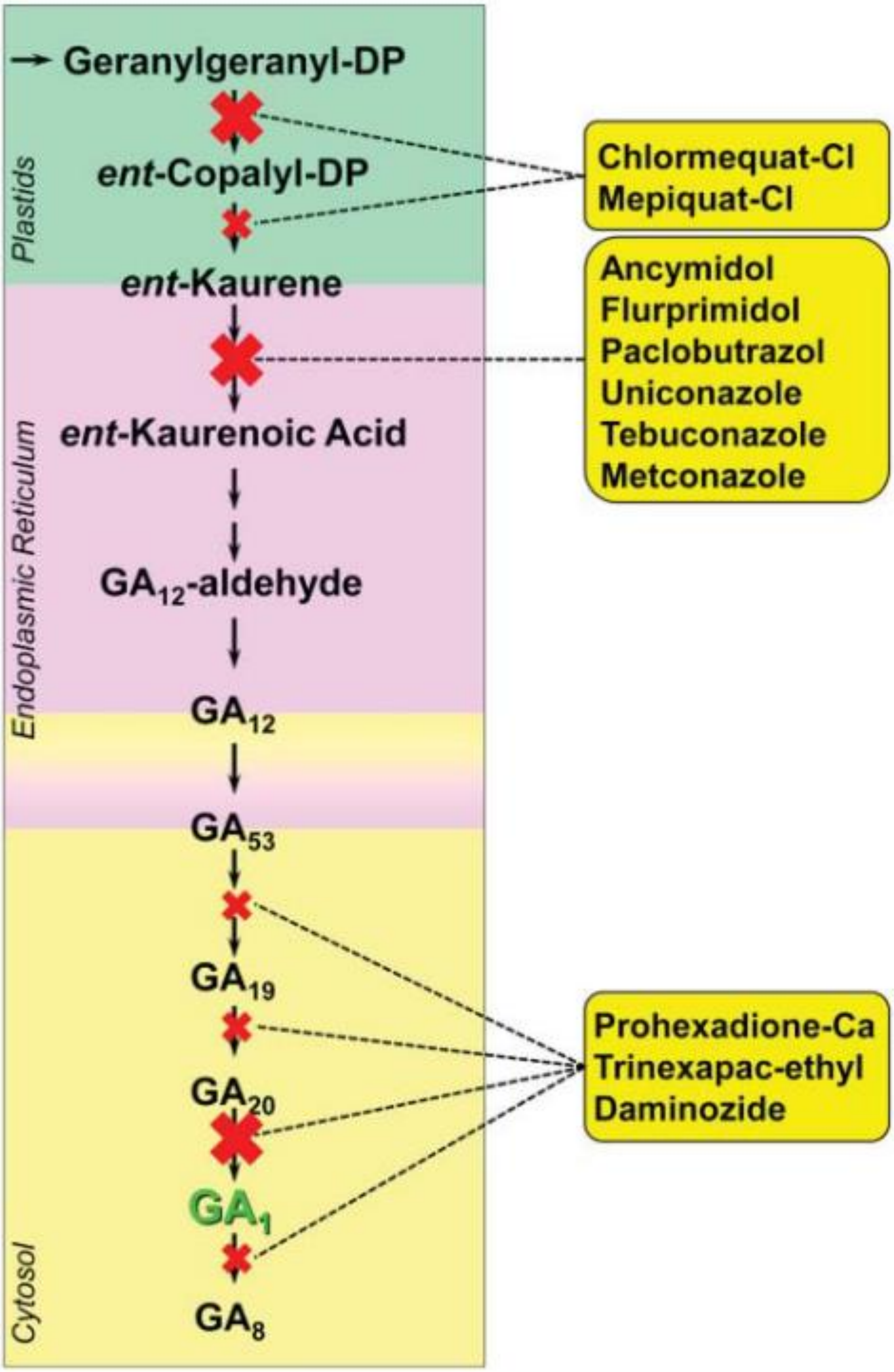


Figure 2: Main steps of gibberellin biosynthesis leading to biologically active GA1 and points of inhibition by some plant growth retardants. The cellular locations of the reactions are indicated by the colours green, red, and yellow (Rademacher, 2017).

Gibberellins may be used to promote early canopy structural biomass and development and thus play a role in changing assimilate supply and demand leading to alterations in the vegetative and reproductive growth of cotton. Considerable research has been conducted to study phase change and gene expression in the model plant, *Arabidopsis thaliana*. The best-known hormone that controls vegetative phase change is gibberellic acid (GA) (Manuela and Xu, 2020). GA contributes to vegetative phase change through the release of SPLs (SQUAMOSA promoter binding protein-like transcription factors) from their inhibitory interaction with the DELLA proteins. SPL genes encode plant-specific transcription factors that play important roles in plant phase transition, flower and fruit development, plant architecture and gibberellins signalling. Therefore, because of the role that gibberellins have in vegetative to reproductive phase transition, flower and fruit development, and canopy architecture, plant growth regulators either promoting or inhibiting gibberellin biosynthesis could be utilised to allow cotton growers greater control over the growth and development of their crops and target optimal environmental conditions through critical stages.

Cytokinins are plant hormones with the ability to stimulate cell division in plant tissue cultures and may delay leaf senescence (yellowing) and prevent abscission (Guinn, 2012). Forchlorfenuron, CPPU (*N*-(2-Chloro-4-pyridyl)-*N'*-phenylurea) is a highly active synthetic cytokinin-like plant growth regulator used to increase berry size in kiwi and grapes. CPPU stimulates periclinal cell division leading to more round or oval shaped berries (Khan and Ali, 2018). CPPU can also increase fruit set and accelerate cell expansion and fruit enlargement (Zeng et al., 2016). In China in 2011, too much CPPU was applied to watermelon crops late in the season, resulting exploding watermelons, caused by the rapid expansion of the fruit. Therefore, CPPU applied to developing cotton bolls may increase expansion rates and boll size, potentially leading to increased yield. However, this has not been previously tested in the field.

Abscisic acid (ABA) is a plant hormone that regulates numerous aspects of plant growth, development, and stress responses. ABA is synthesised in all cells that contain plastids and is transported in the xylem and phloem. ABA plays a major role during water stress and in seed and bud dormancy. During water stress, ABA induces the expression of genes for the synthesis of proteins that protect membranes and other proteins from desiccation damage. ABA closes stomata by depolarising the plasma membrane, increasing cytosolic Ca^{2+} and regulating ion transport. ABA is an antagonist of GA and suppresses GA-induced gene expression, resulting in reduced cell elongation during periods of abiotic stress. ABA may be applied to cotton to induce stomatal closure and temporarily "shut down the plant" during a period of hot, dry weather to conserve water for use later in the season. However, it is not known what rates of chemical should be applied, how long the chemical reduces stomatal conductance of the plant, or what the longer-term effects are on yield of cotton grown in the field.

Ethylene is a hormone that promotes abscission and inhibits cell elongation and growth. There are two mechanisms of action: (a) slower transport and increased destruction of auxin, and (b) stimulation of synthesis of pectinase and cellulase in the abscission zone (Guinn, 2012). If young cotton plants were under abiotic stress, ethylene production in the plant could potentially inhibit growth. Aminoethoxyvinylglycine (AVG) is a naturally occurring amino acid, which inhibits ethylene biosynthesis. It is an irreversible inhibitor of PLP-dependent enzymes, such as ACC synthases, where it effectively blocks the active site of the enzyme. Commercially, AVG is used in harvest management and horticulture (Depaepe and Van Der Straeten, 2017). AVG has been shown to ameliorate waterlogging induced damage in cotton by inhibiting ethylene synthesis and sustaining photosynthetic capacity (Najeeb et al., 2015). Dryland cotton seedlings may experience warmer temperatures and lower water availability that promote ethylene production which could inhibit cell elongation and growth. The application of AVG could inhibit ethylene biosynthesis and thereby promote early-season growth and canopy development. However, there appears to be limited research investigating the early-season use of AVG for dryland cotton systems.

In addition to plant hormones, osmoprotectants are small molecules with neutral charge and low toxicity that act as osmolytes (by regulating osmotic pressure) and help plants survive osmotic stress. Osmoprotectants can be placed in three chemical classes: betaines and associated molecules, sugars and polyols, and amino acids. These molecules accumulate in cells and balance the osmotic difference between the cell's surroundings and the cytosol. Therefore, the synthesis and accumulation of osmolytes may help crop plants to cope with abiotic stress. Glycine betaine (GB) is a quaternary ammonium compound considered to be a strong osmoprotectant against drought stress. Features that contribute to GB being an effective metabolite for alleviating stress are its small size, solubility in water, and not interfering with other metabolites within the cell (Ashraf et al., 2011). When exogenously applied as a foliar spray, GB can easily penetrate through the epidermis of the leaf and move to other organs. It has been found that GB protects molecules in response to abiotic stress via stabilisation of membranes, proteins, and DNA structure or as an intermediate in metabolic and physiological processes. GB biosynthesis also occurs naturally in the chloroplast, through a two-step oxidation of choline to betaine through betaine aldehyde. Crop plants have been shown to accumulate GB in response to abiotic stress, although in many crops, the natural accumulation of GB is lower than sufficient to ameliorate the adverse effects of water deficits. However, studies have shown that externally applied GB may ameliorate the impact of environmental stresses, like drought and high salinity, and improve recovery from stress. Positive effects of applied GB have been measured in wheat, barley, sorghum, and soybean. Sarwar et al. (2006) reported that GB accumulation was positively correlated with seed cotton yield and boll number. Therefore, it is possible that the exogenous application of GB may ameliorate the negative effects of abiotic stresses on cotton and thereby increase yield.

Hormones play an important role in the metabolic processes that influence growth and development of a plant, and the ability of the plant to respond to abiotic stress. There are several plant growth regulators that are available commercially, some used in cotton, and many used across other agricultural and horticultural applications. Many of these have not been tested in field conditions for cotton in Australia. This project investigates the use of plant growth hormones on cotton growth, physiology, and yield, with a particular focus on management strategies that improve the resilience of dryland cotton production in Australia.

General methods

Field experiments were conducted to identify and investigate applications of plant growth regulators (PGRs) to improve crop efficiencies and/or deliver yield and lint quality benefits, with a focus on rain-grown production systems. Unless otherwise specified, all experiments used the Sicot 746B3F cultivar of *Gossypium hirsutum* L., a commercially available, transgenic full season cultivar with normal leaf shape, a compact growth habit, very high yield potential, good disease resistance, high lint quality and excellent adaptation to both irrigated and rain grown management in central and hot production areas.

Field Experiments

Seventeen field experiments were conducted over three cotton seasons, with 14 at the Australian Cotton Research Institute (ACRI), Narrabri, NSW, 2 in Whitton, NSW and 1 in Bongeen, Qld. The PGR's tested in each experiment are summarised in Table 2. All field experiments were set-up as a randomised complete block design with six replications. Cotton was planted into the field and managed using standard farming practices. Applications of hormone treatments were made at various leaf or developmental stages, specified in each experiment (Table 2), using hand-held spray equipment (Ryobi 7.5 L hand-held battery-operated sprayer). PGR foliar treatments and label-recommended surfactants were applied 30 cm above the plant line to ensure good coverage. Plant measurements included time to first square (TTFS), time to first flower (TTFF), weekly measurement of plant height (from the cotyledons) and the number of nodes. Biomass samples were harvested from either 0.5 or 1 linear metre, at developmental stages based on the control. Harvested plants were mapped to measure fruit retention and position. Maturity picks were collected from 1 linear metre to determine the number of days after planting to when the plot reached 60% Open Boll. Yield data was obtained from small plot pickers. Cotton was ginned at Cotton Seed Distributors (CSD) and quality data was obtained from Australian Classing Services, Wee Waa. Comparisons of PGR treatments and relevant experiment materials and methods are detailed in each milestone (summarised in the achievements table).

Climatic conditions: The experiments were conducted over distinct climatic events including drought and wet seasons. Data was accessed from the OzForecast weather stations at ACRI (Chico Myall Vale, 149.6E / 30.2S, 210 m ASL), Bongeen (DDCGI, 151.4E / 27.6S) and

Brigalow (MIA 146.2E / 34.6S, 153 m ASL). Seasonal climatic conditions are summarised in Table 3.

Glasshouse Experiments
Glasshouse experiments were conducted at the Australian Cotton Research Institute (ACRI), Narrabri, NSW. Plants were grown at 32/20^{o}C (day/night) under natural light.

Nutrition/fertiliser applications: Prior to planting, soils in pots were kept saturated with water and a basal fertiliser, 10 g MULTIgro® (Incitec Pivot Fertilisers, Melbourne, Australia), was added to the soil surface of each pot and dissolved into the soil via hand-held irrigation. MULTIgro® is a multi-purpose garden fertiliser with nutrients N, P, K, S and Ca of 13.1, 4.5, 7.2, 15.4, and 2.4%. The soil surface of each pot was covered with approximately 20 mm of moist sand to assist in reducing surface evaporation and ensure even and rapid plant emergence. Sand was maintained moist through daily surface, hand-held irrigation. Following emergence, seedlings at the three-leaf stage were thinned to a single plant per pot. Plants were provided with non-limiting water supply through daily irrigation, where approximately 1200ml of water over a 15 minute period was supplied at 0900 via drip irrigators to saucers underneath pots. To ensure nutrition did not limit plant growth and function, at first flower pots were fertilised with 2 g of Liquifert® K (Incitec Pivot Fertilisers, Melbourne, Australia) and 6.5 g GranAm® (Incitec Pivot Fertilisers, Melbourne, Australia) dissolved in 500 ml water. Liquifert® K is a soluble potassium chloride based fertiliser (50% K), and GranAm® is a granulated ammonium sulphate fertiliser (20.5% N, 23.9% S). At this same time irrigation drippers were moved to the soil surface, ensuring soil water status remained non-limiting. As required, pests (*Bemisia tabaci* Gennadius*, Tetranychus urticae* Koch*, and Frankliniella occidentalis,* Pergande) were controlled with the use of commercial pesticides.

Leaf gas exchange measurements: Photosynthetic rates, stomatal conductance, and transpiration rates were measured on recently fully expanded leaves using a portable open gas exchange system (LI-6400XT, LI-COR). Settings were as follows: CO_2= 410 µmol mol^{-1}, flow rate= 400 µmol s^{-1}, and block temperature= 32^{o}C.

Statistical analyses
Data was analysed by ANOVA using Genstat version 22 (VSN International Ltd). The assumptions of normality and homogeneity of variance were met for all variables and no transformations were applied. Means of treatments were compared using Tukey's HSD or Fishers Least Significant Difference (LSD) at a 5% level of probability.

Table 2: Overview of all the field experiments conducted as part of the CSP2001- Modern systems Agronomy project.

Exp ID	Cotton Season	Location	Experiment	Planting Date	*Trt*	*Water**	*Row Config*	*Variety*	*PGR*	*Rate (a.i.)*	*Application*	*Frequency*	Timing	Surfactant	Surfactant Rate	Application Dates
1	2020-21	ACRI, Narrabri	DLS1	17/09/2020	*1*	Rain grown	2m, alt.	746B3F	Control	-	-	-	-	-	-	
					2	Rain grown	2m, alt.	746B3F	TEP	2000ppm	Spray to drip	Once	4 Leaf	No	N/A	10/11/2020
					3	Rain grown	2m, alt.	746B3F	TEP	2000ppm	Spray to drip	2x7d	4 Leaf	No	N/A	10/11/20, 17/11/20.
					4	Rain grown	2m, alt.	746B3F	TEP	2000ppm	Spray to drip	3x7d	4 Leaf	No	N/A	10/11/20, 17/11/20, 24/11/20.
					5	Rain grown	2m, alt.	746B3F	MC	60ppm	Spray to drip	3x7d	4 Leaf	Yes	3 drops	10/11/20, 17/11/20, 24/11/20.
					6	Rain grown	2m, alt.	746B3F	Mechanical cut to just over 9n at FF	-	-	-	FF	-	-	5/1/21.
					7	Rain grown	2m, alt.	746B3F	GA_3	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/1L	22/10/20, 3/11/20, 17/11/20.
					8	Rain grown	2m, alt.	746B3F	$GA_{4,7}$	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/1L	22/10/20, 3/11/20, 17/11/20.
2	2020-21	ACRI, Narrabri	DLS2	11/11/2020	*1*	Rain grown	2m, alt.	746B3F	Control	-	-	-	-	-	-	
					4	Rain grown	2m, alt.	746B3F	AVG	830ppm	Spray to drip	3x10d	4 Leaf	Yes	0.5ml/1L	17/12/20, 4/1/21, 22/1/21.
					5	Rain grown	2m, alt.	746B3F	AVG	830ppm	Spray to drip	4x10d	4 Leaf	Yes	0.5ml/1L	17/12/20, 4/1/21, 22/1/21, 4/2/21.
					6	Rain grown	2m, alt.	746B3F	AVG	830ppm	Spray to drip	5x10d	4 Leaf	Yes	0.5ml/1L	17/12/20, 4/1/21, 22/1/21, 4/2/21, 11/2/21.
					7	Rain grown	2m, alt.	746B3F	AVG	830ppm	Spray to drip	2x10d	1st stress > FF	Yes	0.5ml/1L	8/2/21, 23/2/21.
					8	Rain grown	2m, alt.	746B3F	GB	$12g.L^{-1}$	Spray to drip	2x10d	1st stress > FF	No	N/A	8/2/21, 23/2/21.
					9	Rain grown	2m, alt.	746B3F	GA_3	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/1L	30/11/20, 17/12/20, 4/1/21.
					10	Rain grown	2m, alt.	746B3F	$GA_{4,7}$	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/1L	30/11/20, 17/12/20, 4/1/21.
3	2020-21	ACRI, Narrabri	Semi Irrigated	11/11/2020	*1*	Semi Irrigated	2m, alt.	746B3F	Control	-	-	-	-	-	-	
					2	Semi Irrigated	2m, alt.	746B3F	AVG	830ppm	Spray to drip	2x10d	1st stress > FF	MAXX	0.5ml/1L	8/2/21.

					3	Semi Irrigated	2m, alt.	746B 3F	GB	12g.L^{-1}	Spray to drip	2x10d	1st stress > FF	No	N/A	8/2/21, 23/2/21.
					4	Semi Irrigated	2m, alt.	746B 3F	CPPU	10ppm	Spray to drip	2x10d	FCB	No	N/A	23/2/21
					6	Semi Irrigated	2m, alt.	746B 3F	AMF	15ml.ha^{-1}	In-furrow	Once	Planting	No	N/A	
4	2020-21	ACRI, Narrabri	Irrigated Sprays not Applied	11/11/2020	1	Irrigated	Solid	746B 3F	Control	-	-	-	-	-	-	Sprays Not Applied
					2	Irrigated	Solid	746B 3F	CPPU	10ppm	Spray to drip	2x10d	FCB			
					3	Irrigated	Solid	746B 3F	AVG + NAA	830ppm+10 ppm	Spray to drip	2x10d	FCB	Yes	0.5ml/1L	
					4	Irrigated	Solid	746B 3F	AMF	15ml.ha^{-1}	In-furrow	Once	Planting	No	N/A	
					5	Irrigated	Solid	746B 3F	$GA_{4,7}$ + 6-Benzyladenine	125ml.ha^{-2}	Spray to drip	3x10d	FS	Yes	0.5ml/1L	
					6	Irrigated	Solid	746B 3F	$GA_{4,7}$ + 6-Benzyladenine	125ml.ha^{-3}	Spray to drip	3x10d	FF	Yes	0.5ml/1L	
5	2020-21	ACRI, Narrabri	DLS3 ABANDONED	ABANDONED	1	Rain grown	2m, alt.	746B 3F	Control	-	-	-	-	-	-	Trial Abandoned
					2	Rain grown	2m, alt.	714B 3F	Varietal change; determinate	-	-	-	-	-	-	
					3	Rain grown	2m, alt.	748B 3F	Varietal change; indeterminate	-	-	-	-	-	-	
					4	Rain grown	2m, alt.	746B 3F	AVG	830ppm	Spray to drip	3x10d	4 Leaf	Yes	0.5ml/1L	
					5	Rain grown	2m, alt.	746B 3F	AVG	830ppm	Spray to drip	4x10d	4 Leaf	Yes	0.5ml/1L	
					6	Rain grown	2m, alt.	746B 3F	AVG	830ppm	Spray to drip	5x10d	4 Leaf	Yes	0.5ml/1L	
					7	Rain grown	2m, alt.	746B 3F	GA_3	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/1L	
					8	Rain grown	2m, alt.	746B 3F	$GA_{4,7}$	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/1L	
6	2020-21	Bongeen, Qld	Bongeen	20/11/2020	1	Rain grown	Single Skip	746B 3F	Control	-	-	-		-	-	
					2	Rain grown	Single Skip	714B 3F	Varietal change; determinate	-	-	-		-	-	
					3	Rain grown	Single Skip	748B 3F	Varietal change; indeterminate	-	-	-		-	-	
					4	Rain grown	Single Skip	746B 3F	TEP	2000ppm	Spray to drip	3x10d	4 Leaf	No	-	18/12/20, 29/12/20, 11/1/21.
					5	Rain grown	Single Skip	746B 3F	Mechanical cut to just over 9n at FF	-	-	Once	FF	-	-	1/2/20.

					6	Rain grown	Single Skip	746B 3F	MC	60ppm	Spray to drip	3x10d	4 Leaf	Yes	3 drops	18/12/20, 29/12/20, 11/1/21.
					7	Rain grown	Single Skip	746B 3F	AVG	925ppm	Spray to drip	3x10d	4 Leaf	Yes	0.5ml/ 1L	18/12/20, 29/12/20, 11/1/21.
					8	Rain grown	Single Skip	746B 3F	AVG + $GA_{4,7}$	925ppm+80 ppm	Spray to drip	3x10d	4 Leaf	Yes	0.5ml/ 1L	18/12/20, 29/12/20, 11/1/21.
7	2020 -21	Whitton, NSW	Whitton	2/10/2020	1	Irrigated	Solid	746B 3F	Control	-	-	-	-	-	-	
					2	Irrigated	Solid	714B 3F	GA_3	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	4/11/20, 13/11/20, 23/11/20.
					3	Irrigated	Solid	748B 3F	$GA_{4,7}$	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	4/11/20, 13/11/20, 23/11/20.
					4	Irrigated	Solid	746B 3F	AVG + GA_3	925ppm + 80ppm	Spray to drip	3x10d	2 Leaf instead of 4L	Yes	0.5ml/ 1L	4/11/20, 13/11/20, 23/11/20.
					5	Irrigated	Solid	746B 3F	AVG + $GA_{4,7}$	925ppm + 80ppm	Spray to drip	3x10d	2 Leaf instead of 4L	Yes	0.5ml/ 1L	4/11/20, 13/11/20, 23/11/20.
					6	Irrigated	Solid	746B 3F	GB	4000ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	4/11/20, 13/11/20, 23/11/20.
8	2021 -22	ACRI, Narrabri	DLS1	17/09/2021	1	Rain grown	2m, alt.	746B 3F	Control	-	-	-	-	-	-	
					2	Rain grown	2m, alt.	746B 3F	TEP	2000ppm	Spray to drip	Once	4 Leaf	No	N/A	1/11/21.
					3	Rain grown	2m, alt.	746B 3F	TEP	2000ppm	Spray to drip	2x7d	4 Leaf	No	N/A	1/11/21, 9/11/21.
					4	Rain grown	2m, alt.	746B 3F	TEP	2000ppm	Spray to drip	3x7d	4 Leaf	No	N/A	1/11/21, 9/11/21, 16/11/21.
					5	Rain grown	2m, alt.	746B 3F	MC	60ppm	Spray to drip	3x7d	4 Leaf	Yes	3 drops	1/11/21, 9/11/21, 16/11/21.
					6	Rain grown	2m, alt.	746B 3F	Mechanical cut to just over 9n at FF	-	-	-	-	-	-	4/1/21.
					7	Rain grown	2m, alt.	746B 3F	GA_3	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	21/10/21, 1/11/21, 9/11/21.
					8	Rain grown	2m, alt.	746B 3F	$GA_{4,7}$	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	21/10/21, 1/11/21, 9/11/21.
9	2021 -22	ACRI, Narrabri	DLS2	21/10/2021	1	Rain grown	2m, alt.	746B 3F	Control	-	-	-	-	-	-	-
					2	Rain grown	2m, alt.	746B 3F	ABA	125ppm	Spray to drip	2x14d	1st stress > FF	Yes	0.5ml/ 1L	5/1/21, 17/1/21.
					3	Rain grown	2m, alt.	746B 3F	ABA	250ppm	Spray to drip	2x14d	1st stress > FF	Yes	0.5ml/ 1L	5/1/21, 17/1/21.
					4	Rain grown	2m, alt.	746B 3F	AVG	830ppm	Spray to drip	3x10d	4 Leaf	Yes	0.5ml/ 1L	23/11/21, 1/12/21, 10/12/21.
					5	Rain grown	2m, alt.	746B 3F	AVG	830ppm	Spray to drip	4x10d	4 Leaf	Yes	0.5ml/ 1L	23/11/21, 1/12/21, 10/12/21, 20/12/21.

					6	Rain grown	2m, alt.	746B3F	AVG	830ppm	Spray to drip	5x10d	4 Leaf	Yes	0.5ml/1L	23/11/21, 1/12/21, 10/12/21, 20/12/21, 30/12/21.
					7	Rain grown	2m, alt.	746B3F	AVG	830ppm	Spray to drip	2x10d	1st stress > FF	Yes	0.5ml/1L	5/1/22, 13/1/22.
					8	Rain grown	2m, alt.	746B3F	GB	$12g.L^{-1}$	Spray to drip	2x10d	1st stress > FF	No	N/A	5/1/22, 13/1/22.
					9	Rain grown	2m, alt.	746B3F	GA_3	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/1L	15/11/21, 23/11/21, 1/12/21.
					10	Rain grown	2m, alt.	746B3F	$GA_{4,7}$	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/1L	15/11/21, 23/11/21, 1/12/21.
10	2021-22	ACRI, Narrabri	Semi Irrigated	21/10/2021	1	Semi Irrigated	2m, alt.	746B3F	Control	-	-	-	-	-	-	
					2	Semi Irrigated	2m, alt.	746B3F	AVG	830ppm	Spray to drip	2x10d	1st stress > FF	Yes	0.5ml/1L	5/1/22, 13/1/22.
					3	Semi Irrigated	2m, alt.	746B3F	GB	$12g.L^{-1}$	Spray to drip	2x10d	1st stress > FF	No	N/A	5/1/22, 13/1/22.
					4	Semi Irrigated	2m, alt.	746B3F	CPPU	10ppm	Spray to drip	2x10d	FCB	No	N/A	8/3/22, 18/3/22.
					5	Semi Irrigated	2m, alt.	746B3F	AVG+NAA	830ppm+10ppm	Spray to drip	2x10d	FCB	Yes	0.5ml/1L	8/3/22, 18/3/22.
					6	Semi Irrigated	2m, alt.	746B3F	AMF	$15ml.ha^{-1}$	In-furrow	Once	Planting	No	N/A	21/10/21.
11	2021-22	ACRI, Narrabri	Irrigated	21/10/2021	1	Irrigated	Solid	746B3F	Control	-	-	-	-	-	-	
					2	Irrigated	Solid	746B3F	CPPU	10ppm	Spray to drip	2x10d	FCB	No	N/A	8/3/22, 18/3/22.
					3	Irrigated	Solid	746B3F	AVG+NAA	830ppm+10ppm	Spray to drip	2x10d	FCB	Yes	0.5ml/1L	8/3/22, 18/3/22.
					4	Irrigated	Solid	746B3F	AMF	$15ml.ha^{-1}$	In-furrow	Once	Planting	No	N/A	
					5	Irrigated	Solid	746B3F	$GA_{4,7}$ + 6-Benzyladenine	$125ml.ha^{-1}$	Spray to drip	3x10d	FS	Yes	0.5ml/1L	20/12/21, 30/12/21, 10/1/22.
					6	Irrigated	Solid	746B3F	$GA_{4,7}$ + 6-Benzyladenine	$125ml.ha^{-1}$	Spray to drip	3x10d	FF	Yes	0.5ml/1L	10/1/22, 17/1/22, 31/1/22.
12	2021-22	ACRI, Narrabri	DLS3	18/11/2021	1	Rain grown	2m, alt.	746B3F	Control	-	-	-	-	-	-	
					2	Rain grown	2m, alt.	714B3F	Varietal change; determinate	-	-	-	-	-	-	
					3	Rain grown	2m, alt.	748B3F	Varietal change; indeterminate	-	-	-	-	-	-	
					4	Rain grown	2m, alt.	746B3F	AVG	830ppm	Spray to drip	3x10d	4 Leaf	Yes	0.5ml/1L	15/12/21, 23/12/21, 5/1/22.
					5	Rain grown	2m, alt.	746B3F	AVG	830ppm	Spray to drip	4x10d	4 Leaf	Yes	0.5ml/1L	15/12/21, 23/12/21, 5/1/22, 13/1/22.

					6	Rain grown	2m, alt.	746B 3F	AVG	830ppm	Spray to drip	5x10d	4 Leaf	Yes	0.5ml/ 1L	15/12/21, 23/12/21, 5/1/22, 13/1/22, 21/1/22.
					7	Rain grown	2m, alt.	746B 3F	GA3	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	6/12/21, 15/12/21, 23/12/21.
					8	Rain grown	2m, alt.	746B 3F	$GA_{4,7}$	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	6/12/21, 15/12/21, 23/12/21.
13	2021-22	Whitton, NSW	Whitton	12/10/2021	*1*	Irrigated	Solid	746B 3F	Control	-	-	-	-	-	-	
					2	Irrigated	Solid	714B 3F	GA_3	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	9/11/21, 22/11/21, 3/12/21.
					3	Irrigated	Solid	748B 3F	$GA_{4,7}$	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	9/11/21, 22/11/21, 3/12/21.
					4	Irrigated	Solid	746B 3F	AVG + GA_3	830ppm + 80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	9/11/21, 22/11/21, 3/12/21.
					5	Irrigated	Solid	746B 3F	AVG + $GA_{4,7}$	830ppm + 80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	9/11/21, 22/11/21, 3/12/21.
					6	Irrigated	Solid	746B 3F	GB	4000ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	9/11/21, 22/11/21, 3/12/21.
14	2022-23	ACRI, Narrabri	DLS1	5/10/2022	*1*	Rain grown	Single Skip	746B 3F	Control	-						
					2	Rain grown	Single Skip	746B 3F	TEP	2000ppm	Spray to drip	Once	4 Leaf	No	N/A	18/11/22.
					3	Rain grown	Single Skip	746B 3F	TEP	2000ppm	Spray to drip	2x7d	4 Leaf	No	N/A	18/11/22, 28/11/22.
					4	Rain grown	Single Skip	746B 3F	TEP	2000ppm	Spray to drip	3x7d	4 Leaf	No	N/A	18/11/22, 28/11/22, 5/12/22.
					5	Rain grown	Single Skip	746B 3F	MC	60ppm	Spray to drip	3x7d	4 Leaf	Yes	3 drops	18/11/22, 28/11/22, 5/12/22.
					6	Rain grown	Single Skip	746B 3F	Mechanical cut to just over 9n at FF	-	Spray to drip			-	-	6/1/23.
					7	Rain grown	Single Skip	746B 3F	GA_3	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	3/11/22, 11/11/22, 21/11/22.
					8	Rain grown	Single Skip	746B 3F	$GA_{4,7}$	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/ 1L	3/11/22, 11/11/22, 21/11/22.
15	2022-23	ACRI, Narrabri	DLS 2	18/10/2022	*1*	Rain grown	Single Skip	746B 3F	Control	-	-	-	-	-	-	
					2	Rain grown	Single Skip	746B 3F	ABA	125ppm	Spray to drip	2x14d	1st stress > FF	Yes	0.5ml/ 1L	25/1/23, 8/2/23.
					3	Rain grown	Single Skip	746B 3F	ABA	250ppm	Spray to drip	2x14d	1st stress > FF	Yes	0.5ml/ 1L	25/1/23, 8/2/23.
					7	Rain grown	Single Skip	746B 3F	AVG	830ppm	Spray to drip	2x10d	1st stress > FF	Yes	0.5ml/ 1L	25/1/23, 3/2/23.
					8	Rain grown	Single Skip	746B 3F	GB		Spray to drip	2x10d	1st stress > FF	No	N/A	25/1/23, 3/2/23.

16	2022-23	ACRI, Narrabri	Irrigated	18/10/2022	*1*	Irrigated	Solid	746B3F	Control	-	-	-	-	-	-	
					2	Irrigated	Solid	746B3F	CPPU	10ppm	Spray to drip	2x10d	FCB	No	N/A	9/3/23, 20/3/23.
					3	Irrigated	Solid	746B3F	AVG + NAA	830ppm+10ppm	Spray to drip	2x10d	FCB	Yes	0.5ml/1L	9/3/23, 20/3/23.
					5	Irrigated	Solid	746B3F	GA$_{4,7}$ + 6-Benzyladenine	125ml/ha	Spray to drip	3 x 10d	FS	Yes		19/12/22, 29/12/22, 9/1/23.
					6	Irrigated	Solid	746B3F	GA$_{4,7}$ + 6-Benzyladenine	125ml/ha	Spray to drip	3 x 10d	FF	Yes		13/1/23, 24/1/23, 3/2/23.
					7	Irrigated	Solid	746B3F	GA$_3$	80ppm	Spray to drip	3x10d	10 days < FS	Yes		8/12/22, 19/12/22, 29/12/22.
17	2022-23	ACRI, Narrabri	DLS 3	29/11/2022	*1*	Rain grown	Single Skip	746B3F	Control	-	-	-	-	-	-	
					2	Rain grown	Single Skip	714B3F	Varietal change; determinate	-	-	-	-	-	-	
					3	Rain grown	Single Skip	748B3F	Varietal change; indeterminate	-	-	-	-	-	-	
					4	Rain grown	Single Skip	746B3F	AVG	830ppm	Spray to drip	3x10d	4 Leaf	Yes	0.5ml/1L	3/1/23, 13/1/23, 20/1/23.
					5	Rain grown	Single Skip	746B3F	AVG	830ppm	Spray to drip	4x10d	4 Leaf	Yes	0.5ml/1L	3/1/23, 13/1/23, 20/1/23, 30/1/23.
					6	Rain grown	Single Skip	746B3F	AVG	830ppm	Spray to drip	5x10d	4 Leaf	Yes	0.5ml/1L	3/1/23, 13/1/23, 20/1/23, 30/1/23, 8/2/23.
					7	Rain grown	Single Skip	746B3F	GA$_3$	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/1L	19/12/22, 29/12/22, 9/1/23.
					8	Rain grown	Single Skip	746B3F	GA$_{4,7}$	80ppm	Spray to drip	3x10d	2 Leaf	Yes	0.5ml/1L	19/12/22, 29/12/22, 9/1/23.

Table 3: Seasonal climate conditions for each location. Values represent monthly minimum and maximum temperatures and total monthly rainfall.

Cotton Season	**Location**	**Relevant Experiment ID**	**Climatic conditions**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**
2020-21	Bongeen, Qld	6	Min Temperature (ºC)	0	7	7	9	12	12	10	0	-2	-1
			Max Temperature (ºC)	32	33	40	41	35	37	35	30	26	24
			Total Rainfall (mm)	0	46	3	32	97	19	30	9	35	13
	ACRI, Narrabri	1,2,3,4,5	Min Temperature (ºC)	2	9	9	12	13	16	13	3	1	2
			Max Temperature (ºC)	29	33	42	44	37	38	34	31	26	23
			Total Rainfall (mm)	12	14	3	114	26	90	145	9	20	43
	Whitton, NSW	7	Min Temperature (ºC)	0	5	7	9	11	10	8	0	0	1

			Max Temperature (°C)	30	32	41	37	39	34	34	32	26	18
			Total Rainfall (mm)	27	52	15	18	73	23	8	0	4	51
2021-22	ACRI, Narrabri	8,9,10,11,12	Min Temperature (°C)	2	6	7	11	16	12	13	8	4	0
			Max Temperature (°C)	28	36	33	38	39	36	35	30	27	21
			Total Rainfall (mm)	23	30	200	20	89	31	39	27	51	1
	Whitton, NSW	13	Min Temperature (°C)	3	5	8	9	16	11	12	8	2	0
			Max Temperature (°C)	27	30	31	40	36	35	34	28	24	18
			Total Rainfall (mm)	34	39	75	8	128	9	41	55	29	12
2022-23	ACRI, Narrabri	14,15,16,17	Min Temperature (°C)		9	8	8	16	13	11	7	0	
			Max Temperature (°C)		31	34	34	38	37	38	28	25	
			Total Rainfall (mm)		118	63	21	39	3	82	24	0	

Table 4: Summary of plant growth regulators tested in cotton during the CSP2001 project.

PGR/Treatments	**Active Constituent**	**Mode of action/Description**	**Relevant Exp ID**
ABA	200 g/kg S-Abscisic acid	Role in seed development and dormancy. **Stomatal response to osmotic stress**, drought, salinity, freezing (as soil dries out, leaf water potential drops, ABA content increases and stomatal resistance increases)	2, 9, 15
AVG	150 g/kg aminoethoxyvinylglycine present as the hydrochloride salt	**Reduces the production of ethylene** by inhibiting the conversion of S-adenosylmethionine (SAM) to ethylene precursor. AVG also inhibits ACC synthesis.	2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 16, 17
CPPU	10 g/L Forchlorfenuron	Phenylurea type cytokinin. Forchlorfenuron **increases cell number and cell size**. Not translocated in plant tissue (e.g., from treated leaves to fruit)	3, 4, 10, 11, 16
AMF	8.73% Total active ingredients Glomus intraradices, Glomus mosseae, Glomus aggregatum, Glomus etunicatum	Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with plant roots and **enhances the ability of the root system to absorb water and nutrients**.	4, 10, 11

	91.27% total inert ingredients		
GA_3	400 g/kg Gibberellic Acid	Accelerates seed germination, increases fruit size of pears and cherries. **Promote longitudinal growth**, the induction of hydrolytic enzymes in germinating seeds, the induction of bolting in long-day plants, **promotion of fruit setting and development**.	1, 2, 5, 7, 8, 9, 12, 13, 14, 16, 17
$GA_{4,7}$	10% GA_4 + GA_7 90% trade secret	Reduces russetting (a superficial disorder in which the fruit surface is interrupted by raised corky outgrowths) in apples, thereby improving size and shape.	1, 2, 5, 7, 8, 9, 12, 13, 14, 17
$GA_{4,7}$ + Benzyladenine	19 g/L Gibberellins A_4 and A_7 19 g/L Benzyladenine (cytokinin)	Reduces russetting (a superficial disorder in which the fruit surface is interrupted by raised corky outgrowths) in apples, thereby improving size and shape.	11, 16
Glycine betaine	97% glycine betaine (*N,N,N*-trimethylglycine) 3% water	A phytohormone-like compound that **adjusts the osmatic balance inside plant cells and tissues exposed to osmotic stress conditions and injury**. GB is synthesised in the chloroplast, peroxisome, and cytoplasm by a two-step oxidation reaction of choline (Hernandez-Leon and Valenzuela-Soto, 2023).	2, 3, 7, 9, 13, 15
MC	380 g/L Mepiquat present as the chloride salt	Gibberellin biosynthesis inhibitor. MC is a quaternary ammonium compound that inhibits cyclases involved in **early stages of GA metabolism**, which lead to the formation of *ent*-kaurene (Rademacher, 2015; Rademacher, 2017).	1, 6, 8, 14
NAA	20 g/L 1-Naphthylacetic Acid present as the sodium salt	A synthetic auxin used in many commercial rooting products to promote rooting from stem and leaf cuttings. Auxins stimulate cell elongation and cell division and are involved in phototropic and gravitropic reactions.	3, 4, 10, 11, 16
TEP	250 g/L Trinexapac-ethyl	**Inhibits late stages of GA biosynthesis**, through disruption of 2-oxoglutaric acid production. TEP also reduces ethylene formation and inhibits production of flavanone 3-hydroxylase, consequently interfering with flavonoid metabolism (Rademacher, 2015).	1, 6, 8, 14

Milestone 1. Are there cotton production challenges where novel/alternative agronomic practices could build resilience?

Milestone 1.1 Identify novel agronomic practices that require research validation

To identify novel agronomic practices that could require research validation, several consultations aimed to understand and identify opportunities for the use of novel plant growth hormones in dryland and water limited systems. This included the participation in Dryland Cotton Research Association (DCRA) and Cotton Seed Distributors (CSD) variety trial field days and industry meetings to engage with stakeholders and to further develop professional relationships initiated by the previous project leader.

A challenge for the Australian cotton industry is to maintain high lint quality and productivity under conditions of unpredictable climatic variability and unreliable water supply, particularly for dryland and water limited production systems. Consultations suggest that there are several novel plant growth hormones that may be useful in cotton production systems but are not currently registered for use in cotton. Altering crop development and canopy architecture through the application of different plant growth regulators may enable cotton growers to strategically manage their crops and facilitate a more resilient cotton system to abiotic stress. Whilst some research has already been conducted, there are opportunities to further understand how plant growth regulators may be utilised to mitigate limited water cropping. Experiments conducted throughout this research project have focussed on better understanding the growth and yield responses of cotton to the application of novel PGRs that could have the potential to build more resilient agricultural systems.

Milestone 1.2 Understand industry use of early-season mepiquat chloride in relation to current industry recommendations.

To understand industry use of early-season mepiquat chloride (MC), we engaged with consultants, growers and CottonInfo representatives throughout cotton growing regions to understand how MC is being used.

In July 2023, an online survey was conducted at the Crop Consultants Association (CCA) meeting to understand how consultants viewed the benefits of early-season MC use and what factors they take into consideration when making MC recommendations. There was a particular focus on capturing insights from consultants who were relatively new to the cotton industry. Ten respondents participated in the survey, with responses spanning different cotton regions. Three respondents had been in the cotton industry more than 5 years, four had been in the industry for 2-4 years and three had been in the industry 0-1 years (Table 5).

Table 5: Online survey engagement with consultants at the Crops Consultants Seminar in July 2023

Primary Cotton Region	Number of respondents	Years in the cotton industry
Central Qld	2	5+
Darling Downs	1	5+
St George/Dirranbandi	1	2-4
Gwydir	1	0-1
Upper Namoi	3	0-4
Lachlan/Murrumbidgee	2	2-4

Participants were asked two questions: *in your opinion, what are the main benefits of early season mepiquat chloride use;* and *what do you take into account when recommending early season mepiquat chloride?*

Responses from our survey showed that consultants considered the major benefit of early season MC to be reduced vegetative growth ranked 1st by 6 out of 10 respondents, followed by increased crop uniformity ranked 1st by 3 out of 10 respondents (Figure 3). This suggests that the primary focus of MC early is to manage canopy architecture.

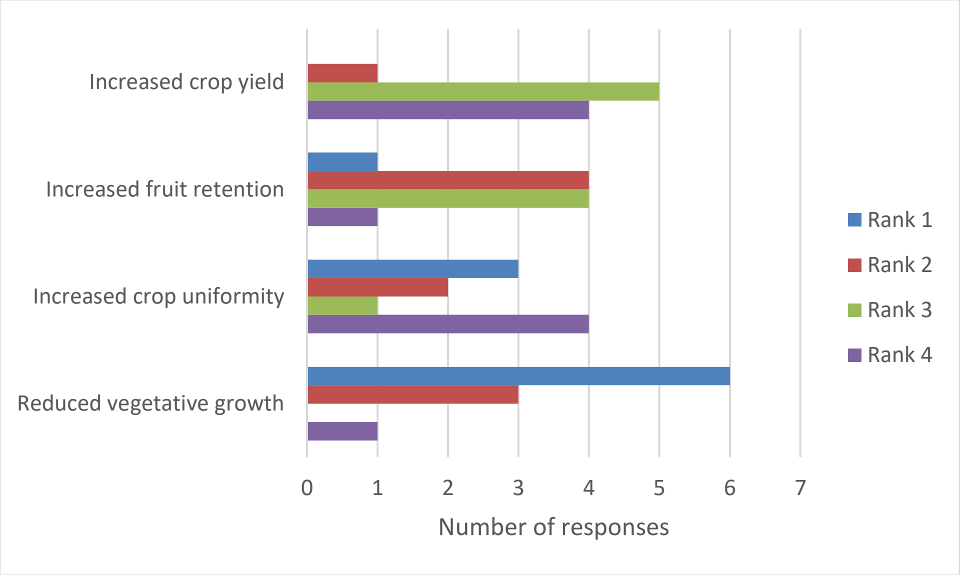


Figure 3: What consultants considered the main benefits of early season mepiquat chloride -use, ranked 1 and 2 (major benefit) to 3 and 4 (minor benefit).

Our survey also showed that consultants primarily take into consideration the weekly measurement of heights and nodes (vegetative growth rate) when making early season MC recommendations, ranked 1st by 6 out of 10 respondents and ranked 2nd by an additional 3 respondents (Table 6). Length of a specific internode also ranked highly as a consideration, with 8 out of 10 respondents ranking specific internode length in the top 3 considerations. This data also suggests that consultants were least likely to base early season MC recommendations on gut feel (ranked 6-8 by 9 out of 10 respondents) and label recommendations (ranked 6-8 by 8 out of 10 respondents). Therefore, it appears that consultants are making early season MC decisions based on plant measurements, rather than using prescriptive or "gut-feel" approaches.

Table 6: What consultants take into account when recommending early season mepiquat chloride , ranked most important (Rank 1) to least important (Rank 8).

	Rank 1	**Rank 2**	**Rank 3**	**Rank 4**	**Rank 5**	**Rank 6**	**Rank 7**	**Rank 8**
Weekly measurement of height and nodes (Vegetative Growth Rate)	6	3	0	1	0	0	0	0
The label recommendations	1	0	0	1	0	2	3	3
Length of specific internode	2	3	3	0	1	0	1	0
General crop inspection	1	0	2	3	2	2	0	0
Field history	0	2	2	0	5	1	0	0
Soil moisture	0	1	1	1	0	2	4	1

Rainfall and temperature forecast	0	1	2	3	2	2	0	0
Gut feel	0	0	0	1	0	1	2	6

Although the survey was limited by a small number of participants, it provided some useful and interesting insights into how the cotton industry was using early season MC in relation to current industry recommendations. These provided some preliminary discussion points to further explore how and why growers and consultants make agronomic decisions across key cotton regions.

We utilised CottonInfo to connect with consultants and growers across key cotton regions. Key regions included the Darling Downs, Moree/Gwydir, Narrabri/Wee Waa, Gunnedah/Aberdeen, and Griffith/Darlington Point. Discussions with consultants in the Darling Downs suggested that there was an interest in improving canopy management to facilitate defoliation efficacy in semi-irrigated, skip row cotton. Discussions at Aberdeen revealed that in the 2022/23 cotton season growers thought that, with hindsight, they should have applied MC earlier and harder than what they did. The growers had the ability to provide "unlimited" water (with respect to crop water requirements) via pivot irrigation, and the warm, humid environment promoted growth of the cotton crop. These growers were interested in applying high rates of MC earlier in the season to restrict vegetative growth and promote yield in a pivot irrigation system.

We attended the Coleambally Grower meeting (November 2023) to better understand the southern region farming system and constraints. Growers and consultants generously shared their knowledge and experience of growing cotton in southern regions, as part of a diverse agricultural system that also included fruit trees such as almonds or prunes (Figure 4 and Figure 5). The shorter season experienced in the southern region had cotton growers attempting to plant in September and establish the crop early. Overall, there was a keen interest in early MC applications and using MC to maintain a small, compact plant that matures early. We also discussed the importance of key dates for last effective flower (LEF) and cut-out that enabled the effective management of maturity of the crop. These engagement activities provided important insights into how and why MC was being applied across several key cotton regions and different cotton systems, and highlighted an appetite for better research on early-season MC strategies across key cotton regions.

Figure 4: Almond trees (left) and cotton fields (right) at Carrathool, NSW (22nd November 2023)

Figure 5: Prune trees (left) and cotton fields (right) at Darlington Point, NSW (23rd November 2023)

Milestone 2. Can cotton production resilience be improved with strategic application of specific hormones/plant growth regulators (PGRs)?

To understand how strategic application of specific hormones/PGRs could affect the resilience of cotton production, three glasshouse experiments were conducted at the Australian Cotton Research Institute (ACRI) and a number of field experiments were conducted during the 2020-21, 2021-22 and 2022-23 cotton seasons. Further detail on the research questions investigated are described in the milestones below.

Milestone 2.1 Extend and validate hormone/PGR approaches deemed as potential opportunities from recently completed thesis research to broaden industry application.

Introduction

Temporarily delaying peak flowering may stabilise cotton yield and improve downside risk associated with crop establishment outside the optimum planting window by avoiding peak flowering during periods of hot conditions. Delays in peak flowering may be achieved by (a) chemically applying gibberellic acid inhibitors to slow vegetative and reproductive growth; (b) chemically removing early fruit via ethylene precursors but retaining the leaf and stem; (c) manually removing early fruit, or whole portions of the plant; and (d) chemically delaying floral initiation and reproductive development but retaining early vegetative growth rates to preserve leaf area and stem growth. Delays in peak flowering may also be achieved by delaying sowing date, however, may not be practical in rainfed systems if growers are able to take advantage of an "early break" in rainfall.

Previous research (Chapter 6 of thesis: "Dynamic Fruiting" Gibberellin Biosynthesis Inhibiting Plant Growth Regulators to Shift Timing of Crop Peak Resource Demand) identified that the application of the gibberellin biosynthesis inhibitor, Trinexapac-ethyl (TEP), may delay reproductive development of cotton (Welsh, 2020). These studies found that with applications of TEP, total and reproductive biomass, and the number and size of green bolls, were decreased in early flowering of cotton. However, further research to understand rate, timing and implications across cotton seasons was required.

Mepiquat chloride (MC), is also a gibberellin biosynthesis inhibitor, and is currently widely used in Australian cotton production systems for the control of excessive vegetative growth and indirectly induce physiological cut-out in high-input irrigated systems. Therefore, early season use of MC may inhibit vegetative growth and/or development of dryland cotton.

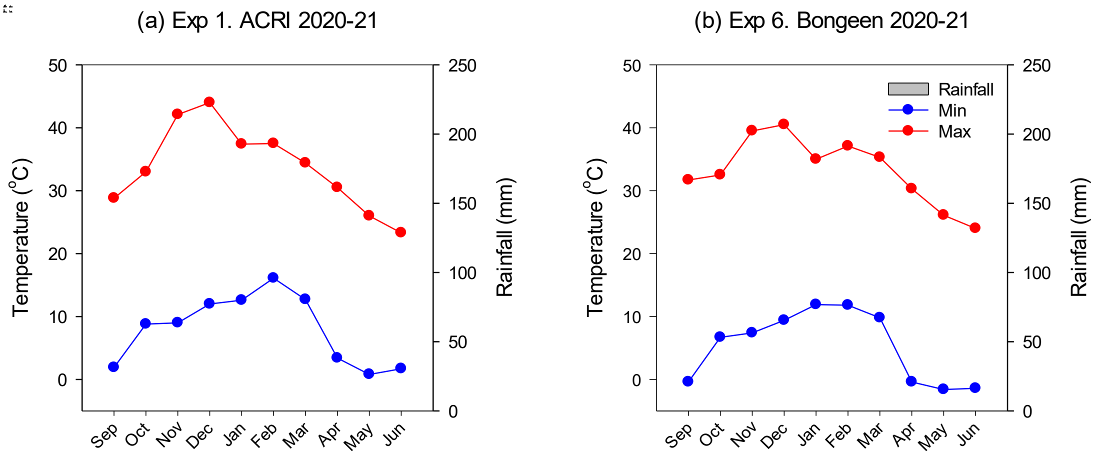
The effects of TEP and MC on dryland cotton production have not been widely tested in the field. The first steps towards broadening this research to industry application involve understanding if the application of gibberellic acid inhibitors can delay development of cotton in the field and the rate of application. This study evaluates the use of TEP, MC and physical fruit removal to delay flowering in rainfed cotton systems.

Research questions

1. Can TEP delay flowering in dryland cotton systems?
2. What are the implications of delayed flowering for maturity, yield and quality?
3. How do delays caused by TEP compare with early-season use of MC or pruning (mechanical cut) of the plant?

Materials and Methods

Field experiments were conducted over three cotton seasons: 2020-21, 2021-22, and 2022-23. In 2020-21, experiments were conducted at ACRI, NSW, and Bongeen, Qld. In the 2021-22 and 2022-23 cotton seasons, field experiments were conducted at ACRI. Experiments in Queensland were abandoned due to COVID-19 restrictions in the 2021-22 season and due to labour limitations in the 2022-23 cotton season. Relevant climate data is shown in Figure 6 and Table 3. Treatments for each experiment are summarised in Table 7. Plant growth regulators were applied at the 4-leaf stage, and the mechanical cut treatment occurred at first flower. Plant development (including the number of days from planting to first square, first flower, and maturity (time to 60% open boll)), yield and quality were measured as summarised in Table 8.


(a) Exp 1. ACRI 2020-21

(b) Exp 6. Bongeen 2020-21

(c) Exp 8. ACRI 2021-22

(d) Exp 14. ACRI 2022-23

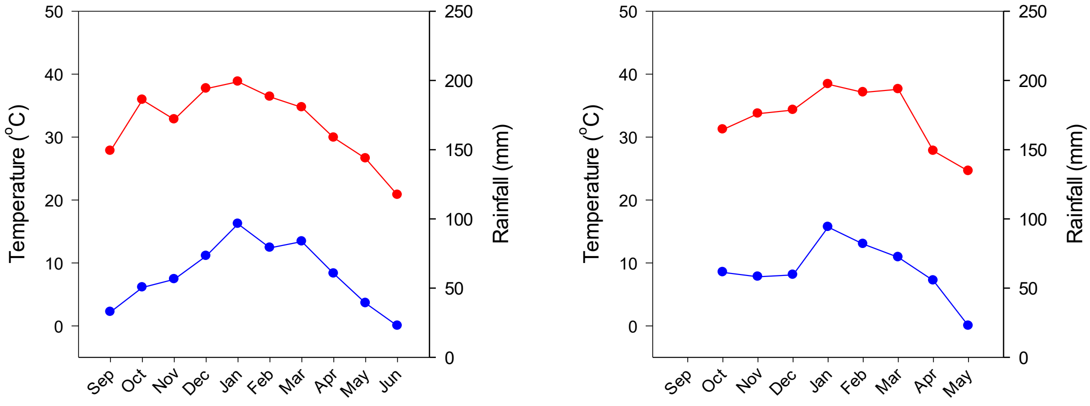


Figure 6: Monthly minimum (blue) and maximum (red) temperatures and rainfall (grey) for experiment 1 (a), experiment 6 (b), experiment 8 (c), and experiment 14 (d).

Table 7: Treatment summary of experiments relevant to Milestone 2.1

Experiment Details	Trt Abbreviation	Treatment	Rate (a.i.; ppm)	Frequency	Surfactant	Surfactant Rate	Application Dates
2020-21 ACRI (Exp 1)							
Cotton Season: 2020-21	Control	Control	-	-	-	-	
Location: ACRI, Narrabri	TEP(1x)	Trinexapac-ethyl	2000	1x	No	N/A	10/11/2020
Experiment: DLS1	TEP(2x)	Trinexapac-ethyl	2000	2x7d	No	N/A	10/11/20, 17/11/20
Planting Date: 17/09/2020	TEP(3x)	Trinexapac-ethyl	2000	3x7d	No	N/A	10/11/20, 17/11/20, 24/11/20
Row Configuration: 2m, alt.	MC(3x)	Mepiquat chloride	60	3x7d	Yes	3 drops	10/11/20, 17/11/20, 24/11/20
	mech.cut	Mechanical cut to just over 9n at FF	-	-	-	-	5/1/21
2020-21 Bongeen (Exp 6)							
Cotton Season: 2020-21	Control	Control	-	-	-	-	
Location: Bongeen, Qld	TEP(3x)	Trinexapac-ethyl	2000	3x10d	No	-	18/12/20, 29/12/20, 11/1/21
Planting Date: 20/11/2020	mech.cut	Mechanical cut to just over 9n at FF	-	Once	-	-	1/2/21
Row Configuration: Single Skip	MC(3x)	Mepiquat chloride	60	3x10d	Yes	3 drops	18/12/20, 29/12/20, 11/1/21
2021-22 ACRI (Exp 8)							
Cotton Season: 2021-22	Control	Control	-	-	-	-	
Location: ACRI, Narrabri	TEP(1x)	Trinexapac-ethyl	2000	1x	No	N/A	1/11/21
Experiment: DLS1	TEP(2x)	Trinexapac-ethyl	2000	2x7d	No	N/A	1/11/21, 9/11/21
Planting Date: 17/09/2021	TEP(3x)	Trinexapac-ethyl	2000	3x7d	No	N/A	1/11/21, 9/11/21, 16/11/21
Row Configuration: 2m, alt.	MC(3x)	Mepiquat chloride	60	3x7d	Yes	3 drops	1/11/21, 9/11/21, 16/11/21
	mech.cut	Mechanical cut to just over 9n at FF	-	-	-	-	4/1/22
2022-23 ACRI (Exp 14)							
Cotton Season: 2022-23	Control	Control	-	-	-	-	
Location: ACRI, Narrabri	TEP(1x)	Trinexapac-ethyl	2000	1 only	No	N/A	18/11/2022
Experiment: DLS1	TEP(2x)	Trinexapac-ethyl	2000	2x7d	No	N/A	18/11/22, 28/11/22
Planting Date: 5/10/2022	TEP(3x)	Trinexapac-ethyl	2000	3x7d	No	N/A	18/11/22, 28/11/22, 5/12/22
Row Configuration: Single Skip	MC(3x)	Mepiquat chloride	60	3x7d	Yes	3 drops	18/11/22, 28/11/22, 5/12/22
	mech.cut	Mechanical cut to just over 9n at FF	-	-	-	-	6/1/23

Table 8: Measurement summary

Relevant Exp ID	Cotton Season	Location	Time to first square (TTFS)	Time to first flower (TTFF)	Maturity	Yield	Quality
1	2020-21	ACRI	×	×	✓	✓	✓
6	2020-21	Bongeen	×	×	×	✓	✓
8	2021-22	ACRI	✓	✓	✓	✓	✓
14	2022-23	ACRI	✓	✓	✓	✓	✓

Results

1. Treatment effects on cotton flowering and development

Squaring (time to first square (TTFS)) and flower (time to first flower (TTFF)) development were measured in Experiments 8 (2021-22) and 14 (2022-23, Table 8). The application of TEP delayed squaring and flowering in both experiments (Table 9). Squares first appeared between 80-89 days after planting (DAP) in Experiment 8 and earlier in Experiment 14 (68-88 DAP). While there was no Control data in Experiment 8, application of TEP (2x) and TEP (3x) significantly delayed squaring in relation to TEP (1x) by 3 and 9 days, respectively. In Experiment 14, the delay in squaring was again significant for TEP (2x) and TEP (3x) in relation to both TEP (1x) and the Control (17 & 19 days and 18 & 20 days, respectively). Consequently, flowering was also delayed by application of TEP. Compared with the Control, TEP (2x) delayed flowering by 5 days in Experiment 8, and by 12 days in Experiment 14. TEP (3x) increased the delay further to 7 and 16 days for Experiments 8 and 14, respectively.

Table 9: Time to first square (TTFS) and time to first flower (TTFF). Values represent the mean and the standard error and change relative to the control. Different letters (within each season and variable) represent significant difference at P< 0.05 using Tukey's HSD. Note that TTFS and TTFF were not measured in the controls in the 2020-21 cotton season.

Cotton Season	Treatment	TTFS (days)	SE	ΔTTFS from Control	TTFF (days)	SE	ΔTTFF from Control
2021-22 season (Exp 8)							
	Control	-	-	-	100^{a}	0.6	-
	TEP (1x)	80^{a}	0.3	-	103^{b}	0.9	3
	TEP (2x)	83a^{b}	1.7	-	105^{bc}	0.9	5
	TEP (3x)	89^{b}	2.5	-	107^{c}	1.0	8
	MC (3x)	-	-	-	98^{a}	0.6	-2
	P-value	0.038			0.001		
2022-23 season (Exp 14)							
	Control	68^{a}	0.9	-	94^{a}	2.1	-
	TEP (1x)	70^{a}	2.1	2	93^{a}	1.5	-1
	TEP (2x)	87^{b}	1.5	20	106^{b}	1.5	12
	TEP (3x)	88^{b}	5.6	20	110^{b}	1.7	17
	MC (3x)	70^{a}	2.6	3	94^{a}	1.9	0
	P-value	0.001			0.001		

2. Treatments effects on cotton maturity, yield and quality

Time to maturity (the number of days after planting to 60% open Boll) was measured in Experiments 1 (2020-21), 8 (2021-22) and 14 (2022—23, Table 8). TEP (3x) delayed maturity across all three seasons but not always significantly (Table 10). In Experiment 1, the delay from the control was 7 days which was marginal, however, in Experiments 8 and 14, maturity delays were significant at 13 and 27 days, respectively. In comparison, TEP

(1x) and TEP (2x) produced only small delays from the Control in Experiment 1 (0-1 day, n.s.), a week in Experiment 8 (6-7 days, n.s.) and a differentiated delay in Experiment 14 (TEP (1x) – 9 days, n.s.); TEP (2x) – 22 days, **). Maturity was delayed by 23 to 36 days compared with the Control in Experiments 14 and 8 due to the severe damage caused to the plants at first flower.

Yield was measured in all four experiments and was highly variable across seasons ranging from 2.1 to 6.3 bales/ha (Table 11). Overall, the application of TEP reduced cotton yield relative to the control plants. At Bongeen (Experiment 6), the application of TEP (3x) reduced yield by 42% relative to the control. In the same season, the reduction at ACRI (Experiment 1) for TEP (3x) was only 17% (n.s.) while fewer applications of TEP (2x & 1x) did little to alter this (18 & 17 % loss compared to Control). In the 2021-22 season (Experiment 8), TEP application did not affect yield. TEP application significantly affected yield in Experiment 14 (2022-23) when TEP (2x) and TEP (3x) reduced yields by 24 and 22%, respectively.

It was difficult to discern quality effects on lint, such as length, strength, micronaire and uniformity, as these can be affected by other factors as well. There was no consistent trend in relation to TEP application (Table 12) with most measured parameters falling into the Australian base grade.

Table 10: Time to maturity (number of days after planting to 60% open boll).

Treatment	**60% open boll (DAP)**	**SE**	**Δ from control**
2020-21 cotton season (Exp 1)			
Control	177^{ab}	1.91	-
TEP(1x)	178^{ab}	1.87	1
TEP(2x)	177^{ab}	2.65	-1
TEP(3x)	184^{b}	1.82	7
MC(3x)	176^{a}	1.61	-2
mech.cut	-	-	-
P-value	0.043		
2021-22 cotton season (Exp 8)			
Control	191^{ab}	4.17	-
TEP(1x)	197^{bc}	2.36	5
TEP(2x)	198^{bc}	1.73	6
TEP(3x)	204^{c}	1.90	13
MC(3x)	185^{a}	2.20	-7
mech.cut	227^{d}	-	36
P-value	0.001		
2022-23 cotton season (Exp 14)			
Control	156^{a}	0.95	
TEP(1x)	165^{abc}	5.21	9
TEP(2x)	178^{bcd}	2.39	22
TEP(3x)	183^{d}	3.18	27
MC(3x)	163^{ab}	2.39	7
mech.cut	179^{cd}	4.82	23
P-value	0.001		

Table 11: Yield (b/ha) of cotton. Values represent the mean, standard error and relative change from the control (%).

Cotton Season	**Treatment**	**Yield (b/ha)**	**SE**	**Δ from Control (%)**
2020-21 Bongeen (Exp 6)				
	Control	3.6^{b}	0.4	NA

	TEP (3x)	2.1^{a}	0.1	-42
	MC	4.0^{b}	0.2	12
	mech.cut	2.6^{a}	0.2	-27
	P-value	0.001		
2020-21 ACRI (Exp 1)				
	Control	3.0	0.4	
	TEP (1x)	2.4	0.4	
	TEP (2x)	2.4	0.2	
	TEP (3x)	2.5	0.2	
	MC	2.8	0.2	
	mech.cut	2.6	0.3	
	P-value	0.855		
2021-22 ACRI (Exp 8)				
	Control	6.0	0.3	
	TEP (1x)	6.0	0.2	
	TEP (2x)	5.9	0.1	
	TEP (3x)	5.5	0.5	
	MC	6.1	0.4	
	mech.cut	6.3	0.2	
	P-value	0.133		
2022-23 ACRI (Exp 14)				
	Control	4.0^{c}	0.1	NA
	TEP (1x)	3.5^{abc}	0.2	-12
	TEP (2x)	3.0^{a}	0.1	-24
	TEP (3x)	3.1^{ab}	0.1	-22
	MC	3.7^{bc}	0.2	-8
	mech.cut	3.6^{abc}	0.2	-11
	P-value	0.001		

Table 12: Lint quality of cotton. Values represent mean. P-value significant at the P< 0.05 level using Tukey's HSD.

Treatment	Staple	Length	Micronaire	Uniformity	Strength	SFI	Elongation
2020-21 Bongeen (Exp 6)							
Control	36	1.12	4.7	82.1^{a}	30.5^{ab}	7.8^{a}	4.9^{ab}
TEP(3x)	36	1.11	4.8	83.4^{b}	309^{ab}	6.4^{a}	5.1^{b}
MC(3x)	37	1.14	4.8	83.0^{ab}	31.8^{b}	6.6^{a}	5.0^{b}
mech.cut	36	1.12	4.8	82.7^{ab}	29.9^{a}	8.0^{a}	4.7^{a}
P-value	0.274	0.206	0.238	0.043	0.010	0.031	0.013
2020-21 ACRI (Exp 1)							
Control	35^{a}	1.10^{a}	4.8^{b}	82.3	30.1^{ab}	9.0	5.2^{ab}
TEP(1x)	35^{a}	1.09^{a}	4.9^{b}	82.0	30.3^{ab}	10.0	5.1^{a}
TEP(2x)	36^{ab}	1.11^{a}	4.8^{b}	82.2	31.2^{b}	8.5	5.2^{ab}
TEP(3x)	36^{ab}	1.11^{a}	4.9^{b}	82.0	30.7^{ab}	9.0	5.1^{a}
MC(3x)	34^{a}	1.07^{a}	4.8^{b}	81.4	29.6^{ab}	9.8	5.2^{ab}
mech.cut	37^{b}	1.16^{b}	3.3^{a}	82.1	29.3^{a}	8.9	5.4^{b}
P-value	0.001	0.001	0.001	0.802	0.046	0.343	0.031
2021-22 ACRI (Exp 8)							
Control	38	1.19^{ab}	4.9^{b}	83.7	31.5	6.3	4.8^{a}
TEP(1x)	38	1.19^{ab}	4.8^{ab}	83.9	31.4	6.0	5.2^{a}
TEP(2x)	38	1.20^{ab}	4.8^{ab}	83.3	30.9	5.2	6.9^{a}
TEP(3x)	38	1.17^{ab}	4.8^{ab}	83.0	30.9	6.1	5.8^{a}

MC(3x)	39	1.21^{b}	4.9^{b}	83.8	31.9	5.7	5.3^{a}
mech.cut	37	1.16^{a}	4.5^{a}	83.6	32.1	5.1	7.3^{a}
P-value	0.063	0.024	0.024	0.617	0.431	0.474	0.037
2022-23 ACRI (Exp 14)							
Control	36	1.10	4.9	82.3	29.4^{a}	8.6	4.2
TEP(1x)	36	1.10	4.9	82.4	29.3^{a}	9.0	4.3
TEP(2x)	35	1.10	4.8	81.9	29.1^{a}	8.6	4.3
TEP(3x)	36	1.11	4.7	82.3	29.9^{ab}	8.3	4.4
MC(3x)	36	1.13	4.9	82.6	31.3^{b}	8.0	4.2
mech.cut	35	1.09	4.9	82.4	30.0^{ab}	8.6	4.3
P-value	0.380	0.102	0.107	0.833	0.016	0.569	0.208

3. Comparison of TEP treatment effects with early-season use of Mepiquat Chloride or pruning (mechanical cut) of the plant

All experiments were also treated with MC (3x – standard application practice) and a mechanical pruning of plants to 9 nodes at first flower, to compare with effects caused by TEP.

In Experiment 8, MC treatments (3x) flowered 2 days (n.s.) earlier than the Control and significantly earlier than all TEP treatments (3, 5 and 8 days for TEP 1x, 2x and 3x, respectively, Table 9). In Experiment 14, MC (3x) began squaring 2 days (n.s.) after the Control, at the same time as TEP (1x), and a significant 12 and 17 days earlier than TEP (2x) and (3x). This pattern and delay extended into flowering.

Across Experiments 1, 8 and 14, the application of MC (3x) had no significant effect on maturity compared with Controls although the trends varied across the seasons. Relative to the control, the application of MC altered maturity between -7 and 7 days (Table 10). In relation to TEP treatment, MC (3x) matured earlier across all three experiments. In Experiment 1, TEP (3x) matured significantly later (8 days) than MC (3x). In Experiment 8, the significant delays compared to MC (3x) were 12, 13 and 19 days for TEP (1x, 2x and 3x), respectively, and in Experiment 14, TEP (2x and 3x) had significant delays of 15 and 18 days.

Mechanical cut treatments significantly delayed maturity in relation to the Control across all experiments: by 36 and 23 days for Experiments 8 and 14, and for Experiment 1, delays were such that 60% maturity was not reached (Table 10). This was due to the crop being defoliated prior to the mechanical cut treatment reaching 60% open boll in Experiment 1. In relation to TEP, mechanical cutting in Experiment 8 also significantly delayed maturity and was 30, 29 and 23 days late compared to the TEP 1x, 2x, and 3x treatments. Mechanical cutting caused no significant delays relative to TEP treatments in Experiment 14.

Relative to the Control, the application of MC (3x) did not significantly affect the yield in any of the experiments though there was a trend across years indicating that MC (3x) had a slight yield advantage over TEP treatments.

Mechanical cutting decreased yield compared to the Control in Experiments 1, 6, and 14 but this was only significant in Experiment 6 (Bongeen, Table 11) with a reduction of 27% (1 bale/ha). Compared to the TEP (3x) treatment, the reduction was also significant at 12% (0.5 bale/ha). The trend in a small yield advantage over TEP treatment was also apparent for the mechanical cutting treatment.

Mechanical cutting had a significant effect on staple, length and micronaire in Experiment 1 in relation to the Control and some TEP treatments (Table 12). Staple was somewhat longer than for medium cotton, length was 37 rather than the base grade of 36 and micronaire was

G4 rather than the base grade of G5. In both Experiments 1 and 14, length was below the base grade of 36, however, there was no discernible pattern with regards to hormone treatment.

Discussion

The effects of TEP on cotton

Our data indicate that early season application(s) of TEP can delay development (TTFS, TTFF, and maturity) of dryland cotton in the field. Our data showed that three applications of TEP delayed flowering more than a single application, with delays in flowering up to 20 days later than control plants. Flow on effects resulted in delays in maturity up to 27 days later than control plants, however, this was largely dependent on the cotton season. These results are consistent with Kupke et al. (2022), where TEP was found to also delay time to flowering in barley.

Application of TEP had the potential to decrease yield compared with control plants. This may be dependent on weather conditions. In particularly cold seasons, the delay in development may result in the plant being unable to mature the fruit prior to the season ending, contributing to significantly lower yields, although there were no detrimental effects on fibre quality. Further research should also explore a broader range of temperatures and environmental conditions to better understand decision-support for applying TEP as a risk management strategy in cotton systems.

The effects of MC on cotton

MC affected growth (data not shown), but not development of cotton. Compared with control plants, there were no significant differences in TTFS, TTFF or maturity across any of the experiments. The effects of MC on yield were variable, however, there were no significant differences in the yield or quality of cotton applied with MC compared to the control. This demonstrates the effect of how MC and TEP interfere with different stages of GA biosynthesis. MC inhibits cyclases involved in early stages of GA metabolism, which lead to the formation of *ent*-kaurene (Figure 2). In comparison, TEP inhibits the late stages of GA biosynthesis (Rademacher, 2015; Rademacher, 2017). Our data highlights the importance of understanding the action of these products, to be able to utilise the products effectively and efficiently. Therefore, MC does not appear to be a suitable gibberellic acid inhibitor for delaying development of early-sown dryland cotton.

The effects of mechanical cut on cotton

The mechanical cut treatment to approximately nine nodes at first flower radically reduced cotton growth and ultimately delayed maturity up to 36 days. The delay in maturity of the bolls resulted in lower yields. Yield was significantly reduced by 27% in ¼ experiments (Experiment 6), however, the trend in the mechanical cut treatment reducing yield compared with the control was evident across ¾ experiments. It is interesting that the mechanical cut treatment for Experiment 8 resulted in the longest delay in time to maturity (36 days) yet had no significant difference in yield compared with the control. This likely reflects seasonal conditions, whereby having sufficient time to mature the crop is crucial when stimulating a developmental delay. Furthermore, the mechanical cut treatment also resulted in some changes to fibre quality parameters. Staple and length of fibre from the mechanical cut treatment were increased in Experiment 1, and micronaire was decreased in both Experiment 1 (3.3) and Experiment 8 (4.5). Optimal micronaire is between 3.8 and 4.5 (Bange et al., 2008). Therefore, there is a risk that delaying maturity using the mechanical cut method may decrease micronaire, due to defoliation of the mechanical cut treatment occurring too early and running out of season length.

Summary

Four field experiments were conducted to investigate if TEP can delay flowering in dryland cotton systems. Our data showed that multiple applications of TEP could delay flowering by up to 20 days, and delay maturity up to 27 days. In comparison, early season application of

MC was ineffective as a method of delaying development, whereas pruning of the plant at flowering had the potential to cause longer delays in maturity, up to 23 days later than the TEP treatments. The application of TEP had the potential to decrease yield, particularly if a cold finish to the season resulted in the plants unable to mature fruit. Understanding how using TEP can shift the timing of peak flowering to avoid periods of high temperatures was not fully tested. The seasons that TEP was tested were cooler and wetter than average, and responses may be different in average or hotter and dryer seasons. Therefore, further research should explore a broader range of temperatures and environmental conditions through both experimental research and modelling to better understand decision-support for applying TEP as a risk management strategy in cotton systems, in conjunction with further consultation with growers and consultants to determine viable agronomic practice.

Milestone 2.2 Develop broader understanding and impact of mechanisms that drive the change between vegetative and reproductive growth and establish whether PGRs can play a role in managing this.

Introduction

Plants have two major stages of development, vegetative and reproductive, with distinct structures produced at each stage. Most of the changes associated with vegetative to reproductive transitions in annual plants are unidirectional; once plants enter the adult vegetative phase they continue with the reproductive phase. In contrast, perennial plants such as cotton, alternate between the adult vegetative and reproductive phases. Hormones have important roles in promoting phase transitions, such as seed germination, seedling morphogenesis and floral induction, which suggests that they may contribute to phase change.

Considerable research has been conducted to study phase change and gene expression in the model plant, *Arabidopsis thaliana*. The best-known hormone that controls vegetative phase change is gibberellic acid (GA) (Manuela and Xu, 2020). GA contributes to vegetative phase change through the release of SPLs (SQUAMOSA promoter binding protein-like transcription factors) from their inhibitory interaction with the DELLA proteins.

SQUAMOSA promoter-binding protein-like (SPL) genes encode plant-specific transcription factors that play important roles in plant phase transition, flower and fruit development, plant architecture and gibberellins signalling. In Arabidopsis, many SPL genes are post-transcriptionally regulated by the microRNA (miRNA), miRNA156. miR156 expression changes during vegetative development with high expression levels in juvenile leaves and low expression levels in adult leaves.

The effects of GA in controlling phase change depend on the species (Raihan et al., 2021). Eriksson et al. (2006) demonstrated that in Arabidopsis, GA_4 is the most active GA in the floral induction. Furthermore, they found that GA_4 was the most abundant of all tested GAs in the shoot apex, and the endogenous GA_4 levels increased up to 100-fold just before floral initiation in Arabidopsis plants. Thus, the implication is that endogenous GA_4 functions as a critical part of the florigenic signal. However, the effects of gibberellic acid or inhibiting gibberellic acid pathways on development of field grown cotton plants are largely unexplored. To gain a better understanding of the mechanisms that cause changes between vegetative and reproductive growth of cotton, our experiments compared the use of two gibberellins (GA_3 and $GA_{4,7}$) and two gibberellin inhibitors (Trinexapac-ethyl, TEP and mepiquat chloride, MC) on the growth and development of cotton.

Described below, experiments 1 and 2 compared two gibberellic acids with gibberellic biosynthesis inhibitors to assess the role that gibberellins may have in the switch from juvenility to floral initiation. Experiments 3 and 4 compared the effects of two gibberellic acids on floral initiation across dryland planting times. A glasshouse experiment explored treatment scenarios through testing optimal application rates of Trinexapac-ethyl. Considerable research has been conducted to explore gene expression and biochemical pathways of gibberellic acid on plants.

Experiments 1 and 2: Comparison of two gibberellic acids with gibberellic biosynthesis inhibitors to assess the role that gibberellins may have in the switch from juvenility to floral initiation

Research Question: Do PGR's either promoting or inhibiting gibberellins affect the switch from vegetative to reproductive growth of cotton?

Materials and Methods

Field experiments were conducted across the 2021-22 and 2022-23 cotton seasons at ACRI, Narrabri. Cotton (cv. Sicot 746B3F) were planted in the dryland sowing 1 (DLS1) experiments. Gibberellic acid (GA_3 and $GA_{4,7}$) and gibberellic acid inhibitors (TEP and MC) were applied either at the 2- or 4- leaf stage as outlined in Table 13. Growth, development, biomass, and plant mapping were measured as outlined in Table 14.

Table 13: Treatment summary addressing Milestone 2.2

Experiment Details	Trt Abbreviation	Treatment	Rate (a.i.; ppm)	Frequency	Timing	Surfactant	Surfactant Rate	Application Dates
2021-22 ACRI (Exp 8)								
Cotton Season: 2021-22	Control	Control	-	-	-	-	-	
Location: ACRI, Narrabri	TEP(1x)	Trinexapac-ethyl	2000	1x	4L	No	N/A	1/11/21
Experiment: DLS1	TEP(2x)	Trinexapac-ethyl	2000	2x7d	4L	No	N/A	1/11/21, 9/11/21
Planting Date: 17/09/2021	TEP(3x)	Trinexapac-ethyl	2000	3x7d	4L	No	N/A	1/11/21, 9/11/21, 16/11/21
Row Configuration: 2m, alt.	MC(3x)	Mepiquat chloride	60	3x7d	4L	Yes	3 drops	1/11/21, 9/11/21, 16/11/21
	GA$_3$	Gibberellic acid 3	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	21/10/21, 1/11/21, 9/11/21
	GA$_{4,7}$	Gibberellic acid 4,7	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	21/10/21, 1/11/21, 9/11/21
2022-23 ACRI (Exp 14)								
Cotton Season: 2022-23	Control	Control	-	-	-	-	-	
Location: ACRI, Narrabri	TEP(1x)	Trinexapac-ethyl	2000	1 only	4L	No	N/A	18/11/2022
Experiment: DLS1	TEP(2x)	Trinexapac-ethyl	2000	2x7d	4L	No	N/A	18/11/22, 28/11/22
Planting Date: 5/10/2022	TEP(3x)	Trinexapac-ethyl	2000	3x7d	4L	No	N/A	18/11/22, 28/11/22, 5/12/22
Row Configuration: Single Skip	MC(3x)	Mepiquat chloride	60	3x7d	4L	Yes	3 drops	18/11/22, 28/11/22, 5/12/22
	GA$_3$	Gibberellic acid 3	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	3/11/22, 11/11/22, 21/11/22
	GA$_{4,7}$	Gibberellic acid 4,7	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	3/11/22, 11/11/22, 21/11/22

Table 14: Measurement summary addressing Milestone 2.2

Relevant Exp ID	Cotton Season	Location	Growth	Development	Biomass	Plant Mapping
8	2021-22	ACRI	×	✓	×	×
14	2022-23	ACRI	✓	✓	✓	✓

Results

Development

Fruit development was altered by the application of PGRs. During the 2022-23 cotton season (Exp 14), days from planting to first square ranged from 68 – 88 DAP (Figure 7). Time to first square was significantly delayed by the application of only some of the Gibberellin inhibitors, TEP (2x) and TEP (3x) ($P< 0.001$). However, TEP (1x) and MC did not significantly delay time to first square (2 – 3 days for both TEP (1x) and MC) compared with the control. The application of gibberellins did not significantly delay squaring compared with the control. However, relative differences compared with the control indicated a consistent delay in squaring, by 6 days with the application of GA$_{4,7}$ and 14 days with the application of GA$_3$. In the 2021-22 cotton season (Exp 8), plants were applied with GA$_3$ squared later than plants applied with TEP (3x) (102 DAP compared with 89 DAP, respectively). It was also noted that plants applied with PGR treatments tended to have greater variability of the mean time to first square than control plants.

..

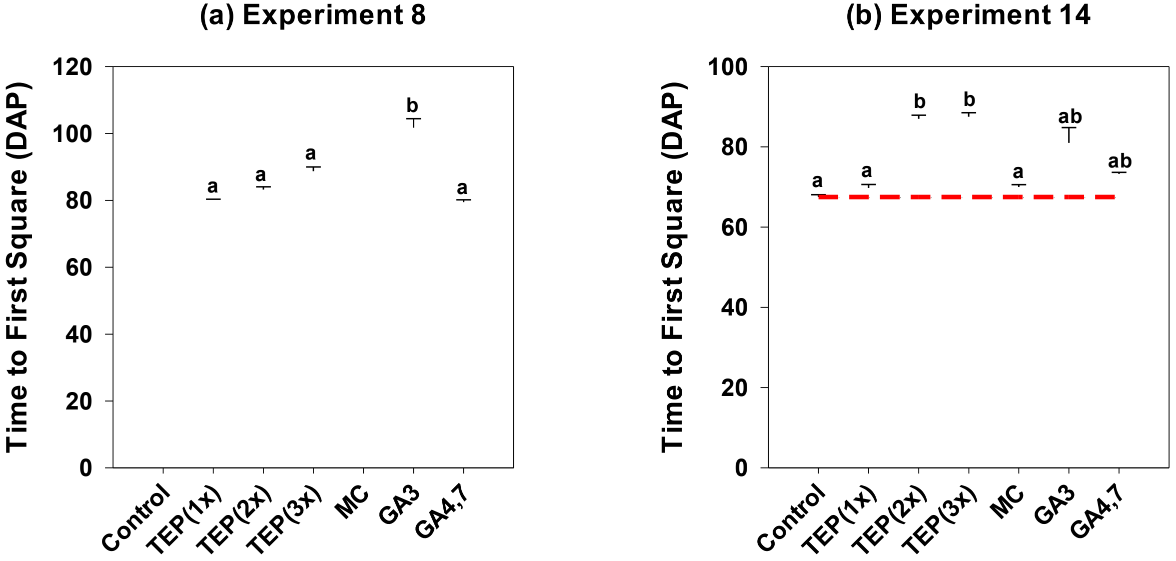


Figure 7: Average number of days from planting to first square (DAP) of cotton applied with gibberellin biosynthesis inhibitor and gibberellin PGR treatments for two cotton seasons (a and b). Values represent mean ± SE. Red dotted line represents the mean of the control. Different letters represent significant differences at the $P< 0.05$ level using Tukey's HSD.

The effect of PGRs on plant growth at squaring

The application of PGRs altered the height and number of nodes of cotton measured at 77 DAP. The application of gibberellin inhibitors reduced plant by 33 – 70%, relative to the control. The response to application of gibberellins was variable. Cotton applied with GA$_3$ were 12% taller, relative to the control. However, the application of GA$_{4,7}$ significantly reduced plant height by 22% compared with the control.

The application of gibberellin inhibitors reduced the number of nodes, relative to the controls, ranging a 1 – 13% reduction. The application of TEP (2x) (-10%), TEP (3x) (-13%) and MC (-10%) significantly reduced the number of nodes compared with the controls. In contrast, the application of either GA$_3$ or GA$_{4,7}$ increased the number of nodes by 6% across both treatments, relative to the control.

The effect of PGRs on vegetative and reproductive growth at squaring

PGR treatment had a significant effect on the vegetative and fruit biomass of cotton ($P< 0.05$). Vegetative biomass of cotton applied with gibberellin inhibitors (TEP or MC) had less vegetative biomass than control plants. Cotton applied with TEP (2x) or TEP (3x) had 66 - 73% less vegetative biomass than control plants ($P< 0.05$, Table 15). Although differences were not significant, vegetative biomass of cotton applied with TEP (1x) and MC were 31 and 33% lower relative to the control. Cotton applied with Gibberellins GA_3 or $GA_{4,7}$ also had significantly less vegetative biomass compared with control plants ($P< 0.05$; 61 and 55%, respectively). The reductions in total vegetative biomass are reflected in lower stem and leaf biomass and less leaf area of cotton that was applied with the PGR treatments (Table 15).

Generally, fruit biomass was decreased with the application of PGRs, except for the TEP (1x) treatment. Cotton treated with TEP (1x) had 34% more fruit biomass relative to the control, however, the difference was not significant. However, plants treated with TEP (3x) had significantly (99%) less fruit biomass than control plants. Fruit biomass of cotton applied with gibberellin inhibitors was generally reduced with the application of PGR compared with the control. Cotton applied with gibberellins also had less fruit biomass relative to the control. The application of GA_3 or $GA_{4,7}$ significantly reduced fruit biomass 92 and 58%, respectively ($P<0.05$; Table 15).

Table 15: Mean biomass for Exp 14 at 77 DAP (21/12/22) of cotton applied with plant growth hormones. Values represent the mean. Different letters represent significant differences at P< 0.05 using Tukey's HSD.

	P-value	**Control**	**TEP(1x)**	**TEP(2x)**	**TEP(3x)**	**MC(3x)**	**GA_3**	**$GA_{4,7}$**
Height (cm plant^{-1})	0.001	14^{d}	10^{bc}	6^{a}	4^{a}	8^{b}	16^{d}	11^{c}
Nodes (plant^{-1})	0.001	12^{bc}	12^{b}	11^{a}	11^{a}	11^{a}	13^{c}	13^{bc}
Leaf Area (cm^2/m)	0.002	2234^{b}	1506^{ab}	672^{a}	498^{a}	1463^{ab}	647^{a}	916^{a}
Leaf (g/m)	0.001	25^{c}	18^{bc}	9^{ab}	6.6^{a}	18^{bc}	8^{ab}	11^{ab}
Stem (g/m)	0.004	18^{b}	12^{ab}	6^{a}	5^{a}	11^{ab}	8^{a}	8^{a}
Total vege biomass (g/m)	0.001	43^{b}	30^{ab}	15^{a}	12^{a}	29^{ab}	17^{a}	19^{a}
Total fruit biomass (g/m)	0.002	0.9^{bc}	1.2^{c}	0.1^{ab}	0.0^{a}	0.8^{bc}	0.1^{ab}	0.4^{abc}
Total biomass (g/m)	0.001	44^{b}	31^{ab}	15^{a}	12^{a}	30^{ab}	17^{a}	20^{a}

At 77 DAP, plants applied with TEP(3x) had 36% greater retention than control plants and GA_3 had 33% greater retention than control plants ($P= 0.003$, Table 16). However, there were no significant differences between control plants and any PGR treatments for total fruiting sites, total fruit, or the position of fruit (P1 or P2+).

Table 16: Plant mapping data at 77 DAP during the 2022-23 cotton season (Exp 14).

	P-value	**Control**	**TEP(1x)**	**TEP(2x)**	**TEP(3x)**	**MC(3x)**	**GA_3**	**$GA_{4,7}$**
Retention (% plant^{-1})	0.003	65^{a}	77^{abc}	78^{abc}	89^{c}	69^{ab}	87^{bc}	75^{abc}
Total fruiting sites (plant^{-1})	0.028	33^{ab}	26^{a}	20^{a}	23^{ab}	23^{ab}	37^{b}	26^{ab}
Total Fruit (plant^{-1})	0.047	23^{ab}	21^{ab}	16^{a}	21^{ab}	17^{a}	33^{b}	20^{ab}
P1 Fruit (plant^{-1})	0.583	8	10	7	8	9	8	9
P2+ Fruit (plant^{-1})	0.682	10	8	6	6	7	8	9

Discussion

The effect of gibberellins on the switch from vegetative to reproductive growth of cotton

Our experiments tested two gibberellin formulations, GA_3 and $GA_{4,7}$, to explore the effects of gibberellins on the switch from vegetative to reproductive growth of cotton. Although there was no significant difference compared with the control, relative differences indicated that the application of either GA_3 or $GA_{4,7}$ may delay time to first square, thus, affecting the switch from vegetative to reproductive growth. This was supported by the biomass and plant mapping data measured at 77 DAP. Our study demonstrated that cotton applied with either GA_3 or $GA_{4,7}$ had less stem and leaf biomass than control plants, resulting in less total vegetative biomass overall at 77 DAP. Although there was no significant difference in total fruit biomass for either GA_3 or $GA_{4,7}$ compared with the control, relative fruit biomass was less than the control. Our data indicate no overall change in the number of fruit, or the position of fruit on the plant. It appears that early fruit set and retention was improved with GA_3, and a trend for improved retention for plants applied with $GA_{4,7}$.

Given that time to first square was also relatively later, this suggests that gibberellic acid pathways may have been disrupted, resulting in both delayed growth and development.

The effect of gibberellin inhibitors on the switch from vegetative to reproductive growth of cotton

Our experiments tested two gibberellin inhibitors, TEP and MC, to explore the effects of gibberellin inhibitors on the switch from vegetative to reproductive growth of cotton. Our results showed that multiple applications of TEP delayed the time to first square, thus affecting the switch from vegetative to reproductive growth of cotton. However, there was no significant difference in the time to first square for plants applied with MC, suggesting that different gibberellic acid pathways were affected. Applications of TEP(2x) and TEP(3x) resulted in plants with less leaf, stem, and consequently, total vegetative biomass than control plants. Plants applied with TEP(1x) or MC(3x) had relatively less vegetative biomass than the control plants, although the differences were not significant. TEP(3x) significantly reduced total fruit biomass, whereas MC the treatment was relatively less than the control. Despite less fruit biomass, TEP(3x) had a greater rate of retention than control plants. Although there were no significant differences in total fruit or position of fruit on the plant, TEP and MC treatments had relatively less fruit than the control plants, indicating a delay in the reproductive growth.

These data suggest that some gibberellin inhibitors may delay the switch from vegetative to reproductive growth in cotton, however, the exact mechanism behind the phase change requires further investigation. It is also important to consider the cumulative application, as our data shows that TEP(2x) and TEP(3x) resulted in greater delays in phase-change than TEP(1x).

Summary

Our experiments investigated the research question "do PGR's either promoting or inhibiting gibberellins affect the switch from vegetative to reproductive growth of cotton?" Two field experiments were conducted to investigate the effect of two gibberellins, GA_3 and $GA_{4,7}$, and two gibberellin inhibitors, TEP and MC, on the transition from the vegetative to the reproductive phase of cotton. Our data showed that the application of the gibberellin inhibitor, TEP, significantly delayed reproductive development and growth. However, relative to the control, the application of gibberellins may also delay reproductive development and growth, thereby suggesting interruption to gibberellic biochemical pathways. Our research highlights that it is important to understand which pathways are being affected, to specifically target desired outcomes. Therefore, there is the potential to refine our understanding of how PGR's that promote or inhibit gibberellic pathways affect vegetative to reproductive phase transitions of cotton. Improving this understanding could enable growers to have additional agronomic strategies to manipulate phase transitions in cotton crops and better target optimal environmental conditions.

Experiments 3 and 4: Comparison of the effects of two gibberellic acids on floral initiation across dryland planting times

Research question: Do gibberellic acids (GA_3 or $GA_{4,7}$) stimulate floral initiation across different planting times in dryland cotton systems?

Materials and Methods

To understand the effects of two gibberellic acids (GA_3 and $GA_{4,7}$) on floral development of dryland cotton, five experiments across two cotton seasons were conducted as outlined in Table 17. Time to first square (TTFS) and time to first flower (TTFF) were measured as shown in Table 18.

Table 17: Treatment summary addressing Milestone 2.2

Experiment Details	Trt Abbreviation	Treatment	Rate (a.i.; ppm)	Frequency	Timing	Surfactant	Surfactant Rate	Application Dates
2021-22 ACRI								
Experiment 8: DLS1	Control	Control	-	-	-	-	-	
Planting Date: 17/09/2021	GA_3	Gibberellic acid 3	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	21/10/21, 1/11/21, 9/11/21
Row Configuration: 2m, alt.	$GA_{4,7}$	Gibberellic acid 4,7	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	21/10/21, 1/11/21, 9/11/21
Experiment 9: DLS2	Control	Control	-	-	-	-	-	
Planting Date: 21/10/2021	GA_3	Gibberellic acid 3	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	15/11/21, 23/11/21, 1/12/21
Row Configuration: 2m, alt.	$GA_{4,7}$	Gibberellic acid 4,7	80	3x10d	2L	Yes	0.5/1ml.L-1	15/11/21, 23/11/21, 1/12/21
Experiment 12: DLS3	Control	Control	-	-	-	-	-	
Planting Date: 18/11/2021	GA_3	Gibberellic acid 3	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	6/12/21, 15/12/21, 23/12/21
Row Configuration: 2m, alt.	$GA_{4,7}$	Gibberellic acid 4,7	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	6/12/21, 15/12/21, 23/12/21
2022-23 ACRI								
Experiment 14: DLS1	Control	Control	-	-	-	-	-	
Planting Date: 5/10/2022	GA_3	Gibberellic acid 3	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	3/11/22, 11/11/22, 21/11/22
Row Configuration: Single Skip	$GA_{4,7}$	Gibberellic acid 4,7	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	3/11/22, 11/11/22, 21/11/22
Experiment 17: DLS3	Control	Control	-	-	-	-	-	
Planting Date: 29/11/2022	GA_3	Gibberellic acid 3	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	19/12/22, 29/12/22, 9/1/23
Row Configuration: Single Skip	$GA_{4,7}$	Gibberellic acid 4,7	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	19/12/22, 29/12/22, 9/1/23

DLS1= dryland sowing 1; DLS2= dryland sowing 2; DLS3= dryland sowing 3

Table 18: Experiments where time to first square (TTFS) and time to first flower (TTFF) measurements were taken

Cotton Season	Experiment	TTFS	TTFF
2021-22 season			
	DLS1 (Exp 8)	×	✓
	DLS2 (Exp 9)	✓	×
	DLS3 (Exp 12)	×	✓
2022-23 season			
	DSL1 (Exp 14)	✓	✓
	DLS3 (Exp 17)	✓	✓

Results

The mean number of days from planting until plants in each treatment reached first square and first flower is shown in Table 19. The application of GA_3 significantly delayed TTFS by 13 days compared with control plants in the 2022-23 cotton season for DLS1 plants ($P< 0.1$). The developmental delay with the application of GA_3 was also evident with an 8–18-day delay in TTFF for DLS1 plants across both cotton seasons ($P< 0.05$). It was observed that plants applied with $GA_{4,7}$ may experience some minor delays in TTFS and TTFF in the DLS1 experiments, however, these delays were not significantly different from the control. Data show no significant difference in TTFS and TTFF for DLS2 or DLS3 plantings across either cotton season.

Table 19: Number of days until time to first square (TTFS) and time to first flower (TTFF) for cotton grown in dryland experiments, sown with different planting dates. Values represent mean ± SE. † represents significance at P< 0.1. Different letters represent significant difference.

Relevant Exp ID	Experiment	P-value	Control	SE	GA_3	SE	ΔGA_3 from Control (days)	$GA_{4,7}$	SE	$\Delta GA_{4,7}$ from Control (days)
	2021-22 season									
8	DLS1-TTFS		NA		102	5.4		80	1.3	
8	DLS1-TTFF	*0.001*	100^{a}	0.6	118^{b}	3.8	18	102^{a}	0.4	2
9	DLS2-TTFS	*0.500*	58	0.7	60	0.0	2	59	0.7	1
12	DLS3-TTFF	*0.926*	67	2.5	68	3.3	1	67	3.1	0
	2022-23 season									
14	DLS1-TTFS	*0.054*†	68^{a}	0.9	81^{b}	3	13	73^{ab}	3.5	5
14	DLS1-TTFF	*0.037*	94^{a}	2.1	101^{b}	1.5	8	98^{ab}	1.1	5
17	DLS3-TTFS	*0.387*	62	1.8	63	1.6	1	58	1.4	-4
17	DLS3-TTFF	*0.658*	84	1.6	85	0.5	1	86	2.6	2

Discussion

Our data suggests the application GA_3 may delay floral initiation of dryland cotton planted early in the season. Given that floral initiation of cotton is also largely temperature dependent (Mauney, 1966), there is the possibility of a temperature by hormone interaction, which could be explored further in future experiments. This data suggests that GA_3 may be worthwhile considering as a hormone to delay fruit development in early planted cotton. However, to properly understand the mechanism behind the delay, application rate, timing and management recommendations requires further investigation.

Summary

Our experiments investigated the research question “do gibberellic acids (GA_3 or $GA_{4,7}$) stimulate floral initiation across different planting times in dryland cotton systems?” We conducted five field experiments to examine development of plants applied with GA_3 or $GA_{4,7}$ in dryland cotton systems. Our data showed that the application of GA_3 significantly delayed development of dryland cotton planted early in the season, however, these responses were not evident in dryland cotton planted later in the season. This suggests there may be important temperature or environmental interactions to understand, which may drive responses across different cotton regions.

Glasshouse experiment: Determining Trinexapac-ethyl treatment scenarios applied to an Australian cotton cultivar to slow vegetative growth or induce vegetative physiological stasis, to manipulate the phase transition to reproductive growth

Research question: Which trinexapac-ethyl (TEP) application rate and frequency are optimal to manipulate phase transition of cotton?

Materials and Methods

A glasshouse experiment was conducted at ACRI during the winter of 2021. Cotton (cv. Sicot 746B3F) was planted in 20L pots on the 23rd April 2021. Plants were grown at 35/15°C (day/night). The experiment was set up in a randomised complete block design with 6 replicates. Trinexapac-ethyl (TEP) was applied at different rates beginning at the 4-Leaf stage, as per Table 20. Hormone applications were applied on the following dates: 18/5/21, 24/5/21, 31/5/21, 7/6/21 and 15/6/21. No surfactant was applied with the hormone treatment.

Table 20: Treatment summary of glasshouse experiments in addressing Milestone 2.2.

Trt No.	*PGR*	*Rate (a.i.)*	*Frequency*	Timing
1	Control	N/A	N/A	N/A
2	TEP	1000ppm	3x7d	4L
3	TEP	2000ppm	3x7d	4L
4	TEP	4000ppm	3x7d	4L
5	TEP	16000ppm	3x7d	4L
6	TEP	2000ppm	1x7d	4L
7	TEP	2000ppm	3x7d	4L
8	TEP	2000ppm	5x7d	4L

Time to first square (TTFS) and time to first flower (TTFF) were monitored. Data was analysed by unbalanced ANOVA due to plant mortality in some treatments. Means of treatments were compared using Fishers least significant difference at a 5% level of probability.

Results

The application of TEP significantly affected TTFS and TTFF (P<0.05, Table 21). Three applications of very high rates of TEP (Trt 5; 16000 ppm) caused plant mortality. Compared with control plants, Trt 4 significantly delayed TTFS by 13 days and TTFF by 41 days, however, 2/6 plants had died prior to first square and 5/6 plants had died by first flower. Compared with the control, applications of 1000ppm TEP (Trt 2) did not significantly delay TTFS or TTFF, although non-significant delays of 3 and 10 days, respectively, were observed.

Compared with control plants, a single application of 2000ppm TEP (Trt 6) did not significantly delay TTFS (1 day) or TTFF (4 days). Three applications of 2000ppm TEP (Trt 3 and Trt 7) significantly delayed TTFS by 8-9 days and TTFF by 26-32 days, compared with the control. Five applications of 2000ppm TEP (Trt 8) delayed TTFS by 10 days and TTFF by 45 days, compared with the control.

Table 21: Time to first square (TTFS) and time to first flower (TTFF) for cotton applied with varying rates of Trinexapac-ethyl (TEP). Values represent mean ± SE. Different letters represent significant difference at P< 0.05, using Fishers least significant difference.

Trt	**TTFS (days)**	**SE TTFS**	**ΔTTFS from**	**TTFF (days)**	**SE_TTFF**	**ΔTTFF from**

			control (days)			control (days)
1	41^{b}	1.0		81^{b}	0.8	
2	44^{bc}	0.8	3	91^{c}	2.6	10
3	49^{cd}	4.2	8	113^{de}	5.0	32
4	54^{d}	5.0	13	122^{ef}	0.0	41
5	0^{a*}			0^{a}		
6	42^{b}	0.7	1	86^{bc}	1.5	4
7	50^{d}	1.8	9	107^{d}	2.6	26
8	51^{d}	1.3	10	127^{f}	0.8	45
P-value	*0.001*			*0.002*		

* Plant mortality

Discussion

The effects of different application rates of TEP on cotton development

Low, medium, and high application rates of TEP were tested with a frequency of three applications every seven days (3x7d, Table 20). Low rates of TEP (i.e., 1000ppm; Trt 2) did not significantly delay development, although small delays relative to the control were observed. Medium rates of TEP (i.e., 2000ppm; Trt 3 and Trt 7) delayed squaring 8-9 days and delayed flowering 26-32 days compared with the control, demonstrating that the initial phase transition can be delayed by a little over a week, but indicating that further development (i.e., flowering) may be delayed for a longer period. Higher rates of TEP (4000ppm; Trt 4 and 16000ppm; Trt 5) were shown to potentially lead to longer delays in development but may also have a lethal effect on cotton plants, thereby indicating that higher doses of TEP are riskier for commercial application.

Different frequencies of a 2000ppm application of TEP on cotton development

In comparing a rate of 2000ppm of TEP applied at different frequencies, application frequency and consequently, total dosage played an important role (Table 21). Our data showed that a single application of 2000ppm TEP was not sufficient to significantly delay development of cotton. Three applications of 2000ppm TEP (Trt 3 and Trt 7) significantly delayed TTFS by 8-9 days and TTFF by 26-32 days, compared with the control. Five applications of 2000ppm TEP (Trt 8) delayed TTFS by 10 days and TTFF by 45 days, compared with the control. Therefore, three or five applications of 2000ppm TEP were found to effectively delay development of cotton without resulting in plant mortality, as observed in Trt 5. Three applications of 2000ppm TEP were sufficient to cause a developmental delay, suggesting this rate and frequency may be optimal for cotton. Our data suggests that additional applications (i.e., five applications) may further delay flowering, without altering TTFS, but a grower would also incur additional chemical and application costs.

Summary

Our experiments investigated the research question "which trinexapac-ethyl (TEP) application rate and frequency are optimal to manipulate phase transition of cotton?" We conducted a glasshouse experiment to assess the effect of different application rate and frequency on the time to development of cotton. Our data shows that TEP applied at the 4-leaf stage delays the timing of crop-level reproductive phase change, with squaring delayed up to 54 days and flowering delayed up to 127 days. However, our data also showed application rate and frequency to be important, with very high rates detrimental to plant survival. Optimal treatments appear to be three to five applications of TEP at a rate of 2000ppm, resulting in delays of both squaring and flowering, but without significant damage to the plants. Further research could verify these results in field conditions and assess alternative timing for the application of TEP.

Milestone 2.2 Summary

To develop broader understanding and impact of mechanisms that drive the change between vegetative and reproductive growth and establish whether PGRs can play a role in managing this, we investigated three research questions: (1) Do PGR's promoting or inhibiting gibberellins affect the switch from vegetative to reproductive growth of cotton; (2) Do gibberellic acids (GA_3 or $GA_{4,7}$) stimulate floral initiation across different planting times in dryland cotton systems; and (3) which TEP application rate and frequency are optimal to manipulate phase transition of cotton. Field and glasshouse experiments were conducted from the 2020-21 to 2022-23 cotton seasons. Data showed significant delays in growth and development of cotton when plants were applied with early applications of gibberellin or gibberellin inhibiting PGRs, suggesting that gibberellic pathways were altered, however, further refinement of timing, rates and other environmental interactions is required. Improving this understanding may enable growers to have additional agronomic strategies to manipulate phase transitions in cotton crops to target optimal environmental conditions in a season.

Milestone 2.3 Develop an understanding of the concept of "crop determinacy" and assess its role in providing resilience in crop production in stressed environments.

Introduction

Cotton is a perennial plant with an indeterminate growth habit. However, the term "determinate" is applied to cultivars that terminate reproductive development comparatively abruptly and do not readily begin a second fruiting cycle (Milroy and Bange, 2003). The degree of determinacy is strongly associated with whether a cultivar is a short or long season type, with short season cultivars widely considered determinate. It has been suggested that cultivars with low determinacy (i.e., more indeterminate) are important for rainfed production because they allow growth to resume more readily following a setback after stress (i.e., water deficit). Therefore, cotton cultivars with differing levels of determinacy could be utilised to improve resilience in rainfed cotton systems.

Abiotic stresses in dryland cotton systems may lead to shedding of young fruit. Shedding of fruit in stressed cotton is linked with higher ethylene accumulation. Therefore, regulating ethylene production could improve cotton yield by limiting fruit abscission. Aminoethoxyvinylglycine (AVG) can regulate ethylene accumulation by blocking its biosynthetic pathway or its action. In a glasshouse study on waterlogged cotton, the application of AVG was shown to block ethylene accumulation in leaves and improve leaf growth (Najeeb et al., 2015).

Gibberellins are a group of plant hormones that stimulate elongation of the stem, flowering, and may be used to promote early canopy structural biomass. There are more than 80 different gibberellins which may exhibit many physiological effects, suggesting that they have more than one primary site of action. Gibberellins may stimulate early growth and canopy development of dryland cotton.

To improve our understanding of the concept of "crop determinacy" and assess its role in providing resilience in crop production in stressed environments, three field experiments were conducted to compare cotton varieties with differing levels of determinacy with two PGR strategies, inhibiting ethylene production and promoting early season growth using gibberellins.

Research question: How do PGRs affect crop determinacy compared with varieties?

Materials and Methods

Field experiments were conducted during the 2020-21, 2021-22, and 2022-23 cotton seasons. These experiments compared three varieties Sicot 746B3F (control), Sicot 714 (determinate), and Sicot 748 (indeterminate) with PGRs aminoethoxyvinylglycine (AVG, an ethylene inhibitor) and gibberellins (GA_3 and $GA_{4,7}$) to alter crop growth and architecture, as outlined in Table 22. Time to first flower, cut-out biomass, plant mapping (retention) and yield were measured (Table 23). Data was analysed by ANOVA using Genstat v19 with a 5% level of probability. Treatment means were compared using either Fishers least significant difference (for unbalanced datasets) or Tukey’s HSD (for balanced datasets).

Table 22: Treatment summary addressing Milestone 2.3

Experiment Details	Trt Abbreviation	Treatment	Rate (a.i.; ppm)	Frequency	Timing	Surfactant	Surfactant Rate	Application Dates
2020-21 Bongeen (Exp 6)								
Cotton Season: 2020-21	Control	Control (Sicot 746B3F)	-	-	-	-	-	
Location: Bongeen, Qld	Determinate	Determinate variety (Sicot 714B3F)	-	-	-	-	-	
Planting Date: 20/11/2020	Indeterminate	Indeterminate variety (Sicot 748B3F)	-	-	-	-	-	
Row Configuration: Single Skip	AVG(3x)	Aminoethovinylglycine	925	3x10d	4L	Yes	0.5/1ml.L^{-1}	18/12/20, 29/12/20, 11/1/21
	AVG+GA$_{4,7}$	Aminoethovinylglycine + Gibberellic acid 4,7	925 + 80	3x10d	4L	Yes	0.5/1ml.L-1	18/12/20, 29/12/20, 11/1/21
2021-22 ACRI (Exp 12)								
Cotton Season: 2021-22	Control	Control (Sicot 746B3F)	-	-	-	-	-	
Location: ACRI, Narrabri	Determinate	Determinate variety (Sicot 714B3F)	-	-	-	-	-	
Experiment: DLS3	Indeterminate	Indeterminate variety (Sicot 748B3F)	-	-	-	-	-	
Planting Date: 18/11/2021	AVG(3x)	Aminoethovinylglycine	830	3x10d	4L	Yes	0.5/1ml.L^{-1}	15/12/21, 23/12/21, 5/1/22
Row Configuration: 2m, alt.	AVG(4x)	Aminoethovinylglycine	830	4x10d	4L	Yes	0.5/1ml.L^{-1}	15/12/21, 23/12/21, 5/1/22, 13/1/22
	AVG(5x)	Aminoethovinylglycine	830	5x10d	4L	Yes	0.5/1ml.L^{-1}	15/12/21, 23/12/21, 5/1/22, 13/1/22, 21/1/22
	GA$_3$	Gibberellic acid 3	80	3x10d	2L	Yes	0.5/1ml.L^{-1}	6/12/21, 15/12/21, 23/12/21
	GA$_{4,7}$	Gibberellic acid 4,7	80	3x10d	2L	Yes	0.5/1ml.L-1	6/12/21, 15/12/21, 23/12/21
2022-23 ACRI (Exp 17)								
Cotton Season: 2022-23	Control	Control (Sicot 746B3F)	-	-	-	-	-	
Location: ACRI, Narrabri	Determinate	Determinate variety (Sicot 714B3F)	-	-	-	-	-	
Experiment: DLS3	Indeterminate	Indeterminate variety (Sicot 748B3F)	-	-	-	-	-	

Planting Date: 29/11/2022	AVG(3x)	Aminoethovinylglycine	830	3x10d	4L	Yes	$0.5/1ml.L^{-1}$	3/1/23, 13/1/23, 20/1/23
Row Configuration: Single Skip	AVG(4x)	Aminoethovinylglycine	830	4x10d	4L	Yes	$0.5/1ml.L^{-1}$	3/1/23, 13/1/23, 20/1/23, 30/1/23
	AVG(5x)	Aminoethovinylglycine	830	5x10d	4L	Yes	$0.5/1ml.L^{-1}$	3/1/23, 13/1/23, 20/1/23, 30/1/23, 8/2/23
	GA_3	Gibberellic acid 3	80	3x10d	2L	Yes	$0.5/1ml.L^{-1}$	19/12/22, 29/12/22, 9/1/23
	$GA_{4,7}$	Gibberellic acid 4,7	80	3x10d	2L	Yes	$0.5/1ml.L^{-1}$	19/12/22, 29/12/22, 9/1/23

Table 23: Summary of the measurements that were taken in each experiment.

Relevant Exp ID	Cotton Season	Experiment	TTFF	Biomass	Retention	Yield
6	2020-21	Bongeen	✗	✗	✗	✓
12	2021-22	DLS3	✓	✓	✓	✓
17	2022-23	DLS3	✓	✓	✓	✓

Results

Time to First Flower

There was no significant difference in time to first flower in the 2021-22 cotton season (P= 0.218, Table 24). During the 2022-23 cotton season, there were no significant differences in time to first flower between varieties or hormonal treatment compared with the control, but the determinate variety flowered ~11 days earlier than the AVG x 3 and AVG x 5 treatment (P= 0.041).

Variety: Variety did not significantly affect TTFF of cotton

AVG: Regardless of application frequency, the application of AVG did not consistently alter TTFF of late-planted cotton, compared with the control.

Gibberellins: GA_3 or $GA_{4,7}$ did not alter TTFF of late-planted dryland cotton, compared with the control.

Table 24: Time to first flower (TTFF) for cotton applied with various hormone treatments across two cotton seasons at ACRI, Narrabri. Values represent mean ± SE. Different letters represent significant difference at P< 0.05, using Fishers least significant difference.

		2021-22 (Exp 12)		**2022-23 (Exp 17)**	
Trt ID.	**Treatment**	**TTFF**	**SE**	**TTFF**	**SE**
1	Control	67	2.5	83.5 ^{ab}	1.6
2	Det.Variety (741B3F)	62	0.7	76.5 ^{a}	1.6
3	Indet Variety (748B3F)	70	0	83.5 ^{ab}	1.7
4	AVG (3x)	63	0.3	88.5 ^{b}	4
5	AVG (4x)	66	1.9	83.25 ^{ab}	0.9
6	AVG (5x)	68	2.3	87.25 ^{b}	2
7	GA_3	68	3.3	84.5 ^{ab}	0.5
8	$GA_{4,7}$	67	3.1	85.5 ^{ab}	2.6
	P-value	*0.218*		*0.041*	

Biomass and Retention

Biomass and retention around cut-out were measured 102 DAP and 110 DAP for the 2021-22 and 2022-23 cotton seasons, respectively. Biomass results shown in Table 25 and are described below.

Variety: In 2021-22 the indeterminant variety was significantly taller than the control, indeterminant varieties and all of the hormonal treatments. The determinant variety had fewer nodes compared to the control variety but not the indeterminant variety. In 2022-23 there were no significant difference in the height or number of nodes between the control plants and the determinate or indeterminate varieties.

AVG: In 2021-22 The application of AVG reduced height, relative to the control. Despite high variability, compared to control plants but there was no difference in 2022-23.,

Gibberellins: There was a lot of variability in the growth response of cotton to gibberellins. In the 2021-22 season, plants with GA_3 applied were taller with an average of two more compared to control plants but were shorter than the determinant variety despite having more nodes. In 2022-23 plants with $GA_{4,7}$ applications had an average of 3 more nodes compared with the control, indeterminant and determinant varieties.

Table 25: Cut-out biomass harvest for two cotton seasons. Values represent means. Different letters represent significant difference at $P< 0.05$, using Tukey's test.

	P-value	**1.Cont**	**2.Det**	**3.Indet**	**4.AVG(3x)**	**5.AVG(4x)**	**6.AVG(5x)**	**7.GA$_3$**	**8. GA$_{4,7}$**
Exp 12. 2021-22 cotton season (102 DAP)									
Height (cm plant^{-1})	0.001	90^{bc}	91^{bc}	99^{d}	84^{a}	83^{a}	84^{a}	94^{c}	86^{ab}
Nodes (plant^{-1})	0.001	22^{b}	21^{a}	22^{ab}	22^{b}	22^{ab}	22^{ab}	24^{c}	21^{ab}
Leaf Area (cm^2/m)	0.214	14206	13975	11797	11630	12463	12656	14900	12468
Leaf (g/m)	0.635	226	232	234	228	210	234	246	252
Stem (g/m)	0.308	341	353	367	333	300	322	377	358
Square (g/m)	0.173	12	5	12	12	10	14	15	13
Green Boll (g/m)	0.059	307	397	309	308	306	297	297	346
Total vege biomass (g/m)	0.455	567	585	601	561	511	556	623	609
Total fruit biomass (g/m)	0.096	319	402	321	320	316	311	312	359
Total biomass (g/m)	0.369	886	987	922	881	827	868	935	968
Exp 17. 2022-23 cotton season (110 DAP)									
Height (cm plant^{-1})	0.001	62^{ab}	64^{ab}	67^{b}	64^{ab}	58^{a}	61^{ab}	62^{ab}	64^{ab}
Nodes (plant^{-1})	0.001	21^{a}	21^{a}	22^{a}	21^{a}	22^{ab}	22^{ab}	23^{ab}	24^{b}
Leaf Area (cm^2/m)	0.223	17737	22921	15688	18820	15153	19210	17439	17004
Leaf (g/m)	0.174	142	136	118	129	143	149	119	124
Stem (g/m)	0.924	199	202	188	186	192	204	184	183
Square (g/m)	0.313	11	5	12	12	15	21	4	20
Total vege biomass (g/m)	0.672	341	338	306	315	336	352	303	307
Total fruit biomass (g/m)	0.590	181	213	155	170	172	157	194	142
Total biomass (g/m)	0.605	522	551	460	486	508	509	497	449

Retention and plant mapping results are show in Table 26.
Variety: During the 2021-22 cotton season, retention rates were not significantly affected by variety. the indeterminate variety had fewer fruiting sites, and fewer total fruit and fewer Position 1 fruit compared to the control plants but was not different to the determinant variety. During the 2022-23 cotton season, there were no significant changes in retention, total fruiting sites, total fruit, or fruiting position for either the determinate (Trt 2) or indeterminate variety (Trt 3) compared with the control. Whilst more indeterminant varieties are generally recommended for dryland production system these data suggests that indeterminate varieties for late sown, dryland cotton might not have a growing season advantage compared with more determinate varieties in some seasons.
AVG: In 2021-22 the AVGx3 treatment had fewer total fruit compared to the control, but there were no difference in any of the AVG treatments in the 2022-23 season.
Gibberellins: There was a lot of variability in the retention and fruiting responses of cotton applied with gibberellins. In 2021-22 there were fewer 1st position fruit in the GA_3 treatments compared to the control. In 2022-23 the only significant differences were higher fruit retention per plant in the $GA_{4,7}$ treatment with higher total fruit numbers and numbers of 2+ position fruit compared to the control and determinant variety. Whilst not statistically significant there was numerically lower fruit retention in GA_3 treatments compared to the control, suggesting that the application of GA_3 in late sown dryland cotton may negatively affect fruit retention. However, retention and fruiting responses of cotton to the application of $GA_{4,7}$ had no differences in the 2021-22 season, and consistent increases in the 2022-23 season, suggesting the need for further investigation.

Table 26: Retention and fruiting sites for cotton plants applied with different plant growth regulators across two cotton seasons. Values represent means. Different letters represent significant difference at $P< 0.05$, using Tukey's test.

	P-value	**1. Control**	**2.Det**	**3.Indet**	**4.AVG(3x)**	**5.AVG(4x)**	**6.AVG(5x)**	**7.GA$_3$**	**8. GA$_{4,7}$**
Exp 12. 2021-22 Cotton Season (102 DAP)									
Retention (plant^{-1})	0.161	66	63	63	65	67	67	58	62
Total fruiting sites (% plant^{-1})	0.001	38^{bc}	34^{ab}	27^{a}	36^{a}	38^{ab}	36^{bc}	46^{c}	38^{bc}
Total Fruit (plant^{-1})	0.003	26^{b}	22^{ab}	16^{a}	23^{ab}	25^{b}	24^{b}	27^{b}	23^{ab}
P1 Fruit (plant^{-1})	0.001	11^{b}	10^{ab}	8^{a}	12^{b}	11^{b}	11^{b}	9^{a}	10^{ab}
P2+ Fruit (plant^{-1})	0.003	14^{ab}	12^{ab}	8^{a}	12^{ab}	14^{ab}	13^{ab}	18^{b}	13^{ab}
Exp 17. 2022-23 Cotton Season (110 DAP)									
Retention (% plant^{-1})	0.001	63^{abc}	49^{ab}	57^{abc}	68^{bc}	66^{bc}	76^{c}	42^{a}	78^{c}
Total fruiting sites (plant^{-1})	0.043	24^{a}	28^{a}	24^{a}	27^{a}	27^{a}	24^{a}	24^{a}	34^{a}
Total Fruit (plant^{-1})	0.001	16^{ab}	14^{a}	14^{a}	20^{ab}	19^{ab}	20^{ab}	11^{a}	27^{b}
P1 Fruit (plant^{-1})	0.013	7^{ab}	7^{ab}	7^{a}	8^{ab}	8^{ab}	7^{ab}	6^{a}	9^{b}
P2+ Fruit (plant^{-1})	0.001	3^{a}	2^{a}	3^{a}	4^{ab}	4^{ab}	4^{ab}	1^{a}	7^{b}

Yield

Yield results are shown in Table 27, and described below.

Variety: There was no significant difference in yield between the control (Sicot 746B3F), determinate and indeterminate varieties across any of the experiments ($P>0.05$).

AVG: There was no significant difference in yield between the Control and AVG application across any of the experiments ($P>0.05$). However, yield of the AVG + $GA_{4,7}$ was 30% lower than the Control in Experiment 6.

Gibberellins: Across both cotton seasons at ACRI, cotton applied with $GA_{4,7}$ had 8.8-9.7% greater relative yield than Control plants. Relative yield response to GA_3 was variable, with 5.2% increase in 2021-22 and a 3.9% decrease in 2022-23.

Table 27: Relative yield of cotton with varying determinacy compared with PGR strategies across three field experiments.

Cotton season	Experiment	Relevant Exp ID	PGR	Yield (b/ha)
2020-21	Bongeen	6	Control (Sicot 746B3F)	3.6^{bc}
2020-21	Bongeen	6	Determinate variety, Sicot 714B3F	4.4^{c}
2020-21	Bongeen	6	Indeterminate variety, Sicot 748B3F	3.8^{c}
2020-21	Bongeen	6	AVG	2.8^{ab}
2020-21	Bongeen	6	AVG + $GA_{4,7}$	2.5^{a}
P-value				*0.001*
2021-22	DLS3	12	Control (Sicot 746B3F)	5.2
2021-22	DLS3	12	Determinate variety, Sicot 714B3F	5.8
2021-22	DLS3	12	Indeterminate variety, Sicot 748B3F	5.6
2021-22	DLS3	12	AVG (3x)	5.2
2021-22	DLS3	12	AVG (4x)	5.1
2021-22	DLS3	12	AVG (5x)	5.7
2021-22	DLS3	12	GA_3	5.5
2021-22	DLS3	12	$GA_{4,7}$	5.7
P-value				0.485
2022-23	DLS3	17	Control (Sicot 746B3F)	3.3
2022-23	DLS3	17	Determinate variety, Sicot 714B3F	4.2
2022-23	DLS3	17	Indeterminate variety, Sicot 748B3F	3.9
2022-23	DLS3	17	AVG (3x)	3.6
2022-23	DLS3	17	AVG (4x)	3.8
2022-23	DLS3	17	AVG (5x)	3.7
2022-23	DLS3	17	GA_3	3.2
2022-23	DLS3	17	$GA_{4,7}$	3.6
P-value				0.743

Discussion

How did variety alter crop determinacy across the experiments?

Overall, yield data suggests that there was no significant difference between varieties in dryland cotton systems. In terms of crop development, growth and fruit retention the lack of consistent differences between these varieties suggests that there is not a great deal of variation in terms of "determinacy" vs. "indeterminacy" in these varieties. Therefore, there is value in follow up research with varieties with greater variation in cotton determinacy to determine how it could be exploited for managing crop stress. The application of various

PGRs may provide some yield benefits, however the greatest relative yield benefits were achieved by utilising varietal differences.

How did AVG alter crop determinacy across the experiments?
Overall, our data indicates that there were no consistent changes in TTFF, biomass, retention rates or yield for plants applied with AVG compared with the controls across three field experiments. This data indicates that the early applications of various rates of AVG did not alter crop determinacy compared with the controls.

How did Gibberellins alter crop determinacy across the experiments?
Overall, there were no consistent changes in TTFF, biomass, retention rates or yield for plants applied with either GA_3 or $GA_{4,7}$ compared with the controls, when tested across two field experiments. This data indicates that the early application of GA_3 or $GA_{4,7}$ did not alter crop determinacy of cotton compared with control plants.

Summary

To develop an improved understanding of the concept of "crop determinacy" and assess its role in proving resilience in crop production in stressed environments, three field experiments were conducted to investigate how PGRs affect crop determinacy compared with variety. Reliable and consistent manipulation of crop determinacy using AVG, GA_3 or $GA_{4,7}$ was not observed throughout these three field experiments conducted. Results reflect seasonal climate, and with retention rates of the Controls between 60-70% suggests that cotton did not experience a stressed environment that resulted in shedding of fruit, and therefore, negates the need to regulate ethylene accumulation. However, there are some responses that are interesting and have potential application in cotton systems, although further research with greater variation in determinacy is required.

Milestone 2.4 Establish whether there are alternative hormones/PGR approaches that can enhance cotton growth in stressed environments.

Introduction

Abscisic acid (ABA) is a plant hormone synthesised in all cells that contain plastids and is transported in the xylem and phloem. ABA plays a major role in seed and bud dormancy, but also during plant water stress. During water stress, ABA induces the expression of genes for the synthesis of proteins that protect membranes and other proteins from desiccation damage. ABA closes stomata by depolarising the plasma membrane, increasing cytosolic Ca^{2+} and regulating ion transport. ABA is an antagonist to gibberellins and suppresses GA-induced gene expression.

ABA may be applied to cotton to induce stomatal closure and temporarily "shut down the plant" during a period of hot, dry weather to conserve water for use later in the season. It is possible that utilising these physiological mechanisms may enhance water use efficiency of crops in water limited environments. However, it is not known what rates of chemical should be applied, how long the chemical reduces stomatal conductance of the plant, or what the longer-term effects are on yield of cotton grown in the field. Glasshouse and field experiments were conducted to explore the effect of ABA on the physiology and yield of cotton (Table 28):

Research questions:

1. How does the application of ABA applied at first flower affect cotton physiology?
2. Can we "protect" plants against water deficit stress during flowering? Can we "shut down" the plant and reduce water use? Is fruit and yield retained?

Table 28. Treatment summary for the ABA glasshouse experiment addressing Milestone 2.4

Cotton Season	Experiment	Trt	PGR	Rate	Frequency	Timing
March 2022	Glasshouse	3	ABA (WW)	125 ppm	1x	First flower
		4	ABA (WD)	125 ppm	1x	First flower
		5	ABA (WW)	250 ppm	1x	First flower
		6	ABA (WD)	250 ppm	1x	First flower
		7	ABA (WD)	125 ppm	2x	First flower
		8	ABA (WD)	250 ppm	2x	First flower

Glasshouse experiment: How does the application of ABA applied at first flower affect cotton physiology?

A glasshouse experiment was conducted in March 2022 to investigate the effect that two rates of ABA have on the physiology of cotton in well-watered and water deficit conditions. This experiment tested the hypotheses (1) Abscisic acid applied at first flower decreases stomatal conductance of cotton; (2) how long does an ABA application affect physiology of cotton plants when applied at first flower; (3) Does a second application of ABA induce the same physiological response?

Materials and Methods

Cotton (cv. Sicot 746B3F) was planted in the glasshouse on the 19th January 2022. Plants were grown at 30/20°C (day/night), fertilised, and well-watered until the beginning of the experiment. The experiment was designed as a randomised block design with six replications.

At the beginning of the experiment at 55 DAP, two water treatments were established. A well-watered treatment (WW) was maintained at 100% field capacity by daily replenishing the water used. For the water deficit treatment (WD), water was withheld until the soil water content dropped below 50% field capacity, and then plants were maintained at 50% field capacity. Pots were weighed daily to gravimetrically calculate water use and replacement.

ABA treatments were applied at first flower. Two application rates were tested, 125ppm or 250ppm. Plants were applied with a single application of ABA (17/3/22; 57 DAP). A second application occurred only for two of the treatments at 63 DAP (23/3/22), as outlined in Table 29.

Table 29: Chemical and water treatments, application rates and timing

Trt Abbreviation	Watering	Product	Rate ppm	Timing	Wetter
WW_Control	WW	Control			
WD_Control	WD	Control			
WW_ABA.125	WW	ABA	125	FF X 1	Yes
WD_ABA.125	WD	ABA	125	FF X 1	Yes
WW_ABA.250	WW	ABA	250	FF X 1	Yes
WD_ABA.250	WD	ABA	250	FF X 1	Yes
WD_ABA2x125	WD	ABA	125	FF X 2	Yes
WD_ABA2x250	WD	ABA	250	FF X 2	Yes

Leaf-level gas exchange (photosynthesis, stomatal conductance, and transpiration) was measured using the Licor 6400XT over 12 days following the first application of ABA. Settings were as follows: CO_2= 410, flow rate = 400, Block temperature = 32°C.

Results

There was no significant ABA by water treatment interactions on photosynthesis, stomatal conductance, or transpiration on any of the days measured (Table 30).

Effect of water-deficit and ABA treatments on cotton physiology

Plants with the water deficit treatment consistently had reduced physiology than plants in the well-watered treatment. The water deficit treatment reduced photosynthesis by 20-48%, reduced stomatal conductance by 45-71% and reduced transpiration by 23-53%, compared with well-watered plants.

Effect of ABA treatments on cotton physiology

The initial ABA treatment had a significant effect on photosynthesis, stomatal conductance, and transpiration on the first two days following the ABA application (i.e., 18/3/22 and 19/3/22, $P<0.05$). On the first day after application (18/3/22), compared with the control, photosynthesis of cotton sprayed with ABA125 was reduced by 25%, stomatal conductance was reduced by 52% and transpiration was reduced by 26%. There was no significant difference in photosynthesis, stomatal conductance, or transpiration between ABA125 and control plants from the second day after application (19/3/22).

For plants applied with ABA250, on the first day after application, photosynthesis was reduced by 31%, stomatal conductance was reduced by 56% and transpiration was reduced by 33% compared with the control. On the second day after application, photosynthesis of ABA250 plants was reduced by 31%, stomatal conductance was reduced by 47% and transpiration was reduced by 32% compared with the control. However, there was no significant difference in physiology with ABA treatments from four days after application (21/3/22).

A second application of ABA seven days after the first application induced a reduction in stomatal conductance for two days (24/3/22 and 25/3/22). The day after the second application of ABA (64 DAP, 24/3/22), stomatal conductance of ABA125.2 was 61% lower than ABA125 and the stomatal conductance of ABA250.2 was 67% lower than ABA250. The following day (65 DAP, 25/3/22), stomatal conductance of ABA125.2 plants and ABA250.2 plants were 65% and 61% lower than control plants, respectively. There was no significant difference between the ABA125 and ABA125.2 or the ABA250 and ABA250.2 treatments the second day. A second application of ABA did not significantly affect photosynthesis or transpiration.

*Table 30. ANOVA results of ABA and water treatments on stomatal conductance of cotton. Values are P-values and * represents significance at P< 0.05.*

Date	Day after planting (DAP)	Day after initial application	ABA treatment	Water	ABA x Water
Photosynthesis					
18/03/2022	58	1	0.038*	0.001*	0.347
19/03/2022	59	2	0.043*	0.001*	0.872
21/03/2022	61	4	0.177	0.001*	0.678
22/03/2022	62	5	0.575	0.001*	0.915
23/03/2022	63	6	0.648	0.001*	0.831
24/03/2022	64	7	0.063	0.001*	0.288
25/03/2022	65	8	0.090	0.002*	0.232
28/03/2022	68	11	0.991	0.007*	0.935
29/03/2022	69	12	0.268	0.001*	0.636
Stomatal Conductance					
18/03/2022	58	1	0.002*	0.001*	0.468
19/03/2022	59	2	0.026*	0.001*	0.539
21/03/2022	61	4	0.060	0.001*	0.937
22/03/2022	62	5	0.366	0.001*	0.639
23/03/2022	63	6	0.158	0.001*	0.516
24/03/2022	64	7	0.024*	0.001*	0.889
25/03/2022	65	8	0.033*	0.001*	0.230
28/03/2022	68	11	0.807	0.001*	0.706
29/03/2022	69	12	0.639	0.001*	1.000
Transpiration					
18/03/2022	58	1	0.042*	0.001*	0.644
19/03/2022	59	2	0.044*	0.001*	0.983
21/03/2022	61	4	0.209	0.001*	0.670
22/03/2022	62	5	0.729	0.001*	0.995
23/03/2022	63	6	0.295	0.001*	0.877
24/03/2022	64	7	0.157	0.001*	0.364
25/03/2022	65	8	0.090	0.001*	0.190

28/03/2022	68	11	0.945	0.002*	0.835
29/03/2022	69	12	0.864	0.003*	0.724

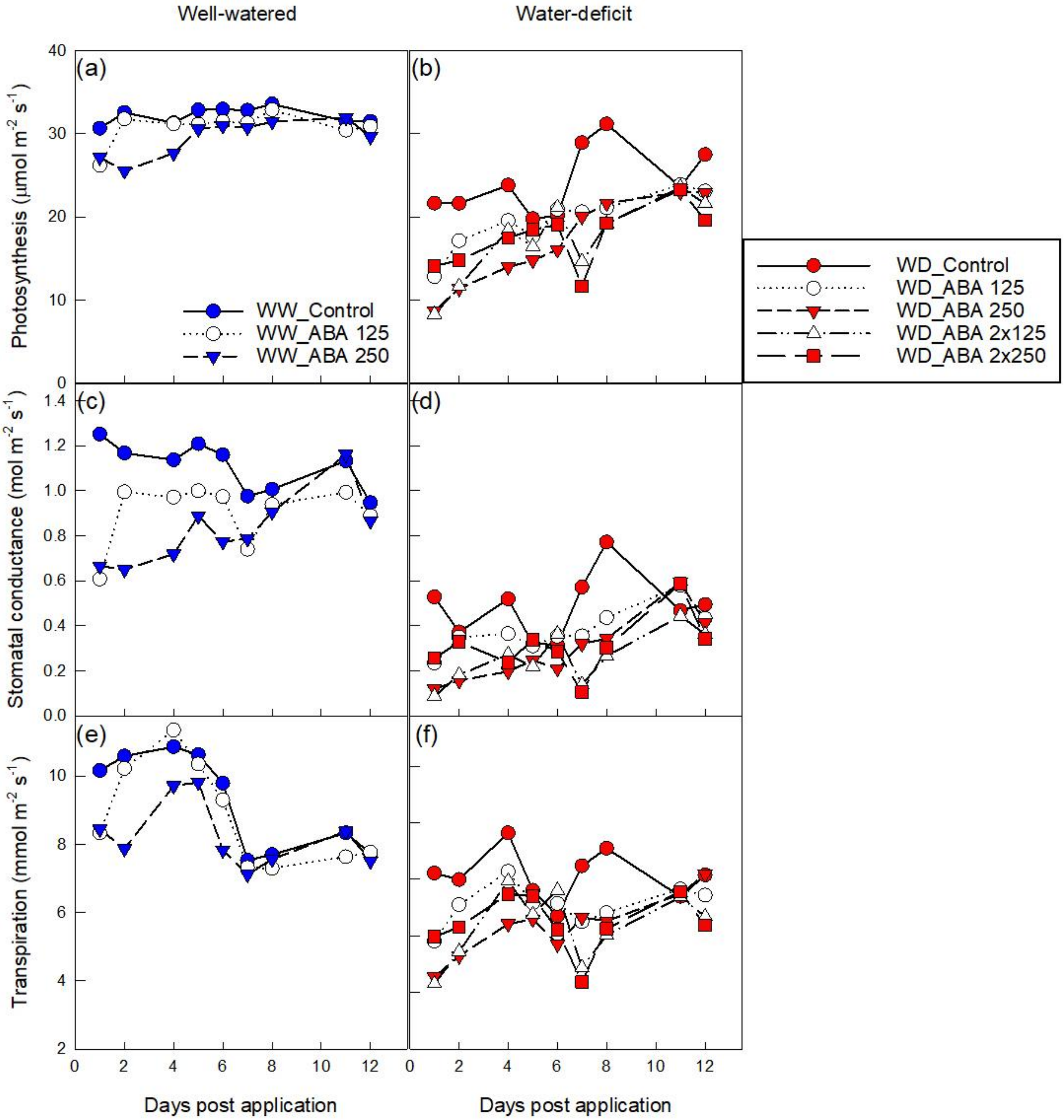


Figure 8. Effect of abscisic acid (ABA) on cotton physiology of well-watered and water-deficit plants. Photosynthesis (a and b), stomatal conductance (c and d) and transpiration (e and f). ABA was applied at either 125 ppm or 250 ppm, with a single application (at 57 DAP) or two applications (at 57 DAP and then 7 days post application).

Discussion

There was no significant ABA by water treatment interactions, suggesting that stomatal conductance of cotton responds to ABA in the same way regardless of soil water availability (Table 30). Our data also showed that cotton with the water deficit treatment consistently had reduced photosynthesis, stomatal conductance, and transpiration than plants in the well-watered treatment.

ABA treatments reduced photosynthesis (25-31%), stomatal conductance (52-56%), and transpiration (25-33%) of cotton for up to two days after application. Cotton sprayed with a lower rate of ABA (ABA125) had a shorter response time than cotton sprayed with a higher rate of ABA (ABA250). Therefore, the application of ABA may induce a short-term reduction in stomatal conductance for approximately 1-3 days, with a heavier application rate lasting longer than a lighter application rate. Unfortunately, leaf-level physiology was not measured at 60, 67 or 68 DAP due to unfavourable weather for physiology measurements (i.e., cloudy, rainy weather). However, by 4 days post application, the was no significant difference in stomatal conductance of plants sprayed with ABA and the controls when applied at first flower.

A second application of ABA, seven days after the first application, induced a reduction in stomatal conductance (61-67%), but not a difference in photosynthesis or transpiration. Compared with cotton that had received a prior ABA treatment, a second ABA treatment was only effective for one day, and effective for up to two days compared with control plants. This indicates that the response time is similar, regardless of whether the plants have been exposed to exogenous ABA previously.

Our experiment also explored the effect of two different rates of ABA, 125ppm and 250ppm, on the physiology of cotton. Our data indicates that both rates effectively reduce stomatal conductance by a similar degree (51-56%), although the effect was slightly longer lasting at the higher application rate (250ppm), with the possibility of reducing stomatal conductance for two to potentially three days.

Therefore, abscisic acid (ABA) applied at first flower temporarily decreases stomatal conductance of cotton for around two days, without having significant lasting physiological effects. These results highlight the importance of appropriate timing of the application, as the physiological effects are not long-lasting. However, this study does not address any longer-term effects on growth or yield.

Summary

A glasshouse experiment was conducted in March 2022 to investigate the effect that two rates of ABA (125ppm and 250ppm) have on the physiology of cotton in well-watered and water deficit conditions, applied at first flower. Our data showed that both rates of ABA effectively reduced stomatal conductance of cotton for one to two days, with the higher rate having a slightly longer-lasting effect. A second application of ABA, seven days after the first application, also reduced stomatal conductance of cotton for up to two days.

Field experiments:

Research question: Can we "protect" plants against water deficit stress during flowering? Can we "shut down" the plant and reduce water use? Is fruit and yield retained?

Materials and Methods

Field experiments were conducted during the 2021-22 and 2022-23 cotton seasons at ACRI, Narrabri. In each season, cotton (cv. Sicot 746B3F) was planted as part of the dryland, normal sowing trials (DLS2, Table 31). Two rates of ABA (125ppm or 250ppm) were applied, with the first application at the first stress after first flower and a second application 14 days later. Plant mapping, biomass and yield and water use were measured.

Table 31: Treatment summary for field experiments addressing Milestone 2.4

Experiment Details	Trt Abbreviation	Treatment	Rate (a.i.; ppm)	Frequency	Timing	Surfactant	Surfactant Rate	Application Dates
2021-22 ACRI								
Cotton Season: 2021-22	Control	Control (Sicot 746B3F)	-		-	-	-	
Location: ACRI, Narrabri	ABA125	Abscisic acid	125	2x14d	1st stress > FF	Yes	0.5/1ml.L^{-1}	5/1/22, 17/1/22
Experiment: DLS2	ABA250	Abscisic acid	250	2x14d	1st stress > FF	Yes	0.5/1ml.L^{-1}	5/1/22, 17/1/22
Planting Date: 21/10/2021								
Row Configuration: 2m, alt.								
2022-23 ACRI								
Cotton Season: 2022-23	Control	Control (Sicot 746B3F)	-		-	-	-	
Location: ACRI, Narrabri	ABA125	Abscisic acid	125	2x14d	1st stress > FF	Yes	0.5/1ml.L^{-1}	25/1/23, 8/2/23
Experiment: DLS2	ABA250	Abscisic acid	250	2x14d	1st stress > FF	Yes	0.5/1ml.L^{-1}	25/1/23, 8/2/23
Planting Date: 18/10/2022								
Row Configuration: Single Skip								

Results

Overall, the application of ABA, either at 125ppm or 250ppm, did not have a significant effect on the growth of cotton (Table 32). In one instance, plants applied with ABA250 were shorter and had more nodes than control plants (P<0.05), however, this trend was not observed in the 2022-23 cotton season.

Table 32: Biomass and plant mapping of cotton applied with two rates of ABA. Values represent the mean. Letters represent significant difference at P< 0.05 using Tukey's HSD.

	P-value	**Control**	**ABA125**	**ABA250**
2021-22 Cotton Season (Cut-out biomass @ 123DAP (21/2/22))				
Height (cm plant^{-1})	0.008	63^{b}	61^{b}	54^{a}
Nodes (plant^{-1})	0.006	23^{a}	23^{a}	29^{b}
Retention (%)	0.239	51	53	58
Total vege biomass (g/m)	0.638	364	341	331
Total fruit biomass (g/m)	0.911	438	428	418
Total biomass (g/m)	0.773	801	768	749
2022-23 Cotton Season (Post treatment biomass @ 125DAP (20/2/23))				
Height (cm plant^{-1})	0.796	55	56	55
Nodes (plant^{-1})	0.033	21^{a}	20^{a}	20^{a}
Retention (%)	0.515	35	39	37
Total vege biomass (g/m)	0.338	245	288	260
Total fruit biomass (g/m)	0.831	293	313	314
Total biomass (g/m)	0.599	539	601	574
2022-23 Cotton Season (60% open biomass @ 148DAP (15/3/23))				
Height (cm plant^{-1})	0.804	55	55	56
Nodes (plant^{-1})	0.191	20	20	21
Retention (%)	0.885	39	41	39
Total vege biomass (g/m)	0.220	215	227	275
Total fruit biomass (g/m)	0.596	403	410	466
Total biomass (g/m)	0.421	618	636	741

There was no significant difference in the yield of dryland cotton applied with ABA across two cotton seasons (P> 0.05, Table 33).

Table 33: Yield of cotton applied with two rates of abscisic acid (ABA; 125ppm or 250ppm) across two cotton seasons at ACRI, Narrabri.

Cotton season	**Experiment**	**PGR**	**Application Rate (ppm)**	**P-value**	**Control Yield (b/ha)**	**PGR Yield (b/ha)**	**Yield relative to Control (%)**	**Yield relative to Control**
2021-22	DLS2	ABA	125	0.772	5.7	5.9	2.7	↑
2021-22	DLS2	ABA	250	0.772	5.7	5.7	-1.3	↓
2022-23	DLS2	ABA	125	0.318	4.1	4	-2.3	↓
2022-23	DLS2	ABA	250	0.318	4.1	4.2	3.3	↑

There was no significant difference in the change in volumetric soil water content (ΔVSWC %) between 62DAP and 192 DAP (P> 0.05, Table 34).

Table 34: ANOVA table ΔVSWC between 62DAP and 192DAP for cotton applied with two rates of ABA. Values represent the mean.

Depth (cm)	P-value	Control	ABA125	ABA250
10	0.478	0.58	0.01	0.01
30	0.733	0.22	0.24	0.05
50	0.235	0.09	0.07	0.15
70	0.461	0.24	0.06	0.10
90	0.449	0.15	0.09	0.10

Discussion

Our data showed that the application of ABA at first stress after first flower did not significantly increase growth or yield of dryland cotton across two cotton seasons. We were also unable to detect changes in soil water content, suggesting that there was no difference in plant water use for plants applied with ABA. These results reflect a possible short-term physiological response to the application of ABA. ABA is hormone that is dynamic in the plant, although ABA uptake was not measured in these experiments, but could be included in any future studies. With the assumption that stomatal conductance was limited following application, as shown by the physiology measurements in our glasshouse study and in literature (Gadallah, 1995; Hu et al., 2022), it is likely that the plant was able to compensate yield for any minor temporary water and/or heat stresses experienced throughout the season resulting in no significant differences in yield. However, environmental conditions in Narrabri during the 2021/22 and 2022/23 cotton seasons were relatively mild, and not representative of hot, dry abiotic stresses that plants can sometimes be exposed to at first flower.

Summary

Two field experiments were conducted at ACRI, Narrabri across the 2021-22 and 2022-23 cotton seasons to investigate the effect that two rates of ABA (125ppm and 250ppm) have on the growth, water use and yield of cotton, applied at first flower. Our study found that little evidence that we can "protect" plants against water deficit stress during flowering using ABA, however, the two seasons were not representative of seasons where the plants were exposed to large water deficits during the flowering period. Our data showed no significant changes in plant water use with the application of ABA, and no changes in the retention of fruit and yield.

Milestone 2.4 Summary

To establish whether there are alternative hormones/PGR approaches that can enhance cotton growth in stressed environments, glasshouse and field experiments were conducted to (1) to investigate the effect that two rates of ABA have on the physiology of cotton in well-watered and water deficit conditions; and (2) understand the effects of ABA on growth, water use and yield of cotton in the field. Data from the glasshouse experiment showed significant reductions in stomatal conductance of cotton applied with ABA for approximately two to three days, with higher application rates having a marginally longer lasting effect. The short-term effects of ABA on cotton physiology likely contributed to no significant changes in plant water use, fruit retention or yield of plants grown in field conditions although the crop was not displaying signs of stress. Thus, ABA was not shown to enhance cotton growth in the level of stress that we were able to achieve in these experiments.

Milestone 2.5 Identify potential new hormone/PGR formulations that could assist cotton agronomy.

Introduction

There are several hormones and PGR formulations that are used in other broadacre and horticultural crops, but currently not used in cotton production. Hormones such as Forchlorfenuron (CPPU), AMF, GA$_{4,7}$ and 6-Benzyladenine, and Glycine Betaine may assist crop management. Further information on each growth hormone is provided below.
Research question: are there any new hormones/PGR formulations identified using a broad screening process that benefit cotton yield?

Materials and Methods

Several experiments were conducted to screen potential new hormone/PGR formulations that could assist cotton agronomy. Experiments were conducted during the 2021-22 and 2022-23 cotton seasons in replicated small plot field trials at ACRI. Products tested included Forchlorfenuron (CPPU), GA$_{4,7}$ and 6-Benzyladenine, and Glycine Betaine. Methods for each hormone/PGR formulation are described below.

Forchlorfenuron (CPPU)

Introduction

Forchlorfenuron, CPPU (*N*-(2-Chloro-4-pyridyl)-*N′*-phenylurea) is a highly active synthetic cytokinin-like plant growth regulator used to increase berry size in kiwi and grapes. CPPU stimulates periclinal cell division leading to more round or oval shaped berries (Khan and Ali, 2018). CPPU can also increase fruit set and accelerate cell expansion and fruit enlargement (Zeng et al., 2016). In China in 2011, too much CPPU was applied to watermelon crops late in the season, resulting exploding watermelons, caused by the rapid expansion of the fruit. Therefore, CPPU applied to developing cotton bolls may increase expansion rates and boll size, potentially leading to increased yield. However, this has not been tested in the field.
Research question: Does CPPU applied at first cracked boll increase cotton yield?

Methods

The effect of CPPU on boll size and yield was tested in the semi-irrigated experiment in 2021-22 season (Exp 10) and in the irrigated experiments in the 2021-22 (Exp 11) and 2022-23 (Exp 16). cotton seasons. In each season, cotton was planted in October and grown in using standard agronomic practices for the region. Two applications of CPPU were applied at First Cracked Boll at a rate of 10ppm, 2 x 10 days apart, as outlined in Table 35. Boll size and final yield was measured across each of the three experiments.

Table 35. Treatment summary for CPPU applications

Experiment Details	**Trt Abbreviation**	**Treatment**	**Rate (a.i.; ppm)**	**Frequency**	**Timing**	**Surfactant**	**Application Dates**
2021-22 ACRI (Exp 10)							
Cotton Season: 2021-22	Control	Control	-	-	-	-	
Location: ACRI, Narrabri	CPPU		10ppm	2x10d	FCB	None	8/3/22, 18/3/22.
Experiment: Semi							
Planting Date: 21/10/2021							
Row Configuration: 2m, alt.							
2021-22 ACRI (Exp 11)							
Cotton Season: 2021-22	Control	Control	-	-	-	-	

Location: ACRI, Narrabri Experiment: Irrigated Planting Date: 17/09/2021 Row Configuration: Solid	CPPU		10ppm	2x10d	FCB	None	8/3/22, 18/3/22.
2022-23 ACRI (Exp16)							
Cotton Season: 2022-23	Control	Control	-	-	-	-	
Location: ACRI, Narrabri Experiment: Irrigated Planting Date: 18/10/2022 Row Configuration: Solid	CPPU		10ppm	2x10d	FCB	None	9/3/23, 20/3/23.

Results

CPPU applied at first cracked boll did not significantly affect boll size of cotton (P> 0.05, Table 36).

Table 36: Boll size of plants applied with CPPU at first cracked boll

Relevant Exp ID	Cotton season	Experiment	P-value	Control (g boll^{-1})	CPPU (g boll^{-1})
10	2021-22	Semi	0.581	4.40	4.31
11	2021-22	Irrigated	0.831	4.44	4.39
16	2022-23	Irrigated	0.393	4.67	4.87

The effect of CPPU on lint yield was variable across experiments. Lint yield of semi-irrigated cotton was reduced by 7.8% with the application of CPPU at first cracked boll in Exp 10 (P< 0.05, Table 37). However, there was no significant difference in irrigated lint yield across the two cotton seasons tested (Exp 11 and Exp 16).

Table 37: Lint yield of plants applied with CPPU at first cracked boll

Relevant Exp ID	Cotton season	Experiment	P-value	Control Lint yield (b/ha)	CPPU Lint yield (b/ha)	Lint yields relative to Control (%)	Lint yields relative to Control
10	2021-22	Semi	0.036	6.0	5.5	-7.8	↓
11	2021-22	Irrigated	0.893	14.1	13.9		
16	2022-23	Irrigated	0.887	14.2	14.2		

Discussion

The application of CPPU applied at first cracked did not increase cotton boll size or cotton yield across any of our three experiments. In the semi-irrigated experiment (Exp 10), our data showed that yield of cotton applied with CPPU at first cracked boll was lower than the control, however, this was not reflected in the irrigated experiments. It could be possible that cell expansion was limited by water deficit, exacerbating a negative water by CPPU interactive effect, but this would need to be tested further. Burke and Sanchez (2018) found that CPPU may increase lateral root production, potentially reducing water stress and increasing yields, however, these applications of CPPU were applied much earlier, at the cotyledon stage.

Summary

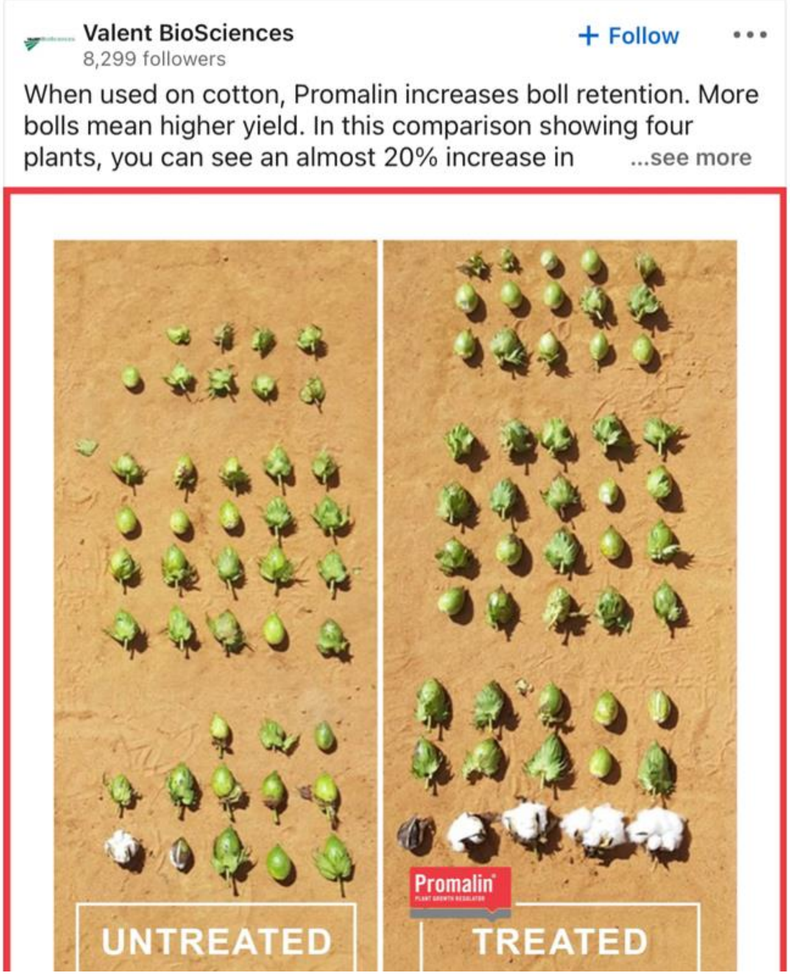
The application of CPPU was tested in three experiments across the 2021-22 and 2022-23 cotton seasons. Our data indicated that CPPU did not increase boll weight or yield of irrigated or semi-irrigated cotton when applied at first cracked boll.

Gibberellins A_4 and A_7 + 6-Benzyladenine ($GA_{4,7}$ + BA)

Introduction

Gibberellins promote the elongation of fruit. Commercially, gibberellins are used to increase fruit size of table grapes. Gibberellins increase the stalks of the grapes so that there is more space for grapes to enlarge in the grape bunches. A mixture of the cytokinin benzyl adenine and $GA_{4,7}$ ($GA_{4,7}$ + BA) is very effective in stimulating apple fruit to increase in size, especially in red Delicious type apples (Veinbrants and Miller, 1981). Delicious apples with a high length:diameter (L:D) ratio and prominent calyx lobes are termed "typey", and are said to have the characteristic Delicious shape. Air temperature during flowering and early fruit development is crucial in determining final fruit shape, with low air temperature after blooming resulting in apples with the characteristic Delicious shape.

Trials in Brazil have reported increased boll size and subsequent increased yield in cotton when applied with a product containing $GA_{4,7}$ + BA at 10 day intervals starting at either match-head square (BBCH 52; first floral buds visible) or at early flowering (5-6 flowers/8m; BBCH 61) (Figure 9). However, the effects on physiology, boll size and yield of cotton applied with $GA_{4,7}$ + BA have not been assessed in Australian production systems.



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When used on cotton, Promalin increases boll retention. More bolls mean higher yield. In this comparison showing four plants, you can see an almost 20% increase in ...see more

Figure 9: A product containing $GA_{4,7}$ + 6-Benzyladenine has been shown to increase boll retention in cotton, leading to greater yields (LinkedIn, 2022).

Research questions: 1. Does the application of $GA_{4,7}$ + BA alter cotton physiology? 2. Does the application of $GA_{4,7}$ + BA increase boll size in irrigated cotton?

Methods

Glasshouse experiments

Cotton (cv. Sicot 746B3F) was planted in the glasshouse on the 19th January 2022. Plants were grown at 30/20°C (day/night), fertilised and well-watered, maintained at 100% field capacity by weighing the pots each day and replenishing the water used. The experiment was designed as a randomised block design with six replications. A single application of $GA_{4,7}$ + BA was applied using handheld spray equipment at a rate of 125ppm at first flower (17/3/22; 57 DAP). Leaf-level gas exchange was measured using a Licor 6400XT, for 12 days after application. Results were analysed in Genstat v 19 using ANOVA, with a 0.05 level of significance.

Field experiments

The application of $GA_{4,7}$ + BA was tested in two irrigated field experiments across two cotton seasons, with two different timings of application (first square, FS and first flower, FF). A treatment summary is shown in Table 38. Boll size and yield were measured.

Table 38: Treatment summary for $GA_{4,7}$ + BA applications in field experiments

Experiment Details	Trt Abbreviation	Treatment	Rate (a.i.; ppm)	Frequency	Timing	Surfactant	Surfactant Rate	Application Dates
2021-22 ACRI (Exp 11)								
Experiment: Irrigated	Control	Control	-	-	-	-	-	
Planting Date: 21/10/2021	$GA_{4,7}$ + BA FS	$GA_{4,7}$ + 6-Benzyladenine FS	$125ml.ha^{-1}$	3x10d	FS	Yes	0.5ml/1L	20/12/21, 30/12/21, 10/1/22.
Row Configuration: Solid	$GA_{4,7}$ + BA FF	$GA_{4,7}$ + 6-Benzyladenine FF	$125ml.ha^{-1}$	3x10d	FF	Yes	0.5ml/1L	10/1/22, 17/1/22, 31/1/22.
2022-23 ACRI (Exp 16)								
Experiment: Irrigated	Control	Control	-	-	-	-	-	
Planting Date: 18/10/2022	$GA_{4,7}$ + BA FS	$GA_{4,7}$ + 6-Benzyladenine FS	$125ml.ha^{-1}$	3x10d	FS	Yes	0.5ml/1L	19/12/22, 29/12/22, 9/1/23.
Row Configuration: Solid	$GA_{4,7}$ + BA FF	$GA_{4,7}$ + 6-Benzyladenine FF	$125ml.ha^{-1}$	3x10d	FF	Yes	0.5ml/1L	13/1/23, 24/1/23, 3/2/23.

Results

Effect of $GA_{4,7}$ + BA on cotton physiology in glasshouse experiments

Results from the glasshouse experiment showed that cotton with $GA_{4,7}$ + BA applied at first flower had 51% lower stomatal conductance than the control plants, only on the second day after application (Figure 10, Table 39). There was no significant difference in stomatal conductance between the control plants and plants applied with $GA_{4,7}$ + BA on any other day of physiology measurement.

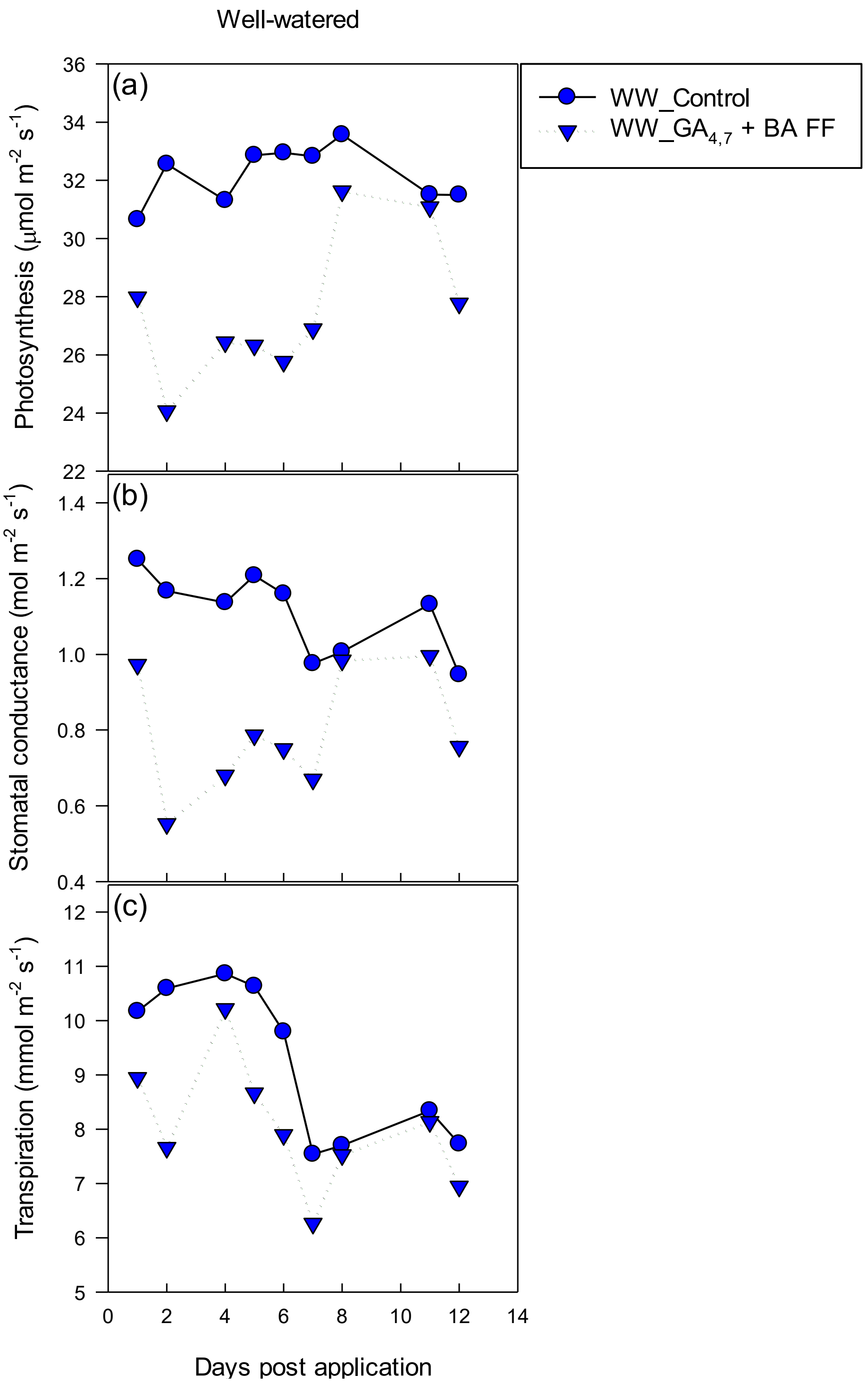


Figure 10. Effect of $GA_{4,7}$ + BA on (a) photosynthesis, (b) stomatal conductance, and (c) transpiration of well-watered plants in the glasshouse. $GA_{4,7}$ + BA was applied at first flower (57 DAP).

*Table 39. ANOVA results of $GA_{4,7}$ + BA on stomatal conductance of cotton grown in the glasshouse. Values are P-values and * represents significance at P< 0.05.*

Date	**Day after planting (DAP)**	**Day after Application**	**$GA_{4,7}$ + BA treatment**
18/3/22	58	1	0.117
19/3/22	59	2	0.012*
21/3/22	61	4	0.157
22/3/22	62	5	0.076
23/3/22	63	6	0.279
24/3/22	64	7	0.093
25/3/22	65	8	0.829

28/3/22	68	11	0.403
29/3/22	69	12	0.117

The effect of $GA_{4,7}$ + BA on boll size and yield in field experiments

Results from the field experiments showed that the application of $GA_{4,7}$ + BA on cotton at either first square or first flower did not have a significant effect on either boll number or boll weight (P>0.05, Table 40).

Table 40: Boll number and weight of cotton applied with $GA_{4,7}$ + BA in the field.

Timing of $GA_{4,7}$ + BA Treatment	Cotton season	Experiment	TrtID	P-value	Control	$GA_{4,7}$ + BA
Boll number (per m^2)						
First Square	2022-23	Irrigated	5	0.636	141	150
First Flower	2022-23	Irrigated	6		141	146
Boll weight (g/m^2)						
First Square	2022-23	Irrigated	5	0.778	4.67	4.78
First Flower	2022-23	Irrigated	6		4.67	4.61

The effect of $GA_{4,7}$ + BA on yield was variable (Table 41). Relative to the control, crops applied with $GA_{4,7}$ + BA at first square had a -0.14 to 3.42% difference in yield, and crops applied with $GA_{4,7}$ + BA at first flower had a -4.03 to 1.14% difference in yield. These results differ from the yield increases reported in Brazil.

Table 41: Lint yield of cotton applied with $GA_{4,7}$ + BA

Timing of $GA_{4,7}$ + BA Treatment	Cotton season	Experiment	Relevant Exp ID	P-value	Control Yield (b/ha)	$GA_{4,7}$ + BA Yield (b/ha)
First Square	2021-22	Irrigated	11	0.655	14.05	14.53
First Square	2022-23	Irrigated	11	0.959	14.16	14.14
First Flower	2021-22	Irrigated	16	0.891	14.05	14.21
First Flower	2022-23	Irrigated	16	0.091	14.16	13.59

Discussion

Cotton with $GA_{4,7}$ + BA applied at first flower in the glasshouse had 51% lower stomatal conductance than the control plants, only on the second day after application. There was no significant difference in stomatal conductance between the control plants and plants applied with $GA_{4,7}$ + BA on any other day of physiology measurement. No sustained changes in leaf-level gas exchange functioning likely contributed to no significant changes in boll number, boll weight or final lint yield, as observed in the field experiments. These results contrast with reports from Brazil and other countries (Figure 9), where application of $GA_{4,7}$ + BA resulted in greater boll size, contributing to increased yields. Contrasting results may be due to differing environmental conditions such as water, air temperature or radiation, or differences in timing or rates of application. Further exploration could investigate if alternative rate and timing of application had any effect on boll size and yield in Australian irrigated systems.

Summary

Glasshouse and field experiments were conducted to assess the effects of $GA_{4,7}$ + BA on physiology, boll size and yield in irrigated cotton. Overall, our data showed no significant effect on stomatal conductance, boll size or yield of cotton.

Glycine Betaine (GB)

Introduction

The biosynthesis of glycine betaine (GB) in plants is often triggered by abiotic stress, with increased GB production in response to water deficits. GB is abundant mainly in chloroplast where it plays a vital role in adjustment and protection of thylakoid membrane, thereby maintaining photosynthetic efficiency. GB is very hygroscopic (absorbs moisture from the air), and may be applied as an osmoprotectant (Clendennen and Boaz, 2019). It is believed that osmoprotectants like GB protect the plants from dehydration and maintain osmotic balance in the cells during a stress event, by protecting cellular structures and enhancing photosynthesis and growth in water deficit conditions.

Crop plants have been shown to accumulate GB in response to abiotic stress, although in many crops the natural accumulation of GB is lower than sufficient to ameliorate the adverse effects of water deficits. However, studies have shown that externally applied GB may ameliorate the impact of environmental stresses, like drought and high salinity, and improve recovery from stress. Positive effects of applied GB have been measured in wheat, barley, sorghum, and soybean. Sarwar et al. (2006) reported that GB accumulation was positively correlated with seed cotton yield and boll number. Therefore, it is possible that the exogenous application of GB may ameliorate the negative effects of abiotic stresses on cotton and thereby increase yield.

Research question: Does the application of GB increase cotton yield?

Methods

The effect of GB on lint yield was tested in six dryland and semi-irrigated experiments across three cotton seasons (Table 42).

Table 42: Treatment summary for Glycine Betaine (GB) applications in field experiments

Experiment Details	Trt Abbreviation	Treatment	Rate (a.i.; ppm)	Frequency	Timing	Surfactant	Application Dates
2020-21 ACRI (Exp 3) Cotton Season: 2020-21 Location: ACRI, Narrabri Experiment: Semi Planting Date: 11/11/2020 Row Configuration: 2m, alt.	Control	Control	-	-	-	-	
	GB	Glycine Betaine	4000	3x10d	2L	None	8/2/21, 23/2/21.
2020-21 Whitton (Exp 7) Cotton Season: 2020-21 Location: Whitton, NSW Experiment: Irrigated Planting Date: 2/10/2020 Row Configuration: Solid	Control	Control	-	-	-	-	
	GB	Glycine Betaine	4000	3x10d	2L	None	4/11/20, 13/11/20, 23/11/20.
2021-22 ACRI (Exp9) Cotton Season: 2021-22 Location: ACRI, Narrabri Experiment: DLS2 Planting Date: 21/10/2021 Row Configuration: 2m, alt.	Control	Control	-	-	-	-	
	GB	Glycine Betaine	4000	3x10d	1st stress > FF	None	5/1/22, 13/1/22.
2021-22 ACRI (Exp 10) Cotton Season: 2021-22 Location: ACRI, Narrabri Experiment: Semi Planting Date: 21/10/2021 Row Configuration: 2m, alt.	Control	Control	-	-	-	-	
	GB	Glycine Betaine	12g.L^{-1}	3x10d	1st stress > FF	None	5/1/22, 13/1/22.
2021-22 Whitton (Exp 13) Cotton Season: 2021-22 Location: Whitton, NSW Experiment: Irrigated Planting Date: 12/10/2021 Row Configuration: Solid	Control	Control	-	-	-	-	
	GB	Glycine Betaine	4000ppm	3x10d	2L	Yes	9/11/21, 22/11/21, 3/12/21.
2022-23 ACRI (Exp15) Cotton Season: 2022-23 Location: ACRI, Narrabri Experiment: DLS2	Control	Control	-	-	-	-	
	GB	Glycine Betaine		2x10d	2L	None	25/1/23, 3/2/23.

Planting Date:
18/10/2022
Row Configuration: Single Skip

Results

There was no significant difference in yield of cotton applied with GB compared with the control (P> 0.05, Table 43). Numerical decreases in relative yield were observed in two experiments, with a 6.1 % to 9.7% reduction (P< 0.1).

Table 43: Yield of cotton applied with Glycine Betaine (GB).

Relevant Exp ID	Cotton season	Experiment	P-value	Control Yield (b/ha)	GB Yield (b/ha)	Yield relative to Control (%)	Yield relative to Control
3	2020-21	Semi	0.815	6.6	6.8		
7	2020-21	Whitton	0.696	18.5	18.7		
9	2021-22	DLS2	0.087	5.7	5.2	-9.7	↓
10	2021-22	Semi	0.056	6.0	5.6	-6.1	↓
13	2021-22	Whitton	0.666	12.6	13.2		
15	2022-23	DLS2	0.524	4.1	3.9		

Discussion

Our results showed that the application of GB did not have a consistent significant effect on the yield of cotton across six field experiments spanning different years and water treatments. Yield response relative to the controls was also variable. No significant yield improvements with the application of GB may be due to experiments conducted in seasons that were relatively mild with minimal abiotic stress events. In contrast, Ramazanoglu et al. (2024) found that the application of GB increased yield of seed cotton by approximately 0.45 bales/ha. However, these results were only based on one cotton season of data in Türkiye where varieties, soil types and inputs are different from Australian production systems. Interestingly, results reported by Ramazanoglu et al. (2024) also show a significant GB by water deficit interaction, suggesting that the magnitude of benefit from the GB application could be greater in 100% irrigated systems. Therefore, there may be a potential to explore the application of GB across differing production systems in Australia.

Summary

The application of GB was tested in six experiments across three cotton seasons and varying water stress conditions. Our data indicated that the application of GB did not significantly increase yield of cotton when applied at the 2-leaf stage, or when applied at first stress after first flower, although the mild environmental conditions throughout these seasons may not have been enough to induce abiotic stresses on the plants.

Milestone 2.5 Summary

Several potential new hormone/PGR formulations that could potentially assist cotton agronomy were broadly screened for yield improvements across field experiments across multiple years. PGR's tested included Abscisic acid (ABA), Forchlorfenuron (CPPU), Gibberellin $A_{4,7}$ and 6-Benzyladenine ($GA_{4,7}$ + BA) and Glycine Betaine (GB). Our data showed inconsistent yield responses for the respective application timings and rates for products tested. These results may have been partially due to mild environmental conditions and a lack of hot, dry temperatures to fully test yield responses across a range of climatic conditions.

Enhanced capability/capacity in cotton cropping systems agronomy (Milestone 4)

Milestone 4.2 Develop oversight and leadership on industry related issues pertaining to cotton agronomy in a systems context.

The Australian cotton production manual was reviewed in February 2022. The manual is very comprehensive, and no changes were suggested for the 2022 cotton season. As more data becomes available on the implications of early-season MC use across regions with the new proposed project, there may be an opportunity to update the Australian cotton production manual with additional information and case studies.

Milestone 4.3 Communicate results of research to industry.

Preliminary results and experimental concepts have been discussed and reported at grower field days, the Australian Cotton Conferences 2022 and 2024, and industry events. Outcomes of these experiments will continue to be presented more broadly via conference presentations, the CRDC Spotlight magazine, and The Australian Cottongrower, where appropriate. We are currently working on a communication engagement plan. We will link with CottonInfo and resources will be invested in extension to communicate results of this research to industry over the next 12 months.

Milestone 4.5. Engage with growers, consultants and CottonInfo representatives to conduct grower/consultant-led research to understand the effects of various alternative early-season mepiquat chloride application strategies on cotton growth and yield.

Introduction

We engaged with growers, consultants and CottonInfo representatives and conducted grower/consultant-led research activities to understand the effects of early-season MC application strategies on cotton growth and yield. Prior to the 2023-24 cotton season, we explored collaborative research experiments with growers and consultants across several cotton regions, spanning the Darling Downs, the Gwydir Valley, the Namoi, and the Murrumbidgee.

Through discussion with consultants and growers across cotton regions, questions emerging around the use of early-season MC were:

- How early should we apply MC?
- How "hard" can we apply MC?
- Is there a difference in varieties and how we should use MC?
- Can we manage the canopy and make defoliation easier?

Methods

Experiments were established at Cecil Plains, Qld; Togo Station at Wee Waa, NSW; Aberdeen, NSW; Griffith, NSW and Darlington Point, NSW. Each experiment was tailored to address the specific questions asked by the grower/consultant. Details of each experiment are outlined in Table 44. Due to seasonal environmental conditions, the grower elected not to apply early season MC to the trials at Darlington Point and the trial was abandoned.

Table 44: Experiment details for grower and consultant-led mepiquat chloride experiments during the 2023-24 cotton season.

Experiment Details	Treatments	Rate RX380TM	Application Dates
Experiment: Cecil Plains, Qld	1. Control		
Variety: Sicot 746B3F	2. High Rate MC	60ml/ha	11/01/2024

Planting Date: 2/11/23 Row Configuration: Single Skip	3. Grower	120ml/ha	23/02/2024		
Experiment: Wee Waa, NSW	1. Control				
Variety: Sicot 746B3F	2. High Split application	60ml/ha	19/12/2023	09/01/2024	25/01/2024
Planting Date: 25/10/23 Row Configuration: Solid, furrow irrigated	3. Low Split application	25ml/ha	19/12/2023	09/01/2024	25/01/2024
Experiment: Aberdeen, NSW	1. Control				
Varieties: Sicot 606B3F (planted: 9/10/23) Sicot 748B3F (planted: 15/10/23) Row Configuration: Solid, pivot irrigated	2. High Split application	60ml/ha	02/01/2024	11/01/2024	
Experiment: Griffith, NSW	1. Control				
Variety: Sicot 748B3F	2. High Split application	60ml/ha / 100ml/ha / 100ml/ha	07/12/2024	28/12/2023	28/01/2024
Planting Date: 4/10/23 Row Configuration: Solid, furrow irrigated	3. Low Split application	25ml/ha / 60ml/ha / 100ml/ha	07/12/2024	28/12/2023	28/01/2024

Results and Discussion

- *How early and "hard" should we apply MC?*

Overall, there was no significant difference in yield across three locations with either low or high early season MC applications (P>0.05, Table 45). At Cecil Plains, yield of skip row cotton ranged from 9.1 to 9.6 bales/ha with a trend for an additional 0.5 bales/ha in Trt 3. At Wee Waa, yield ranged from 11.7 to 11.9 bales/ha. At Griffith, yield ranged from 11.4 to 13.2 bales/ha. Although the difference was not significant, compared with unsprayed cotton the application of early season MC improved yield by 1.2 to 1.8 bales/ha.

These results show that early season MC applications improved yield in Griffith, but there was less of an advantage across longer season regions. This suggests that early season MC applications may be a particularly important management strategy for shorter season growing regions. However, our results also indicate that the management strategy also requires a good understanding of the implications of MC. Comparing high (Trt 2) and low (Trt 3) rates of MC at Griffith shows a 0.5 bale/ha reduction in relative yield with Trt 2 compared with Trt 3 (Figure 11). This suggests that yield penalties may occur with very high early or prescriptive application of MC on cotton.

Table 45: Yield (b/ha) of cotton applied with early season MC.

Location	P-value	Trt1 (Control)	SE	Trt2 (High)	SE	Δ Trt 2 vs. Trt1	Trt3 (Low)	SE	Δ Trt 3 vs. Trt1
Cecil Plains	0.749	9.1	0.3	9.2	0.7	0.1	9.6	0.2	0.5
Wee Waa	0.951	11.8	0.6	11.9	0.7	0.1	11.7	0.6	-0.2
Griffith	0.140	11.4	0.9	12.6	0.6	1.2	13.2	0.5	1.8

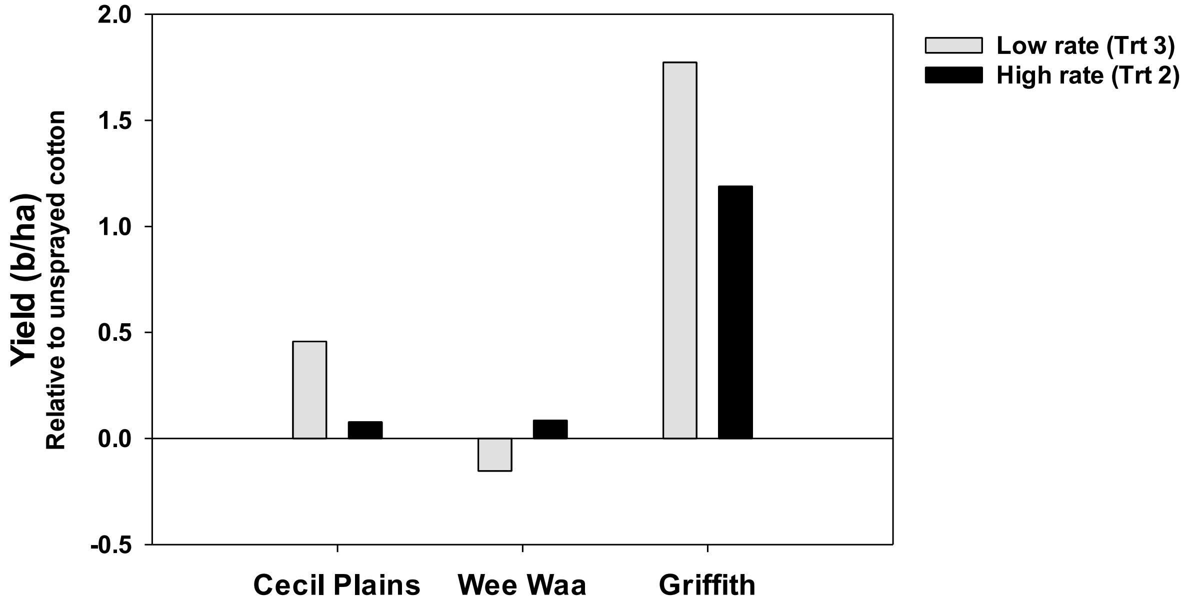


Figure 11: Relative yield (bales/ha) of cotton applied with two alternative mepiquat chloride strategies across three locations spanning the Australian cotton industry. Note that differences were not significant (P>0.05).

- *Is there a difference in varieties and how we should use MC?*

The Aberdeen trial compared early applications of high rates of MC on Sicot 748B3F and Sicot 606B3F. There was no significant variety x MC treatment interaction or MC treatment effect on yield of cotton (P>0.05, Table 46), indicating that there was no significant difference in these two varieties determining differing early-season MC strategies. Averaged across MC treatments, Sicot 748B3F yielded 12.1 bales/ha compared with Sicot 606B3F which yielded 8.3 bales/ha (P= 0.001, Table 46). Although differences were not significant at the 5% level (P= 0.092), there was a numerical difference indicating there may be potential for early-season MC management to improve yield by 1 bale/ha across both varieties.

Table 46: ANOVA results showing treatment effects on yield for the trial at Aberdeen, NSW during the 2023/24 cotton season

Parameter	Mean Yield (b/ha)	P-value
Variety Effects		0.001
Sicot 606B3F	8.3	
Sicot 748B3F	12.1	
MC Treatment Effects		0.092
Control	9.7	
Mepiquat	10.7	
Variety x MC Trt Interactive Effects		0.980

Control x Sicot 606B3F	7.7
Mepiquat x Sicot 606B3F	8.8
Control x Sicot 748B3F	11.6
Mepiquat x Sicot 748B3F	12.7

- *Can we manage the canopy and make defoliation easier?*

From each plot, leaves remaining on plants from 1m of defoliated plants at Cecil Plains were collected, measured using the leaf area machine and the dry mass weighed. Results showed significant differences between MC treatments, with Trt 2 having both a greater leaf area and greater leaf mass than Trt 3 (Table 47). These results indicate that we can alter leaf canopy, but further research is needed to refine methodology and gain a deeper understanding of canopy size and the effects on defoliation.

Table 47: Effects of mepiquat chloride treatments on leaf area and leaf weight of skip-row cotton after defoliation at Cecil Plains, Qld during the 2023/24 cotton season.

MC Trt	Total remaining leaf area (cm2/m)	SE	Total remaining leaf weight (g/m)	SE
1 (Control)	2624^{ab}	94	18.4^{a}	0.8
2 (High MC)	3898^{b}	257	28.2^{b}	2.6
3 (Grower)	1983^{a}	489	14.8^{a}	3.7
P-value	0.017		0.013	

Engagement activities with growers and consultants throughout the mepiquat chloride studies

Throughout the season, we actively engaged with growers, consultants and CottonInfo representatives. Katie presented at the Coleambally grower meeting (November 2023), Coleambally field day (CottonInfo/CSD February 2024), Griffith field walk (Summit Ag, March 2024), and Mullaley field day (Upper Hunter Namoi Cotton Growers Association, April 2024) (Figure 12). A panel discussion at the CCA seminar (Tamworth, June 2024) was presented by the consultants, Sandra and Katie. Unfortunately, the growers were unable to participate in the CCA seminar panel discussion due to a clash with cotton picking. Katie also presented results and outcomes from this research at the Australian Cotton Conference, CSD mastering cotton forum (August 2024): https://csd.net.au/blogs/catch-up-on-the-2024-csd-mastering-cotton-forum/. Furthermore, we are continuing conversations with consultants, CottonInfo and Melanie Jenson regarding additional communication strategies such as CottonInfo newsletters and CRDC Spotlight articles for these trials.

Summary

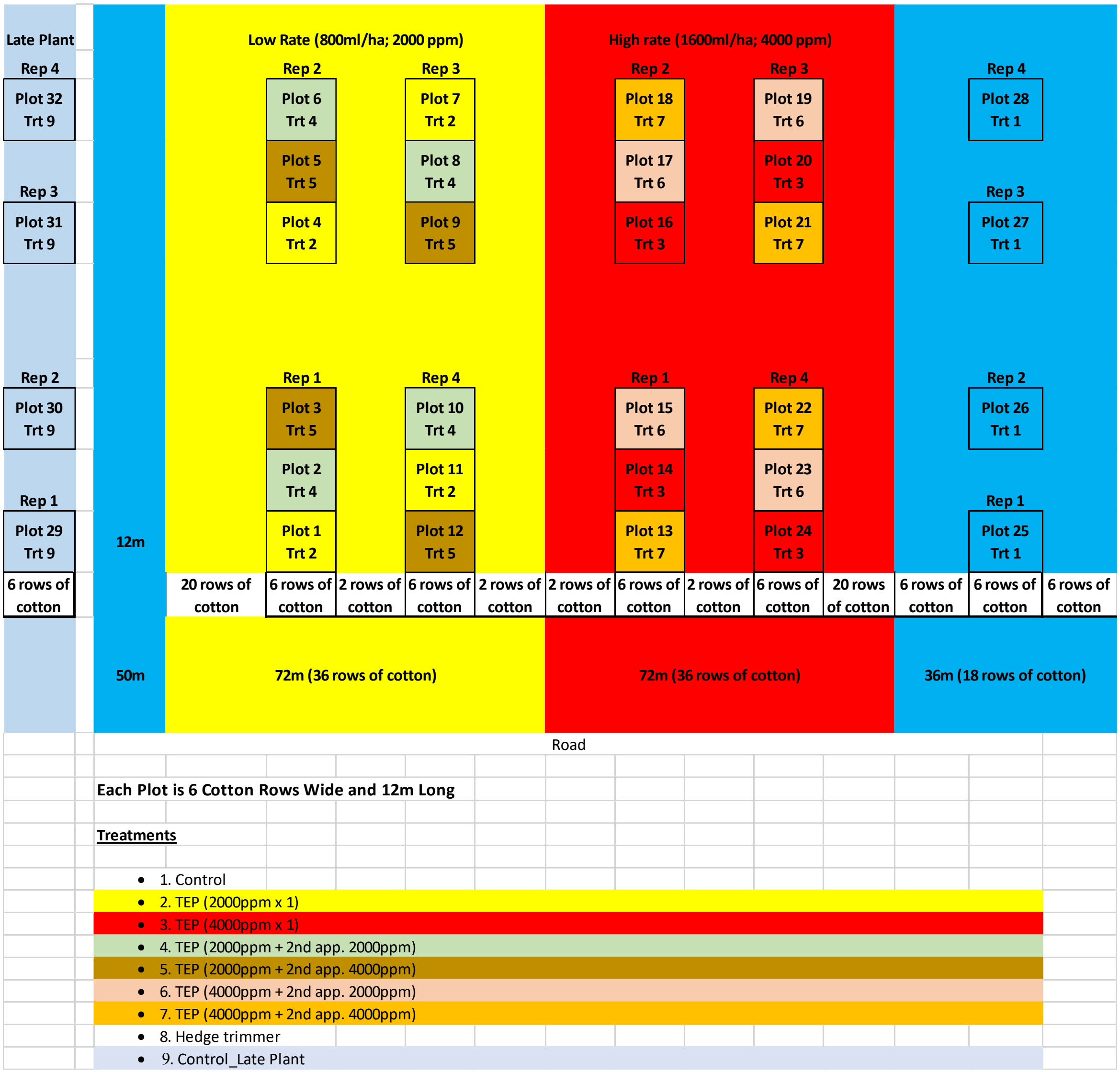
Grower/consultant-led experiments were conducted at Cecil Plains, Aberdeen, Wee Waa and Griffith to better understand the effects of alternative early-season application strategies on cotton growth and yield. Although there were no significant differences in yield, results showed that early-season MC applications may benefit relative yield, however, there may be some losses in relative yield with very high rates of MC. Preliminary results have been presented at four field days during the 2023/24 cotton season, at the CCA seminar in conjunction with consultants across the regions, and at the Australian Cotton Conference, August 2024.

Figure 12: Photos from field day engagement activities during the 2023/24 cotton season

Milestone 4.6 Engage with growers and consultants to conduct a field demonstration of selected novel PGR approaches (based on CSP2001 data and grower interest) that can enhance cotton growth. This will require consultation with growers and technical providers.

A grower/consultant-led field demonstration to investigate the use of Trinexapac-ethyl (TEP) was conducted at Terry Hie Hie during the 2023/24 cotton season. Due to late rainfall dictating a late start to the season for dryland growers, this study was conducted later than our previous TEP trials at ACRI. Cotton variety Sicot 748B3F was planted on the 18th November 2023 in a double-skip configuration. The first spray was applied by the grower via a 36m spray rig on the 17/12/23 at either a low (2000ppm; 0.8L/ha) or high (4000ppm; 1.6L/ha) rate. A randomised block design with four replicates of each treatment was overlayed incorporating treated strips, as shown in Figure 13. A late planted control (i.e., no TEP application) was also measured as a comparison throughout the experiment. However, this complicated the experimental design and was thus removed from the analyses. Growth (height and nodes), development (time to first square (TTFS), time to first flower (TTFF) and time to maturity) and final yield were measured. Data was analysed by ANOVA using Genstat v22. Effects were tested at the 0.05 level of significance, and means were compared using Tukey's HSD. This experimental design enabled us to test several research questions:

1. Do one or two applications of two rates of TEP affect development and yield of cotton?
2. How do different application rate combinations influence development and yield of cotton?



Each Plot is 6 Cotton Rows Wide and 12m Long

Treatments

- 1. Control
- 2. TEP (2000ppm x 1)
- 3. TEP (4000ppm x 1)
- 4. TEP (2000ppm + 2nd app. 2000ppm)
- 5. TEP (2000ppm + 2nd app. 4000ppm)
- 6. TEP (4000ppm + 2nd app. 2000ppm)
- 7. TEP (4000ppm + 2nd app. 4000ppm)
- 8. Hedge trimmer
- 9. Control_Late Plant

Figure 13: Experimental layout for Trinexapac-ethyl treatments at Terry Hie Hie during the 2023/24 cotton season. Note that treatment 8 was dropped from the experiment.

Do one or two applications of two rates of TEP affect development and yield of cotton?

The application of TEP significantly delayed development compared to the control. TTFF was significantly delayed by 7 days, with two applications of high rates of TEP (TTFF occurring at 78 days after planting (DAP) for Trt 7 compared with 71 DAP for Trt 1) (P= 0.045, Table 48).

The application of TEP also delayed time to maturity (days from planting to 60% open) (P= 0.001). Compared with the control, two TEP treatments delayed maturity by 8 days. Trt 7 reached maturity at 160 DAP and the control reached maturity at 152 DAP. TEP treatment effects relative to the control were variable, with maturity ranging from 149 to 160 DAP.

Cotton in Trt 3 and Trt 4 had 1.2 to 1.3 bales/ha greater yield than the control (P=0.017, Table 48). Relative to the control, Trt 2 and Trt 7 increased yield 0.3 to 0.5 bales/ha.

The application of TEP did not have a significant effect on TTFS compared with the control (Trt 1) However, relative to the control, cotton sprayed with TEP reached squaring 1-4 days later.

Table 48: Time to first square (TTFS), time to first flower (TTFF), time to first maturity (60% open boll) and yield (bales/ha) for cotton treated with one or two rates of TEP. Values represent mean days after planting (DAP), standard error (SE) and change relative to the control. Different letters within each variable represent significant difference at P< 0.05 using Tukey's HSD.

Treatment	TTFS (DAP)	SE	ΔTTFS from Control	TTFF (DAP)	SE	ΔTTFF from Control	60% open boll (DAP)	SE	Δ 60% open boll from Control	Yield (bales/ha)	SE	Δ yield from Control
1	50	0.5		71^{a}	0.6	N.A.	152^{a}	1.4	N.A.	5.0^{a}	0.3	N.A.
2	53	1.2		73^{ab}	1.1	3	151^{a}	0.9	-2	5.5^{ab}	0.3	0.5
3	51	1.4		72^{ab}	2.4	2	149^{a}	0.7	-3	6.3^{b}	0.0	1.3
4	52	0.9		76^{ab}	1.0	5	155^{ab}	2.1	3	6.2^{b}	0.4	1.2
7	54	1.3		78^{b}	1.3	7	160^{b}	1.1	8	5.3^{ab}	0.1	0.3
P-value	0.159			0.045			0.001			0.017		

Table 49: Time to first square (TTFS), time to first flower (TTFF), time to first maturity (60% open boll) and yield (bales/ha) for cotton treated with different application rate combinations of TEP. Values represent mean days after planting (DAP), standard error (SE) and change relative to the control. Different letters within each variable represent significant difference at P< 0.05 using Tukey's HSD.

Treatment	TTFS (DAP)	SE	ΔTTFS from Control	TTFF (DAP)	SE	ΔTTFF from Control	60% open boll (DAP)	SE	Δ 60% open boll from Control	Yield (bales/ha)	SE	Δ yield from Control
1	50	0.5		71^{a}	0.6	N.A.	152^{a}	1.4	N.A.	5.0	0.3	
2	53	1.2		73^{ab}	1.1	3	151^{a}	0.9	-2	5.5	0.3	
3	51	1.4		72^{ab}	2.4	2	149^{a}	0.7	-3	6.3	0.0	
4	52	0.9		76^{ab}	1.0	5	155^{ab}	2.1	3	6.2	0.4	
5	53	1.1		77^{ab}	1.0	6	160^{b}	1.8	8	5.8	0.5	
6	52	0.5		75^{ab}	2.0	4	152^{a}	2.0	0	6.3	0.6	
7	54	1.3		78^{b}	1.3	7	160^{b}	1.1	8	5.3	0.1	
P-value	0.145			0.035			0.001			0.169		

How do different application rate combinations influence development and yield of cotton?

Yield across all treatments ranged from 5.0 to 6.5 bales/ha and averaged 5.9 bales/ha. Whilst the application of TEP did have a significant effect on the development parameters, DAP and DAP to 60% open boll ($P < 0.05$), the differences there were not really any discernible differences in rate combinations (Table 49).

Discussion

A grower/consultant-led field demonstration using the novel PGR, Trinexapac-ethyl (TEP) was conducted during the 2023/24 cotton season at Terry Hie Hie. This experiment explored two research questions (1) do one or two applications of two rates of TEP affect development and yield of cotton; and (2) how do different application rate combinations influence development and yield of cotton? Our results showed that the application of TEP consistently delayed time to first flower, with some delays also seen in time to maturity (60% open boll). Delays in development is consistent with results that we observed during experiments from 2020 to 2023 at ACRI, despite the later planting time in the experiment at Terry Hie Hie.

Our data showed that yield of cotton applied with TEP were relatively higher compared with control cotton, with a significant increase of more than 1 bale/ha in some treatments. Improved growth with application of TEP has also been shown in Eucalyptus, which could lead to use of TEP as a PGR in Eucalyptus plantations (Bacha et al., 2024). In contrast, previous experiments at ACRI showed that a combination of substantial delays in development and cold ends to the season resulted in lower yields. Therefore, before yield benefits to the industry can be realised, a better understanding is required of how manipulating GA pathways affects floral initiation and source-sink mechanisms in cotton. This may also involve a better understanding of how TEP interacts with environmental conditions to affect length of developmental delay and yield consequences.

Engagement activities with growers and consultants

Results were presented at the Bundy Field Day (Dryland Cotton Research Association; April 2024). Novel plant growth regulator research presented in the Winter 2024 CRDC Spotlight magazine (pg 30).

Summary

A grower and consultant-led field demonstration testing the effects of a novel PGR for cotton, Trinexapac-ethyl (TEP) on the growth and yield of cotton was conducted during the 2023/24 cotton season at Terry Hie Hie. Our results showed that the application of TEP consistently delayed time to first flower, with some delays also seen in time to maturity (60% open boll). Our data also showed that yields of cotton applied with TEP were relatively greater compared with unsprayed control cotton, with a significant increase of more than 1 bale/ha in some treatments. Preliminary results have been presented at a field day during the 2023/24 cotton season and in the Winter 2024 edition of the CRDC Spotlight magazine. Results have also been presented at the Australian Cotton Conference 2024.

Milestone 5. Fulfil CRDC reporting requirements during the course of the project

Milestone 5.1 Submit milestone/progress reports to CRDC to provide an update on project activities. Six-monthly progress reports have been submitted to CRDC to provide an update on project activities and preliminary experimental results.
Milestone 5.2 Deliver final project report documenting project outcomes. A final project report documenting project outcomes has been submitted to CRDC in October 2024.
Milestone 5.3 Evaluation and reflection report on grower led trials.

Grower and consultant-led trials were conducted during the 2023/24 cotton season as part of Milestones 4.5 and 4.6. Engagement activities and research outcomes are detailed in the relevant sections above. Additional participant feedback from the MC trials is detailed below:

What have you learnt from participating in the collaborative MC trials during the 2023/24 cotton season?

Two main outcomes I have learnt this season, or really confirmed my thinking, were 1. applying MC in an irrigated scenario really needs to be done no further than 7 days out from an irrigation if wanting to see benefits. We did find no negative side effects from the pump breakdown to yield, but we also didn't see the benefit either because of it. However, it did bring the trial strips in for defoliation a week earlier than the rest of the block, and this was also observed in a dryland trial the season before. So, MC application early is helping to 'bring crops in' earlier than without it.

Do you have any comments on the style/approach of the research that was conducted?

No negative comments. Katie and crew were brilliant in working out a trial layout that was easy for everyone involved.

What questions do you have following on from this research?

Would be nice to complete early season application trials in the CSD variety trials so that MC responses in new varieties are available to industry as new varieties come onto the market. A total review of how MC is used in newer varieties is very much needed, and on a valley-by-valley approach, not one size fits all, particularly in the regions growing on cotton. Would like to look at the early applications in dryland and semi-irrigated skip row again. And for a period of time, as each season is different.

Any other comments/suggestions/feedback etc.

We are way overdue for an update on MC and how it works in newer varieties. But I cannot stress enough, while some principles will apply across all valley's, more research in each valley is needed to best see what use patterns suit each valley the best. It also needs to be long term research being that we tend not to have the same seasons year in year out. The more information we have, the more comfortable we will be using MC and using it in a way that will give us beneficial results.

What have you learnt from participating in the collaborative MC trials during the 2023/24 cotton season?

That regardless of rate quite often a crop will compensate and continue to grow once MC has been metabolised. No significant leaf area differences between treated and untreated is very interesting.

Do you have any comments on the style/approach of the research that was conducted?

The trial set up was good. Only bad thing is that there weren't more sites. To fully understand the MC interactions this work needs to be done across multiple sites with varying soil types etc.

What questions do you have following on from this research?

We didn't break the system this season with high rates. Are we growing too much biomass? Can yield be supported with a much smaller canopy. What are the farming system benefits from having a smaller crop that matures slightly quicker. This past season was a very good example that delayed harvest really impacts returns and profitability of the following crop.

Five grower/consultant led trials (four early season MC and one novel PGR) were conducted during the 2023/24 cotton season. These trials provided an opportunity for a greater understanding of current practice and potential PGR use across key cotton regions in irrigated, semi-irrigated, and dryland systems. Engagement with growers and consultants facilitated industry collaborations, and opportunities for field demonstrations expanded outreach of the research. Overall, there is a growing interest in early season MC application that will require further research and dissemination to ensure that plant growth, and thus yields, are not limited. Growers and consultants have also expressed an interest in better understanding novel PGRs such as TEP for altering crop development and enabling alternative tools to minimise production risk for dryland systems.

Part 4 – Summary for public release

This summary is designed to provide a short overview of the project for all interested parties. It will be published on Inside Cotton, CRDC's digital repository, along with the full final report (if suitable for public release). The summary may also be published on grow$^{AG.}$, a collaborative platform that showcases Australian agrifood research, development, and extension projects that are current or have been completed since 1 July 2018. Please complete all fields, ensuring that this exceeds no more than two pages.

Project title:	***Modern Systems Agronomy for Resilient Cotton Production***	
Project details:	CRDC project ID:	CSP2001
	CRDC goal:	*1. Increase productivity and profitability on cotton farms*
	CRDC key focus area:	*1.1 Optimised farming systems*
	Principal researcher:	*Katie Broughton*
	Organisation:	*CSIRO*
	Start date:	*1/7/2020*
	End date:	*30/9/2024*
Objectives	- *Are there cotton production challenges where novel/alternative agronomic practices could build resilience?* - *Can cotton production resilience be improved with strategic application of specific hormones/plant growth regulators (PGRs)?* - *Enhanced capability/capacity in cotton cropping systems agronomy.* - *Fulfil CRDC reporting requirements during the course of the project.*	
Background	Australian rainfed cotton production occurs in warm regions with intermittent and highly variable rainfall throughout the growing season. Predominate areas for rainfed cotton production are northern New South Wales and Southern Queensland (Bange et al., 2005). Environmental variability affects the area of land planted to cotton, and crop yields. Recently, rainfed cotton production has expanded, partly due to improved varietal choices and partly in response to high prices and thus increased profitability with cotton cropping compared with alternative summer crops such as sorghum (Godfrey et al., 2023). Agronomic management practices can reduce the risk associated with such variable in-crop rainfall. Dryland cotton growers can use planting row configuration to manage their growing costs and maintain yield and fibre quality. Skip-row sowing configurations decrease plant population and can increase soil water availability, thereby reducing the downside risk in years with low rainfall. Time of planting is also an important consideration, and sowing dates of rainfed crops are varied according to soil water availability dictating planting opportunities. The length of sowing windows in dryland crops are often longer than for irrigated crops as the length of growing season is less than dryland cotton. Therefore, season length in a region will be a major factor in	

	cultivar choice. Cultivars with phenological plasticity have been shown to benefit rainfed environments. In rainfed systems with high climate variability and long season growth, it has been found that cultivars that are less determinate (i.e., more indeterminate) are preferential in delivering higher yields (Stiller et al., 2005), because of their ability to regrow after significant stress (Bange et al., 2016). Identifying cultivars more suited to rainfed systems is one focus of the CSIRO cotton breeding program (Conaty et al., 2022). It has been identified that plant growth hormones (also known as plant growth regulators) may provide additional agronomic management strategies that could be beneficial to Australian dryland cotton growers. However, there are a number of different hormones to consider, with varied functions within the plant. Plant hormones help regulate the plant by responding to various signals from the plant and environment. Hormones are regulated in different plant tissues during the different developmental stages. Many plants synthesise hormones in all parts of the plant, including in the roots, stems, and leaves. There are five major plant hormones: auxin, cytokinin, gibberellin, abscisic acid, and ethylene. Each type of hormone has a specific function, with several secondary functions. Generally, auxins, gibberellins and cytokinins act as growth stimulators, whereas abscisic acid and ethylene act as growth inhibitors. Therefore, hormones play an important role in the metabolic processes that influence growth and development of a plant, and the ability of the plant to respond to abiotic stress. There are several plant growth regulators that are available commercially, some used in cotton, and many used across other agricultural and horticultural applications. This project investigates the use of plant growth hormones on cotton growth, physiology, and yield, with a particular focus on management strategies that improve the resilience of dryland cotton production in Australia.
Research activities	Field experiments were conducted to identify and investigate applications of plant growth regulators (PGRs) to improve crop efficiencies and/or deliver yield and lint quality benefits. Seventeen field experiments were conducted from 2020 to 2023, with 14 experiments at Narrabri, NSW, 2 experiments in Whitton, NSW and 1 in Bongeen, Qld. From 2023 to 2024, an additional 5 grower/consultant led experiments were conducted across key cotton regions. Most field experiments were set up as a randomised complete block design with six replications, except the grower/consultant led trials which required adaptation to each system.
Outputs	*Please detail the overarching outputs from this research projects: what did the project find/discover/create – be it new knowledge, technical advances etc.* The overarching outputs from this research project are improved understanding of the application of various plant growth regulators (PGRs) for cotton growth and yield in various Australian cotton production systems. We conducted field experiments over four

	consecutive cotton seasons (2020 to 2024) and two glasshouse experiments. Results from field studies identified that the early application of Trinexapac-ethyl (TEP) consistently delayed the development of cotton. Additional research is required to further develop our understanding of fundamental physiology and the underlying principles that we may be able to exploit for agronomic management across variable seasons. Field trials during the 2023-24 cotton season have shown no negative yield results for cotton applied with mepiquat chloride (MC) across most regions. However, relative yield losses were observed with high rates of MC in Griffith, indicating that early-season application rates of MC are important. It would be beneficial to conduct further research so that grower/consultant perceptions are not reliant on a single year of data. Additional outputs from this research have been industry engagement through collaborative field studies, presentations at field days and grower meetings, CCA seminars, conferences, and CRDC Spotlight and Australian Cottongrower articles.
Impacts	*Please detail the impact and implications that your research will have for the Australian cotton industry, including any best practice recommendations.* Hormones play an important role in metabolic processes that influence growth and development of a plant. Several plant growth regulators are currently used in Australian cotton systems, such as MC for the management of the crop canopy. Other plant growth regulators are not currently registered for use in cotton but may benefit the cotton industry through improving the resilience of dryland cotton production in Australia. Throughout this project, a wide range of plant growth regulators were screened in rainfed systems. There is interest in further investigating the use of novel plant growth regulators, specifically Trinexapac-ethyl (TEP), as this research suggests that crop development can be delayed with early-season application. This may enable growers to manage the timing and development of their crops once they are established and in the ground. Further research is needed to register these products for use in cotton before these agronomic strategies can be implemented in the farming system. However, the effective use of PGRs may provide an additional tool to build greater resilience in the system to overcome the challenges of high variability in dryland systems. Demonstration workshops have also generated much interest in greater understanding of early season canopy management using MC and maximising yield and profitability through effective resource management and ensuring effective defoliation at the end of the season. Better understanding of agronomic strategies using multi-

	faceted approaches will ensure profitable and sustainable cotton systems in the future.
Key publications	*Extension resources:* CRDC Spotlight magazine article (Winter 2024) Australian Cottongrower article (Dec 23-Jan 24 issue) DCRA update (May 2024)

References

Ashraf, M., Akram, N.A., Al-Qurainy, F. and Foolad, M.R. (2011) Chapter five - drought tolerance: Roles of organic osmolytes, growth regulators, and mineral nutrients. In: D. L. Sparks, editor Advances in agronomy. Academic Press. p. 249-296.

Bacha, A., Santos, R., de Souza Rodrigues, J., Carrega, W., Carrera, E., Grey, T. and Alves, P.L. (2024) Trinexapac-ethyl dose–response curve for eucalyptus growth and hormonal crosstalk between leaf and shoot apical bud. Journal of Plant Growth Regulation: 1-19. doi:10.1007/s00344-024-11404-w.

Bange, M.P., Baker, J.T., Bauer, P.J., Broughton, K.J., Constable, G.A., Luo, Q., Oosterhuis, D.M., Osanai, Y., Payton, P., Tissue, D.T., Reddy, K.R. and Singh, B.K. (2016) Climate change and cotton production in modern farming systems. doi:10.1079/9781780648903.0000.

Bange, M.P., Carberry, P.S., Marshall, J. and Milroy, S.P. (2005) Row configuration as a tool for managing rain-fed cotton systems: Review and simulation analysis. Australian Journal of Experimental Agriculture 45: 65-77. doi:10.1071/ea03254.

Bange, M.P., Caton, S.J. and Milroy, S.P. (2008) Managing yields of high fruit retention in transgenic cotton (*gossypium hirsutum* l.) using sowing date. Australian Journal of Agricultural Research 59: 733-741. doi:10.1071/ar07423.

Burke, J.J. and Sanchez, J. (2018) Cppu (n-(2-chloro-4-pyridinyl)-n′-phenylurea) enhancement of cotton yields. Journal of Cotton Science 22: 117-125.

Clendennen, S.K. and Boaz, N.W. (2019) Chapter 14 - betaine amphoteric surfactants—synthesis, properties, and applications. In: D. G. Hayes, D. K. Y. Solaiman and R. D. Ashby, editors, Biobased surfactants (second edition). AOCS Press. p. 447-469.

Conaty, W.C., Broughton, K.J., Egan, L.M., Li, X., Li, Z., Liu, S., Llewellyn, D.J., MacMillan, C.P., Moncuquet, P., Rolland, V., Ross, B., Sargent, D., Zhu, Q.-H., Pettolino, F.A. and Stiller, W.N. (2022) Cotton breeding in australia: Meeting the challenges of the 21st century. Frontiers in Plant Science 13. doi:10.3389/fpls.2022.904131.

Conaty, W.C., Johnston, D.B., Thompson, A.J.E., Liu, S., Stiller, W.N. and Constable, G.A. (2018) Use of a managed stress environment in breeding cotton for a variable rainfall environment. Field Crops Research 221: 265-276. doi:https://doi.org/10.1016/j.fcr.2017.10.012.

Depaepe, T. and Van Der Straeten, D. (2017) Ethylene. In: B. Thomas, B. G. Murray and D. J. Murphy, editors, Encyclopedia of applied plant sciences (second edition). Academic Press, Oxford. p. 403-410.

Eriksson, S., Böhlenius, H., Moritz, T. and Nilsson, O. (2006) Ga4 is the active gibberellin in the regulation of leafy transcription and arabidopsis floral initiation. Plant Cell 18: 2172-2181. doi:10.1105/tpc.106.042317.

Gadallah, M.A.A. (1995) Effect of water stress, abscisic acid and proline on cotton plants. Journal of Arid Environments 30: 315-325. doi:https://doi.org/10.1016/S0140-1963(05)80006-0.

Godfrey, S.S., Nordblom, T.L., Anwar, M.R., Ip, R.H.L., Luckett, D.J. and Bange, M.P. (2023) Untangling the complex mix of agronomic and economic uncertainties inherent in decisions on rainfed cotton. Crop and Pasture Science: -. doi:https://doi.org/10.1071/CP22145.

Guinn, G. (2012.) Hormonal relations during reproduction.

Gupta, R. and Chakrabarty, S.K. (2013) Gibberellic acid in plant. Plant Signaling & Behavior 8: e25504. doi:10.4161/psb.25504.

Hernandez-Leon, S.G. and Valenzuela-Soto, E.M. (2023) Glycine betaine is a phytohormone-like plant growth and development regulator under stress conditions. Journal of Plant Growth Regulation 42: 5029-5040. doi:10.1007/s00344-022-10855-3.

Hu, W., Zhang, J., Wu, Z., Loka, D.A., Zhao, W., Chen, B., Wang, Y., Meng, Y., Zhou, Z. and Gao, L. (2022) Effects of single and combined exogenous application of abscisic acid and melatonin on cotton carbohydrate metabolism and yield under drought stress. Industrial Crops and Products 176: 114302. doi:https://doi.org/10.1016/j.indcrop.2021.114302.

Khan, A.S. and Ali, S. (2018) Chapter 9 - preharvest sprays affecting shelf life and storage potential of fruits. In: M. W. Siddiqui, editor Preharvest modulation of postharvest fruit and vegetable quality. Academic Press. p. 209-255.

Kupke, B.M., Tucker, M.R., Able, J.A. and Porker, K.D. (2022) Manipulation of barley development and flowering time by exogenous application of plant growth regulators. 12. doi:10.3389/fpls.2021.694424.

Manuela, D. and Xu, M. (2020) Juvenile leaves or adult leaves: Determinants for vegetative phase change in flowering plants. International Journal of Molecular Sciences 21: 9753.

Mauney, J.R. (1966) Floral initiation of upland cotton gossypium hirsutum l. In response to temperatures. Journal of Experimental Botany 17: 452-459. doi:10.1093/jxb/17.3.452.

Milroy, S. and Bange, M. (2003.) Determinancy in cotton: Measurement and potential implications. 11th Australian Agronomy Conference.

Najeeb, U., Atwell, B.J., Bange, M.P. and Tan, D.K.Y. (2015) Aminoethoxyvinylglycine (avg) ameliorates waterlogging-induced damage in cotton by inhibiting ethylene synthesis and sustaining photosynthetic capacity. Plant Growth Regulation 76: 83-98. doi:10.1007/s10725-015-0037-y.

Rademacher, W. (2015) Plant growth regulators: Backgrounds and uses in plant production. Journal of Plant Growth Regulation 34: 845-872. doi:10.1007/s00344-015-9541-6.

Rademacher, W. (2017) Chemical regulators of gibberellin status and their application in plant production. Annual plant reviews online. p. 359-403.

Rademacher, W. and Brahm, L. (2012) Plant growth regulators. Ullmann's encyclopedia of industrial chemistry.

Raihan, T., Geneve, R.L., Perry, S.E. and Rodriguez Lopez, C.M. (2021) The regulation of plant vegetative phase transition and rejuvenation: Mirnas, a key regulator. Epigenomes 5. doi:10.3390/epigenomes5040024.

Ramazanoglu, E., Kılınçoğlu, N., Beyyavas, V., Cevheri, C.İ., Sakin, E. and Çelik, A. (2024) Glycine betaine application improved seed cotton yield and economic returns under deficit irrigation. Journal of King Saud University - Science 36. doi:10.1016/j.jksus.2024.103445.

Salazar-Cerezo, S., Martínez-Montiel, N., García-Sánchez, J., Pérez-y-Terrón, R. and Martínez-Contreras, R.D. (2018) Gibberellin biosynthesis and metabolism: A convergent route for plants, fungi and bacteria. Microbiological Research 208: 85-98. doi:https://doi.org/10.1016/j.micres.2018.01.010.

Sarwar, M.K.S., Ullah, I., Ashraf, M. and Zafar, Y.J.P.J.o.B. (2006) Glycinebetaine accumulation and its relation to yield and yield components in cotton genotypes grown under water deficit condition.

Stiller, W.N., Read, J.J., Constable, G.A. and Reid, P.E. (2005) Selection for water use efficiency traits in a cotton breeding program: Cultivar differences. Crop Science 45: 1107-1113. doi:https://doi.org/10.2135/cropsci2004.0545.

Veinbrants, N. and Miller, P. (1981) Promalin improves the shape of delicious apples in victoria %j australian journal of experimental agriculture. 21: 623-630. doi:https://doi.org/10.1071/EA9810623.

Welsh, C.J. (2020) Promoting resilience in rain grown cotton systems with plant growth regulators. University of Sydney.

Zeng, H., Yang, W., Lu, C., Lin, W., Zou, M., Zhang, H., Wan, J. and Huang, X. (2016) Effect of cppu on carbohydrate and endogenous hormone levels in young macadamia fruit. PloS one 11: e0158705. doi:10.1371/journal.pone.0158705.