



FINAL REPORT 2016

For Public Release

Part 1 - Summary Details

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

CRDC Project Number: **DAQ1501**

**Project Title: Management of Solenopsis mealybug in
Bollgard® cotton**

Project Commencement Date: 1 July 2014 **Project Completion Date:** 30 June 2017

CRDC Research Program: 1 Farmers

Part 2 – Contact Details

Administrator: Mr Stuart Makings, Principal Co-ordinator (External Funding)

Organisation: Department of Agriculture & Fisheries

Postal Address: PO Box 102, Toowoomba, QLD 4350

Ph: 07 4529 4286 **Fax:** 07 4688 1190 **E-mail:** stuart.makings@daf.qld.gov.au

Principal Researcher: Dr Richard Sequeira, Principal Scientist

Organisation: Department of Agriculture & Fisheries

Postal Address: Locked mail bag 6, Emerald, QLD 4720

Ph: 07 4991 0810 **Fax:** 07 4983 7459 **E-mail:** richard.sequeira@daf.qld.gov.au

Supervisor: Dr Richard Sequeira, Principal Scientist

Organisation: Department of Agriculture & Fisheries

Postal Address: Locked mail bag 6, Emerald, QLD 4720

Ph: 07 4991 0810 **Fax:** 07 4983 7459 **E-mail:** richard.sequeira@daf.qld.gov.au

Signature of Research Provider Representative: _____

Date Submitted: _____

Part 3 – Final Report

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

Background

1. Outline the background to the project.

The cotton mealybug (CMB), *Phenacoccus solenopsis*, also known as the solenopsis mealybug, is a highly polyphagous and invasive global insect pest of cotton. Damage to cotton by this pest includes limiting yield potential (plant stunting and/or mortality) and marketability through effects on lint quality (boll characteristics and lint contamination).

CMB is believed to be native to North America and was first reported from cotton growing areas in Texas in the early 90s. CMB has since been recognised as a globally invasive species, and poses a significant threat to the world's major cotton industries in Asia (India, Pakistan), Brazil and China. In Australia, economically damaging outbreaks of CMB on cotton were reported from the Burdekin and Emerald region of Central Queensland in 2010.

It is well established from research done overseas and in Australia that CMB is easily controlled by naturally occurring predators (lacewings, various coccinellid beetles) and a parasitic wasp in agroecosystems where beneficial arthropod populations are not exposed to the detrimental effects of disruptive agents such as broad-spectrum insecticides. In Australian cotton production systems where a number of key insect pests often require chemical control, anecdotal reports from growers and consultants indicate a potential link between control of other sucking pests (e.g. mirids, aphids) using broad-spectrum insecticides, and the severity of CMB infestations, presumably mediated through the effects of the insecticides used on beneficial arthropod communities. Such a putative cause-effect relationship is consistent with the rising pest status of CMB since 2010, as evidenced by the recurrence of infestations and expansion of its distribution into southern Queensland and New South Wales in 2017.

Following the CMB outbreak of 2010, RD&E investments by the Cotton Research & Development Corporation (CRDC) and other agencies between 2009 and 2014 were aimed at investigating aspects of CMB field biology/ecology, the nature of damage and identifying ecologically sustainable management options in Australian cotton production systems. The outputs of CRDC investments in CMB research (e.g. DAQ1204 2011-2014) showed that if mealybug populations were left unchecked they could cause substantial damage to cotton yield and quality. Although DAQ1204 addressed some aspects of mealybug ecology (e.g. damage and distribution within plants), additional research was required to develop a sound IPM management strategy for this pest.

The outputs of DAQ1204 showed that most newly developed “soft” (selective) chemical insecticides currently approved for use within cotton production systems varied in control efficacy from ineffective to having only suppressive effects. An older organophosphate, methidathion (Supracide®) was shown to be effective but highly hazardous from an environmental and user safety perspective, and therefore incompatible with modern IPM-friendly cotton production systems. Thus, cost effective and IPM compatible chemical control of CMB as and when required was viewed as a high priority for the cotton industry.

This project was designed to continue the lines of investigation commenced in DAQ1204 and provide research outcomes to underpin the successful implementation of IPM in cotton production systems with CMB as an important and recurring element of the pest spectrum.

Objectives

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

The broad objective of the project was to address CMB RD&E that met the needs of industry. Specifically, the work program was designed to achieve two distinct but interrelated objectives, viz. (1) to better understand selected aspects of CMB field ecology, and (2) to develop practical and effective curative options to manage/control CMB activity at a whole paddock level as well as in the more

common situations where infestations were characterised by “hot spots” and the risk of contagion/spread needed to be managed.

Objective 1: Establish and maintain colonies of CMB

Milestones: 1.1 Successfully maintained CMB colonies

Achievement: Achieved

Objective 2: Characterise infield spatial distribution and test functionality of early detection sampling protocols

Milestones: 2.1 Study sites/paddocks with current/historical infestation of CMB identified

Milestones: 2.2 CMB research plan (CMBRP) and Activities Plan for 2015 – 2017 developed

Achievement: Achieved

Three study sites with historical CMB problems were identified in the first half of 2015 for field research – Site1, Site2 and Site3. A mealybug research plan and an activities plan were developed in consultation with CRDC and stakeholders.

Milestones: 2.3 Assessment of CMB spatial incidence and detectability in three cotton crops/paddocks (CMBRP A1) completed

Milestones: 2.4 Assessment of CMB survival and overwintering in two cotton paddocks (CMBRP A2) completed

Milestones: 2.5 Assessment of CMB survival and overwintering on feral (non-cotton) host plants in and around two cotton paddocks (CMBRP A3) completed

Achievement: Activities related to 2.3, 2.4 and 2.5 were conducted in 2015 and 2016 but did not yield useful data

Activities focussed on CMB incidence and population characterization in the field (CMBRP protocol A1; milestone 2.3) were initiated at the three study sites in the 2015/16 and 2016/17 cotton seasons. Establishment of a study site with commercial cotton in the Kingaroy/Byee area was not practical or feasible and was dropped from the work plan. Activities to characterize CMB in-crop survival/overwintering (CMBRP protocol A2; milestone 2.4) were conducted at sites 1 and 2 before and after cotton harvest. Monitoring of georeferenced host vegetation (CMBRP protocol A3; milestone 2.5) along the boundaries of a cotton paddock with a prior history of CMB infestations at the three sites was conducted throughout 2015 and a part of 2016 (see Results section (pages 11, 12) for photos of georeferenced sampling sites).

Milestones: 2.6 Assessments of juvenile/adult dispersal in the laboratory/glasshouse (CMBRP protocol A4) completed; additional trials to be undertaken if necessary after consultation with CRDC

Achievement: Achieved

Two glasshouse experiments to characterise the dispersal potential of CMB by wind and voluntary movement through the canopy were conducted in 2015.

Objective 3: Investigate chemical and biopesticide options for CMB population control

Milestones: 3.1 Assessment of at least one biopesticide option at Kingaroy RS completed (collaboration with Dr. Robert Mensah – NSW DPI)

Milestones: 3.2a Assessment of best bet chemical insecticide options at Kingaroy RS completed

Milestones: 3.2b Potential assessment of best bet chemical insecticide options at Emerald RS completed (subject to feasibility)

Achievement: Achieved

Over the duration of the project, the program of activities resulted in the completion of eight glass house trials and eight field trials of insecticide efficacy against CMB. The glass house trials included chemical and biological insecticides; promising options identified in the glass house trials were further tested in field trials at Qld Department of Agriculture & Fisheries research facilities in Kingaroy and Emerald. A number of biological insecticides, including a fungal product obtained from Dr. Robert Mensah (NSW DPI, Narrabri), were tested in the glasshouse trials between December 2015 and April 2016.

Objective 4: Preparation and submission of final report

Milestones: 4.1 All trial data analysed and Final Report prepared and submitted

Achievement: Achieved by this report

Objective 5:	Silverleaf whitefly (SLW) support to industry
Milestones: 5.1	Establish industry advisory/liason group and organise for mid and end season SLW review
Milestones: 5.2	Review SLW threshold and associated extension material annually based on feedback from advisory group
<u>Achievement:</u>	Achieved
Objective 6:	Effective communication and extension of results
Milestones: 6.1	Communication of results and implications to stakeholders
<u>Achievement:</u>	Achieved

Methods

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

The project's activities were grouped under its two major components, viz. (a) field studies aimed at developing a better understanding of key features of CMB ecology, and (b) identification/development of IPM options with an emphasis on insecticidal control.

The methodology used in the ecological studies and experiments undertaken (milestones 2.3-2.6) was consistent with standard scientific procedures and guidelines recommended for surveys and insect sampling using visual observation and enumeration techniques. The second component of the project involved glasshouse and field trials of biological and chemical insecticides for efficacy against CMB. There were a number of methodological challenges that had to be overcome in order to ensure successful delivery of contracted outputs and outcomes.

The logistics of CMB culturing in the glasshouse/insectary and supply of experimental test populations was a significant methodological challenge in the initial stages of the project because of host plant preferences, the damage done by CMB feeding to host plants, and limitations imposed by temperature dependence in the growth rate of suitable host plants. For example, although there are over 150 plant species reported as being hosts of CMB, very few are suitable for culturing large populations of CMB in the insectary or glasshouse. Cotton is among the most suitable but is relatively slow growing.

A significant limiting factor in the conduct of CMB research in the past has been the ability to mass produce CMB in culture to meet the needs of intensive field and glass/poly house research programs. The methodology used in previous research involved the use of cotton seedlings as the primary food source for CMB cultures. In this project, a new process for rearing CMB in the glass house was developed. The process involves rearing CMB on readily available nursery plants such as tomatoes and transferring them to cotton at short notice or as required. This process allows for rapid up or down scaling CMB cultures with an on-demand supply of plants from a commercial supplier in bulk such that large numbers of insects can be produced as required for manually seeding field plots for experimentation. This method of mass rearing and transferring CMB to field plots was instrumental in the successful planning and implementation of field trials in Emerald and Kingaroy.

Manipulation of CMB for experimental purposes is difficult for several reasons. Their sessile feeding habit and soft bodies makes them highly prone to injury and death if manually moved from their host plant in culture to experimental plants in the glasshouse or field plot. Adult CMB will not readily move of their own accord once they have settled into a feeding position, even if the plant part they are feeding on is excised and allowed to dry. Overlapping of generations and cryptic feeding sites (terminals and bracts of squares, flowers and bolls) make enumeration and standardisation of experimental populations difficult. These, in turn, have implications for the methodology that can be used in experimental trials and the statistical tools required. For example, assessment of CMB population density using standard assessment protocols is often impractical which means that alternatives such as binomial, multinomial techniques and/or the use of density scores becomes necessary; this is evident from the insecticide efficacy trials (see *Insecticide Efficacy Trials Report 2015-2017*, attachment 1) in which population scoring has been used where standard enumeration (counting) was deemed impractical and inefficient.

The analysis of non-standard (and usually non-normal) data requires the use of sophisticated statistical tools that often make interpretation of the results more challenging.

Another significant methodological challenge for the project from the outset was the lack of established and proven methodology for the assessment of insecticide efficacy against CMB. Literature reports of such work conducted in India, Pakistan and China highlight the use of unrealistic rates of highly toxic and environmentally damaging chemical insecticides, and inconsistency in methodology. It was clear that CMB management in Australian cotton production systems had to be based on IPM compatible and environmentally sustainable systemic insecticidal options (CMB have a waxy outer shell that makes products with contact activity largely ineffective).

The specific challenge in obtaining consistent and robust results from insecticide efficacy trials against CMB was the requirement to simultaneously optimize a number of key parameters, viz. product rate and at least two application parameters – application volume required to maximise product uptake by the plant, and equipment settings, e.g. pressure, nozzle type, specification and configuration. Identification of a solution to the parameter optimisation problem required a factorial approach and considerable field experimentation that was beyond the scope of this project. The project team addressed the parameter optimisation challenge by using a best-bet, evaluation and logic based elimination approach that is reflected in the number of methodological changes made in trial/assessment protocols during the course of the project's glasshouse and field trials program.

The detailed methodology for milestones under objective (3) is reported in *Insecticide Efficacy Trials Report 2015-2017* (attachment 1). A summary is provided below.

Glasshouse trials were all conducted at the Department of Agriculture research facility at Tor Street, Toowoomba. CMB reared on potted cotton plants were used in all assessments. A two metre, hand-held spray boom with droppers was used for spraying (see photographs below).



Cotton plants used in glasshouse insecticide efficacy assessments



Hand-held spray boom used to treat experimental plants

Field trials were extensions of glasshouse trials in which promising products were further tested for efficacy in small plots that had previously been seeded with CMB. Field trial conducted in the 2015-16 cotton season (two at Kingaroy RS and two at Emerald RS) are not included in this report because they included (off-label) treatment options that were being considered for registration at the time but subsequently dropped from the research program. In the 2016-17 cotton season, the two final field trials conducted in the project were aimed at partitioning the relative contribution of chemical insecticide treatments and naturally occurring beneficial arthropods to overall mortality of CMB under field conditions. The methodology used in the 2016-17 “chemical insecticide x beneficial insect interaction” trials differed from previous trials in that large field cages (2m x 1.5m x 1.5m) were deployed as a means of separating one treatment from another and controlling access by beneficial insects to experimental CMB populations. The experimental design was a randomised block design with chemical treatment, application frequency (single or double knock) and cage status (open or closed) as the main

factors. Open cages (OC) allowed predators (coccinellids and lacewings) access to CMB on the test plants whereas closed cages (CC) excluded all predatory insects.

Results

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

Objective 1: Establish and maintain colonies of CMB
Milestones: 1.1 Successfully maintained CMB colonies

The new CMB mass rearing procedure was instrumental in the development of capacity to successfully engineer field populations and densities at field research sites (Kingaroy and Emerald Research Stations), as required. This development considerably enhanced the project's capacity to screen chemical options for efficacy under field conditions.

Objective 2: Characterise infield spatial distribution and test functionality of early detection sampling protocols
Milestones: 2.1 Study sites/paddocks with current/historical infestation of CMB identified
Milestones: 2.2 CMB research plan (CMBRP) and Activities Plan for 2015 – 2017 developed

Three study sites/farms with historical CMB problems were identified in the first half of 2015 for field research. A mealybug research plan and an activities plan were developed in consultation with CRDC and stakeholders.

Milestones: 2.3 Assessment of CMB spatial incidence and detectability in three cotton crops/paddocks (CMBRP A1) completed

Activities focussed on CMB incidence and population characterization in the field (CMBRP protocol A1; milestone 2.3) were initiated at the three study sites in the 2015/16 and 2016/17 cotton seasons. Establishment of a study site with commercial cotton in the Kingaroy/Byee area was not practical or feasible and was dropped from the work plan. These activities aimed at detecting early, low density founding populations of CMB on cotton did not yield useful data in that CMB were not detected in the sampling area but hot spots were detected elsewhere in the paddock and farm later in the season. This result highlights the highly unpredictable (seemingly chaotic) spatial population dynamics of CMB.

Milestones: 2.4 Assessment of CMB survival and overwintering in two cotton paddocks (CMBRP A2) completed

Activities to characterize CMB in-crop survival/overwintering (CMBRP protocol A2; milestone 2.4) were conducted at study sites 1 and 2 before and after cotton harvest. These activities were aimed at verifying anecdotal reports and observations of CMB surviving in the soil and on the roots of cotton plants, and their ability to overwinter in the paddock until the next crop was planted. While there have been credible reports and visual observations of CMB found on the roots of cotton plants from industry sources and previous surveys done in DAQ1204, these could not be confirmed in this project; CMB were not detected in the root zone of tagged plants within hotspots. It was recognised at the outset that isolating CMB from soil and soil debris was a challenging activity that could be described more aptly as "looking for a needle in a haystack." Thus, while the probability of success for this activity was low it was necessary from a scientific and practical IPM perspective to confirm qualitative information from other sources (as indicated above) on the importance of this mechanism of survival in determining the spatial incidence of CMB in cotton crops/paddocks.

Milestones: 2.5 Assessment of CMB survival and overwintering on feral host plants in and around at least two cotton paddocks (CMBRP A3) completed

Monitoring of CMB host vegetation (CMBRP protocol A3; milestone 2.5) along the boundaries of the three study sites was conducted throughout 2015 and early 2016. Indicator weeds (volunteer cotton,

pigweed) with resident CMB populations were tagged and monitored at georeferenced sites at regular intervals (see photographs below).

The host vegetation monitoring activities did not yield useful data primarily because dry conditions killed off almost all vegetation of interest, including tagged plants at georeferenced sampling sites, during the autumn and winter of 2015. Interestingly, CMB were again detected on some weeds that reappeared at some of the 2016 georeferenced sampling sites following rainfall in 2017. However, it could not be clearly established where the CMB re-infestations had originated from (survival in the soil or dispersed by wind/water from nearby source populations).

The lack of useful data from the above activities (milestones 2.3-2.5) in 2015 underscores the difficulty of undertaking ecological assessments based on natural occurrences of this insect pest in commercial cotton fields, as indicated above, and highlights the need for manipulative studies under controlled conditions. Given the lack of success in addressing the field ecology aspects of the project, they were discontinued after consultation with the CRDC and the resources redirected towards obtaining better results in the evaluation of chemical control options.

Milestones: 2.6 Assessments of juvenile/adult dispersal in the laboratory/glasshouse (CMBRP protocol A4) completed; additional trials to be undertaken if necessary after consultation with CRDC

Two preliminary glasshouse experiments for characterising the dispersal potential of CMB by wind and voluntary movement through the canopy were conducted in 2015.

Objectives

The specific objectives of the experiments were to characterise dispersal of mealybugs by quantifying the relative importance of (i) movement by crawling that is facilitated by plant contact, (ii) movement facilitated by wind gusts, and (iii) population density on the plant and its implications for dispersal by crawling and wind gusts.

Background and rationale

CMB has proved difficult to control and knowledge of its ecology and dispersal mechanisms within the cotton crop is limited. Anecdotal observations and literature reports indicate that crawling and wind-mediated movement are important mechanisms for the dispersal of mealybugs. However, to date there is little hard evidence in the literature to show how important these factors are in determining patterns of spatial and temporal abundance. Pilot experiments in the laboratory have indicated that mealybugs are clearly capable of dispersing via crawling and wind gusts.

EXPERIMENT 1.

Methods

This experiment was designed to generate baseline data on the interaction between mealybug density on the plant and wind-mediated dispersal (Table 2.6.1). Each factor combination was tested three times. Tests were conducted in the glasshouse on an open flat bench. In each test, a single potted cotton plant (squaring stage) with mealybugs was placed on a table in front of a black cardboard attached to a wall. The cardboard was painted with a thin layer of “Tanglefoot Tangle-Trap”.

A pedestal fan was placed at the opposite end of the table facing the plant and sticky board so that all three items are in a straight line. The distance from the test plant to the sticky board was 40 cm. The distance from the plant to the fan was 165 cm. Continuous wind generated by the pedestal fan was directed towards the plant and the sticky board (Fig. 2.6.1). Mealybug density on each plant prior to fan treatments was recorded. After exposure to wind treatment, the number of mealybugs on the sticky board was counted.

Table 2.6.1. Combinations (factor levels) of wind speed and mealybug density tested in Experiment (1).

Wind speed	Mealybug density on test plant	
1.5 knots	Low (≤ 10 adults)	High (≥ 50 adults)
3.5 knots	Low (≤ 10 adults)	High (≥ 50 adults)

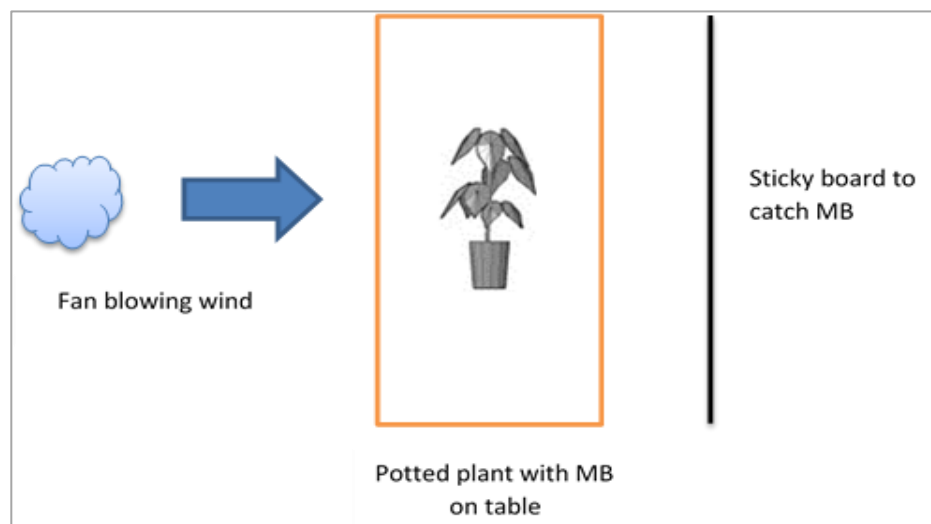


Fig. 2.6.1. Layout of Experiment 1.

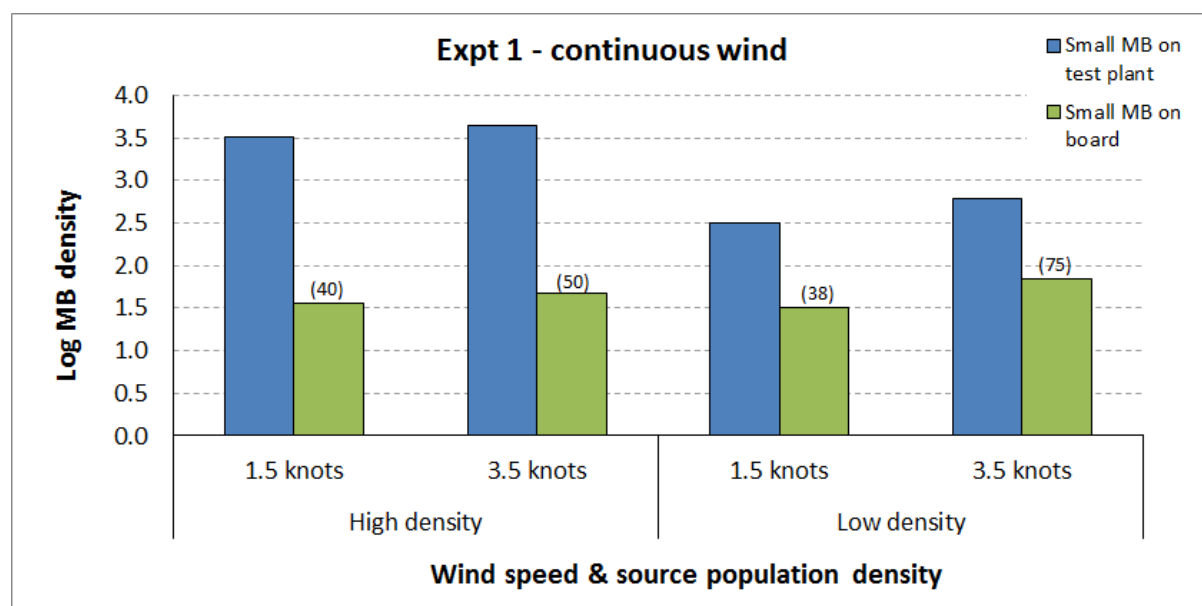


Fig. 2.6.2. The number of small (1st and 2nd instar) mealybugs blown off test plants by wind and captured on a sticky board in relation of original density on the test plants. Numbers in brackets represent actual total MB count (from three replicates) on the sticky board.

Testing was done between 1 pm and 2 pm. To simulate natural wind gust conditions, the fan was run for 3 x 10 minute intervals with a 10 minute break between each (standardised using electrical timer on power cord). Wind speed was measured using a handheld wind meter. Six wind speed measurements were taken per treatment from various positions of the plant (back of pot high in plant, lower in plant, from side leaves, etc.) and average wind speed was recorded. The number of mealybug on the board was recorded. A photographic record of each treatment was maintained.

Results

The number of mealybugs blown off the test plant was similar across density and wind speed factors (Fig. 2.6.2). The most noteworthy result is that even a gentle breeze (1.5 knots) can dislodge and disperse considerable numbers of small mealybugs from infested plants.

EXPERIMENT 2.

Methods

The next investigation in the sequence was designed to quantify the relative importance of crawling and wind-mediated movement in overall dispersal. Testing was conducted in the glasshouse with infested and clean, squaring stage cotton plants, placed on two flat benches (Fig. 2.6.3).

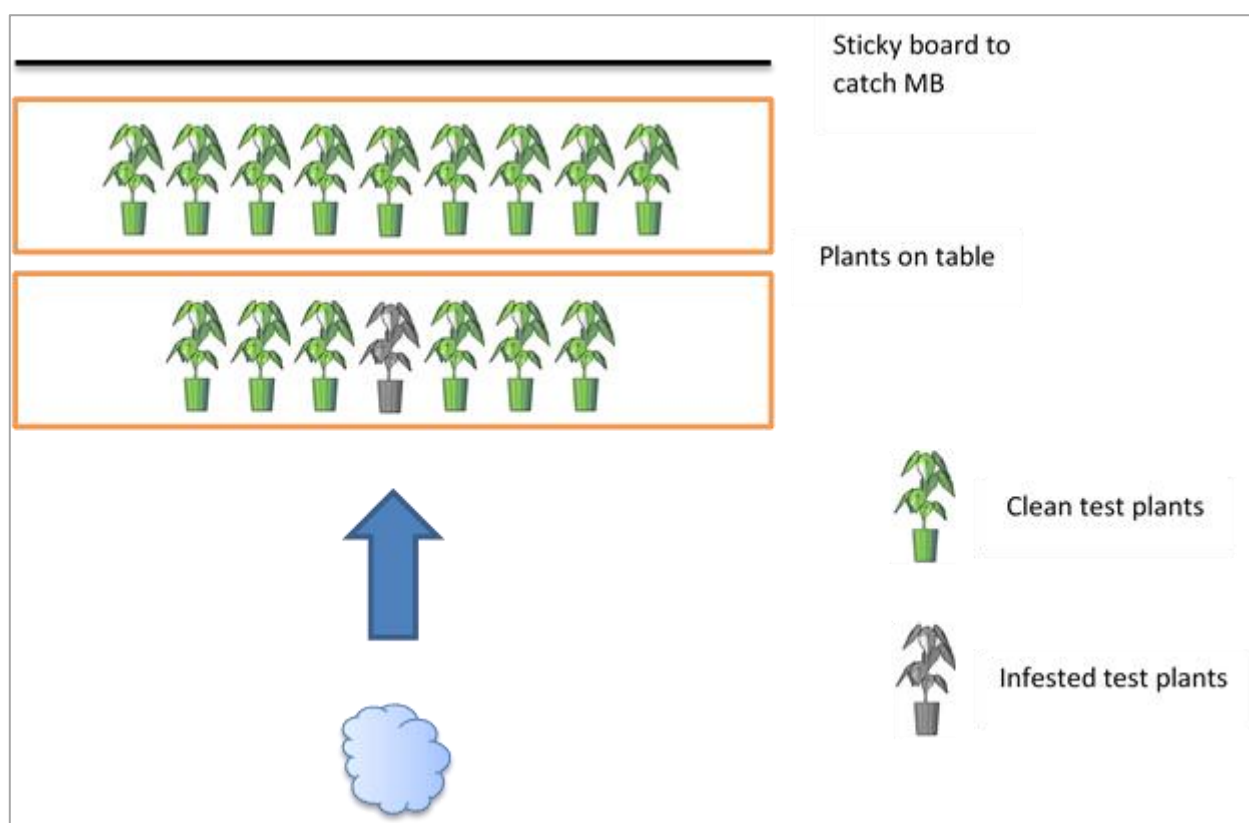


Fig. 2.6.3. Layout of Experiment 2.

The back row of 11 clean test plants (Fig. 2.6.3) was arranged so that pots were aligned in a row and leaves were just touching. The front row was separated from the back row by 60 cm and had one mealybug infested test plant in the middle with three clean test plants on either side in a row so that leaves were touching.

A large sheet of black cardboard stretching the length of the bench was placed behind the table, attached to a wall. The sheet was 40 cm from the row of plants and painted with a thin layer of “Tanglefoot Tangle-Trap”. A pedestal fan was placed 165 cm away, in a straight line with the infested plant (see Fig. 2.6.3) and the sticky board.

Mealybugs were counted for each infested test plant prior to fan treatment and recorded as either large or small. The clean test plants were checked to ensure they were mealybug free prior to treatments. The timing of wind treatment was between 1 pm and 2 pm. To simulate natural wind gust conditions, the fan was run for 3 x 10 minute intervals at the medium speed setting with a 10 minute break between each (standardised using an electrical timer). Wind speed was measured using a handheld wind meter. Six wind speed measurements were taken per treatment from various positions of the sentinel plant (back of pot high in plant, lower in plant, from side leaves, etc.) and average wind speed was recorded. Additional wind speed measurements were recorded from each of the test plants in the back row,

recorded from the centre of the pot. Following wind treatment, the number of mealybug on the board was recorded.

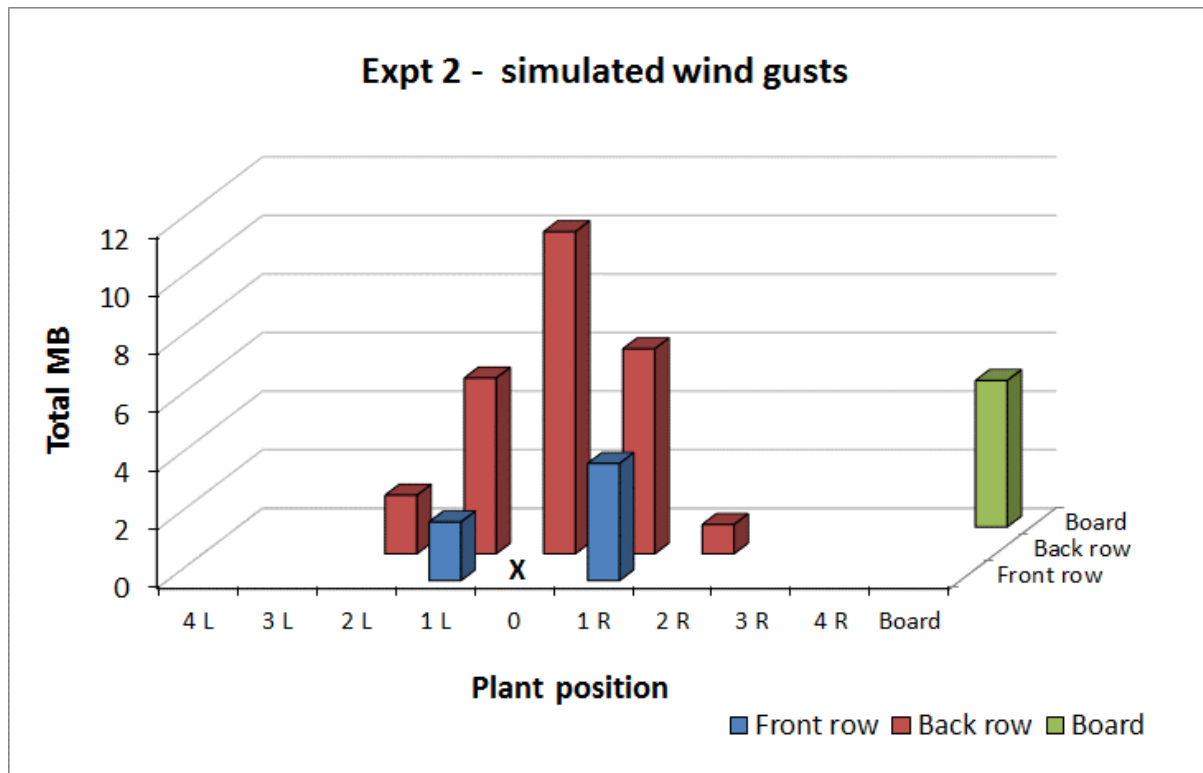


Fig. 2.6.4. Dispersal of small (1st and 2nd instar) mealybugs by crawling (blue columns, front row) or displacement by wind (red columns, back row) from a total of six infested test plants (X) to uninfested plants that were adjacent (to the left (L) or right (R)) or downwind of the infested test plants. The green column (Board) indicates the total number of small mealybugs blown off the infested test plants that were captured on the sticky board (see experimental layout in Fig. 2.6.3).

Results

Dispersal of mealybugs in the front row, to the left or right of the infested test plants (marked by X in Fig. 2.6.4) and perpendicular to the direction of the wind, could have occurred only by crawling. Dispersal from the infested test plants to uninfested plants in the back row could have occurred only as a result of displacement and transportation by wind. The results of this experiment show that at wind speeds of around 3.5 knots, dispersal by crawling does occur but is relatively minor compared to wind dispersal (Fig. 2.6.4). The position of uninfested plants in relation to that of the source plant is important in that dispersal of mealybugs is greatest directly downwind.

The results of Experiments 1 and 2 confirm the importance of wind dispersal in the field ecology of CMB.



Georeferenced sampling sites for CMB on weeds and volunteer host plants at study site 1 (Field 1). Top row: Volunteer cotton along the head ditch (2015). Middle row: volunteer cotton along the head ditch (2015) and late season CMB hotspot on pigweed along the southern drain; Bottom: late season CMB hotspot along the southern edge (2016).



Georeferenced sampling sites for CMB on weed and volunteer host plants in 2015 and 2016. Top row: Wild sunflower and volunteer cotton hosts along the boundary of a cotton field at study site 2 (2015). Middle row: A large patch of pigweed hosting CMB along the tail drain (2015) and volunteer cotton along the boundary of a cotton field (2016) at study site 2. Bottom row: Volunteer cotton and a heavy infestation of CMB hosting weeds in the drainage channel along the tail drain at study site 3 (2016).

Objective 3: Investigate chemical and biopesticide options for CMB population control

From March 2015 to June 2017, the project team completed eight glass house trials and eight field trials of insecticide efficacy against CMB. The glass house trials included chemical and biological insecticides; promising options identified in the glass house trials were further tested in field trials at Qld Department of Agriculture & Fisheries research facilities in Kingaroy and Emerald.

Milestones: 3.1 Assessment of at least one biopesticide option at Kingaroy RS completed (collaboration with Dr. Robert Mensah – NSW DPI)

A number of biological insecticides, including a fungal product obtained from Dr. Robert Mensah (NSW DPI, Narrabri), were tested in the glasshouse trials between December 2015 and April 2016, but showed no discernible efficacy against CMB.

Milestones: 3.2a Assessment of best bet chemical insecticide options at Kingaroy RS completed
Milestones: 3.2b Potential assessment of best bet chemical insecticide options at Emerald RS completed (subject to feasibility)

The project has successfully identified curative and suppressive options with varying levels of efficacy which are anchored within an IPM strategy for CMB (see below) developed as an output of this project. The curative component of the CMB management strategy includes two distinct chemical platforms based on currently registered maximum label rates of (a) spirotetramat (e.g. Movento®) either alone or in conjunction (tank mixed) with efficacy enhancing additives, and (b) sulfoxaflor (e.g. Transform®) either alone or in conjunction with efficacy enhancing additives. The suppressive component of the strategy includes the product buprofezin (currently available for use on CMB under permit in New South Wales) which has efficacy on early instar CMB and has been identified as having the potential to provide a good IPM fit in relation to management of other sucking pests (e.g. whiteflies).

Overall progress in addressing objective 3 and associated milestones was significantly hampered by Bayer Australia's decision in July 2016 to withdraw support for an off-label rate of spirotetramat (600 mL/ha) previously supported by the company for the first two years of the project. Early glasshouse trials had identified this off-label rate as the most promising treatment option for CMB control. Bayer's decision was based on the potential increased risk posed by the higher product rate (relative to the 400 mL/ha rate currently registered in cotton) to foraging bees. Bayer's decision prompted refocussing of efficacy assessment on the currently registered maximum label rates of all candidate products (e.g. spirotetramat @ 400 mL/ha, sulfoxaflor @ 400 mL/ha), but gave the project only 12 months to collect sufficient data to support regulatory approval for the prospective CMB treatment options.

The project team is currently working with Bayer Australia to ensure that the efficacy data available at the end of the project can be used to facilitate a label change to include CMB on the Movento product label. Similarly, the project team is working with Dow Agrosiences Australia to facilitate a change to the Transform product label to include CMB. Transform is currently available for use on CMB in cotton under permit (APVMA PER85052).

The results of the efficacy trials for the products of interest (spirotetramat, sulfoxaflor and buprofezin) are summarised below. Glasshouse and field trials conducted at off-label rates of products have been omitted from the summary below but are included in the detailed trials efficacy report (Attachment 1).

In previous research (DAQ1204) spirotetramat was identified as a molecule of interest with regard to efficacy against CMB. Spirotetramat is also widely regarded as a good IPM option due to its efficacy against other sucking pests, notably the silverleaf whitefly (SLW), *Bemisia tabaci* biotype MEAM1, which is now considered a major pest of cotton in Australia and often found to co-occur in crops infested by CMB. In this project, spirotetramat was evaluated in a series of glasshouse and field assessments, beginning with glasshouse trial (1) in which standard industrial parameter settings (water volume, nozzle settings) commonly used for insecticide application in cotton were used.

Results from the first glasshouse assessment featuring a single application of insecticide confirmed the finding of variable and commercially unacceptable control of CMB from previous research. In subsequent glasshouse assessments, a “double knock” tactic (two sequential applications of the same treatment option 12-14 days apart) along with different adjuvants, inclusion of a crop oil (Biopest® or Canopy®) as a tank mix partner, different water volumes, different nozzle types (flat fan versus turbo twin jet) and configurations (with and without nozzle droppers) were deployed to find the most effective combination of parameters required to maximize individual product efficacy.

The switch from XR flat fan to Turbo twin jet nozzles and the use of higher water volumes (≥ 200 L/ha) resulted in improved control. The most significant improvements in control efficacy resulted from the switch from a single application of the treatment option to a double knock tactic, followed by the addition of crop oils @ 5% (v/v) to all spirotetramat treatment options.

The efficacy of spirotetramat treatments in field trials was equivalent to or better than that observed in the corresponding glasshouse treatments. This was primarily due to additional mortality of CMB in field plots as a result of beneficial insect activity, even though the latter was minimised by the use of pyrethroid based insecticides such as bifenthrin between post treatment assessments.

The performance of sulfoxaflor was similar to that of spirotetramat. The addition of crop oil as a tank mix partner did not improve overall treatment efficacy and in some instances may have had a slightly antagonistic effect on the performance of sulfoxaflor. In the four trials in which buprofezin was included, it provided highly significant control of CMB relative to the unsprayed control but generally resulted in a significantly lower level of control (suppression) in comparison to spirotetramat and sulfoxaflor.

Based on the results of the glasshouse and field assessments, spirotetramat and sulfoxaflor are unlikely to provide $>50\%$ mortality of adult CMB from a single application without supplementary mortality from beneficial insects and other ecosystem services. When used within a double knock tactic (two sequential applications of the same product 12-14 days apart), the expected level of adult CMB control is 60-70% for sulfoxaflor and 70-75% for spirotetramat. Inclusion of crop oil at 5% (v/v) with the latter is expected to increase the expected mortality level to 90-95%.

The Australian cotton industry is increasingly reliant on relatively “soft”, IPM friendly chemistry to manage a range of pests without adversely impacting on beneficial insect communities and flaring key pest species such as SLW and CMB. The outputs of this project show that spirotetramat, sulfoxaflor and buprofezin are clearly compatible with beneficial insect populations, as evidenced by the virtually 100% control of CMB in the chemical+ beneficial insect combination field trials in Emerald and Kingaroy even after two applications of the first two (see Attachment 1 for details).

An decision support framework for CMB management in cotton featuring guidelines for the deployment of the key chemical insecticides identified in this project and discussed above, in the context of crop development stages and other factors, is presented below.



Management of cotton mealybug (CMB, *Phenacoccus solenopsis*)

In-crop options and recommendations:

- Upon first detection, mark infested plants/spots.
- Monitor regularly for CMB and **key beneficials** - lacewings, *Cryptolaemus*, 3-banded ladybird, *Aenasius parasitoid*.
- Beneficial insects are highly effective in keeping CMB populations in check; chemical insecticides should be used for CMB control only in very specific circumstances (see below) and as a last resort if beneficials are absent or at very low densities (early in the season or due to spraying for other insect pests, eg. mirids/whiteflies) and there is a growing risk of CMB infestation/damage.
- **If detection is in the seedling/squaring stages when the cotton plant is highly susceptible and before the seasonal build-up of beneficial insects occurs, CMB infestation carries a significant risk of plant death; chemical intervention may be required (see Table A below for options and expected efficacy).**
- **Good coverage is critical** for effective control of CMB with chemical insecticides; depending on plant size, higher water volumes, up to 250-300 L/ha, will increase efficacy of any rate.
- For ground-rig spray application, the **use of droppers** at alternate positions on the boom (3 nozzles per row of cotton) in conjunction with **Turbo Twinjets (TTJ60-110)** is highly recommended.
- Under certain circumstances and for specific insecticides, sequential application 14 days apart may be required to achieve a satisfactory level of CMB control – contact the Cottoninfo team and your REA for advice.

(A): From flowering to 4 weeks from first defoliation

Infestation Characteristics	Spatial distribution	Other pests at or above threshold	Management option
Individual plants and/or small groups of plants – infested plants may be stunted but NOT SEVERELY AFFECTED	Isolated/scattered <u>throughout field</u> (crop)	Yes - mirids	<ul style="list-style-type: none"> • Aim to control mirids without flaring CMB; sulfoxaflor (Transform) has efficacy (40-60% control) on CMB; efficacy may be rate dependent. • Increasing water volume to 250-300 L/ha will increase efficacy of any rate.
		Yes - SLW	<ul style="list-style-type: none"> • Spirotetramat (Movento) (+ 3-5% oil v/v) - very effective on CMB and has efficacy on SLW. • Buprofezin (Applaud) has suppressive effects on CMB (stops population growth) and is available for use in cotton under permit (PER83180, contact CA prior to use). It also has activity on SLW.
		No	<ul style="list-style-type: none"> • Continue monitoring; Conserve beneficials. • Sequential applications of crop oils (Biopest/Canopy) @ 5% v/v can suppress CMB populations as standalone application
	Confined to one or more clearly identifiable <u>sections</u> of field (crop)	Yes – mirids, SLW	<ul style="list-style-type: none"> • Manually remove and destroy infested plants where possible, avoid spreading to other parts of the field. • Continue monitoring; Conserve beneficials. • Sequential applications of crop oils (Biopest/Canopy) @ 5% v/v can suppress CMB populations as standalone application. • Sulfoxaflor and [spirotetramat + 5% oil v/v] options as above for spot/field section treatment.
		No	<ul style="list-style-type: none"> • Manually remove and destroy infested plants where possible, avoid spreading to other parts of the field. • Continue monitoring. • Sequential applications of crop oils (Biopest/Canopy) @ 5% v/v can suppress CMB populations as standalone application.
		No	<ul style="list-style-type: none"> • Manually remove and destroy infested plants where possible, avoid spreading to other parts of the field. • Continue monitoring. • Sequential applications of crop oils (Biopest/Canopy) @ 5% v/v can suppress CMB populations as standalone application.

Infestation hotspots with dead or dying plants	Isolated/scattered throughout field (crop)	Yes - mirids	<ul style="list-style-type: none"> Aim to control mirids without flaring CMB; sulfoxaflor has efficacy (40-60% control) on CMB; efficacy may be rate dependent. Increasing water volume to 250-300 L/ha will increase efficacy of any rate.
		Yes - SLW	<ul style="list-style-type: none"> Spirotetramat (+ 5% oil v/v)- very effective on CMB (90-95% control) and has efficacy on SLW; Buprofezin has suppressive effect on CMB (stops population growth). It also has activity on SLW.
		No	<ul style="list-style-type: none"> If one or more key beneficials are present – CONSERVE; continue monitoring. Crop oils (Biopest/Canopy) @ 5% v/v are non-disruptive; sequential applications can suppress MB populations as standalone application. If beneficials absent or in very low numbers, recommended option for whole field is spirotetramat + 5% oil; sulfoxaflor and buprofezin can be used but will provide only partial or suppressive control; aim to conserve and build beneficial numbers.
	Confined to one or more clearly identifiable sections of field (crop)	Yes – mirids, SLW	<ul style="list-style-type: none"> If key beneficials are present, minimise mirid and SLW sprays on infested section of field where possible; conserve beneficials and continue monitoring. If mirid/SLW spraying is required, use sulfoxaflor or spirotetramat options, as above.
		No	<ul style="list-style-type: none"> If key beneficials are present, minimise other pest sprays on infested section of field where possible; conserve beneficials and continue monitoring. If key beneficials are absent or in very low numbers, spirotetramat + 5% oil (v/v) is recommended option.

(B) Mealybug (CMB) management options: 0 - 4 weeks from first defoliation

Infestation Characteristics	Spatial distribution	Time to 1 st defoliation	Management option
Individual plants and/or small groups of plants – infested plants may be stunted but NOT SEVERELY AFFECTED	Isolated/scattered throughout field (crop)	3 - 4 wks	<p>In the absence of other pests (eg. SLW), no intervention required:</p> <ul style="list-style-type: none"> Allow beneficials to build and control CMB. Prior to crop harvest, destroy all non-crop vegetation that may harbour CMB bordering the crop (head, tail drains, sides) to minimise CMB survival and carry-over to next crop/season. Practice excellent field/farm hygiene and crop residue management. <p><u>If spraying for SLW is necessary:</u></p> <ul style="list-style-type: none"> Pyriproxifen (Admiral) is very effective on SLW, soft on beneficials and will not flare (no efficacy on) CMB. Buprofezin has suppressive effect on CMB (stops population growth). It also has activity on SLW. High water volumes >150 L/ha will increase efficacy of both products. <p>DO NOT USE BROAD SPECTRUM PRODUCTS FOR SLW KNOCKDOWN</p>

	Confined to one or more clearly identifiable sections of field (crop)	0 – 2 wks	<p>As above.</p> <p>In the absence of other pests (eg. SLW), no intervention required;</p> <ul style="list-style-type: none"> Manually remove and destroy infested plants where possible.
Infestation hotspots with dead or dying plants	Isolated/scattered throughout OR confined to one or more clearly identifiable sections of field (crop)	3 - 4 wks	<p>This situation is the result of failure to detect and manage CMB early, and/or disruption of beneficials.</p> <p>In the absence of other pests (eg. SLW), intervention is not recommended.</p> <ul style="list-style-type: none"> Allow beneficials to build and control CMB. Prior to crop harvest, destroy all non-crop vegetation that may harbour CMB bordering the crop (head, tail drains, sides) to minimise CMB survival and carry-over to next crop/season. Practice excellent field/farm hygiene and crop residue management. <p><u>If spraying for SLW is necessary:</u></p> <ul style="list-style-type: none"> Pyriproxifen (Admiral) is very effective on SLW, soft on beneficials and will not flare (no efficacy on) CMB. Buprofezin has suppressive effect on CMB (stops population growth). It also has activity on SLW. High water volumes >150 L/ha will increase efficacy of both products. <p>DO NOT USE BROAD SPECTRUM PRODUCTS FOR SLW KNOCKDOWN</p>
		0 – 2 wks	<p>This situation is the result of failure to detect and manage CMB early, and/or disruption of beneficials:</p> <ul style="list-style-type: none"> Intervention with chemicals not recommended. Allow beneficials to build and control CMB. Prior to crop harvest, destroy all non-crop vegetation that may harbour CMB bordering the crop (head, tail drains, sides) to minimise CMB survival and carry-over to next crop/season. Practice excellent field/farm hygiene and crop residue management.

End-of-season options and recommendations for a CMB infested crop:

- At harvest, leave picking of the crop or infested sections for last.
- If possible lift picker heads above CMB host spots or infested sections.
- After harvest, destroy crop residue thoroughly and root cut to a depth of 10 cm or more to minimise the survival of plant material in the soil that can harbour CMB; escapes when root cutting are common when volunteers occur just outside of the plant row or when equipment is set up poorly.
- Consider pupae busting if you are planning on growing cotton again in the same or neighbouring field within 1-2 years.
- Everyone entering the farm should practice Come Clean Go Clean principles - brush down clothing after entering a field with mealybugs and ensure farm equipment is cleaned down after entering fields with mealybugs.
- If you have a contractor coming on to the property, inspect the machinery at the gate! Provide contractors with wash down facilities that they can use before and after completing work on the property.
- Make sure your Come Clean Go Clean requirements are communicated to workers, contractors and other people visiting the farm.

Objective 5: Silverleaf whitefly (SLW) support to industry

Milestones: 5.1 Establish industry advisory/liaison group and organise for mid and end season SLW review

A teleconference with industry representatives and extension personnel was held in January 2016 to discuss the need and process for an industry advisory/liaison group. The main outcome of the teleconference was the decision that a formalised liaison group and scheduled meetings were not required. Contribution to/participation by the project leader in needs based industry meetings and extension/communication activities organised by the CRDC, CottonInfo and Crop Consultants Australia (CCA) to disseminate SLW management advice and new/revised recommendations was deemed to be sufficient.

Milestones: 5.2 Review SLW threshold and associated extension material annually based on feedback from advisory group

The threshold matrix and guidelines for management of SLW in Australian cotton crops were reviewed and revised in March/April 2015, 2016 and 2017. The project leader (Dr. Richard Sequeira) has been working with CSIRO researchers (Dr. Lewis Wilson, Dr. Simone Heimoana and Ms Tanya Smith) to validate the current sampling and threshold recommendations for southern Queensland and New South Wales. The findings of a 2017 analysis by Dr. Richard Sequeira of a multi-season data set on SLW distribution and abundance from the McIntyre and Gwydir valleys collected by CSIRO staff has been instrumental in guiding new investment by the CRDC in RD&E aimed at improving SLW management in cotton.

Objective 6: Effective communication and extension of results

Milestones: 6.1 Communication of results and implications to stakeholders

Dr. Richard Sequeira was invited to and presented at the 2016 and 2017 CCA annual meetings on SLW and other crop protection issues. Dr. Sequeira presented the results of the SLW sampling and thresholds matrix validation work conducted by CSIRO staff in NSW at the CCA meeting in July 2017. Dr. Sequeira's analysis and presentation have contributed to feedback from the cotton industry to the CRDC on the need to update/revise the current SLW management framework, including thresholds and sampling guidelines.

A summary of the CMB outputs from this project was presented by Dr. Sequeira at the World Cotton conference in Brazil (May 2016), the Australian Cotton Research Conference in Canberra in September 2017, and several regional IPM meetings organised by CottonInfo staff in 2017. Dr. Moazzem Khan presented a summary of the findings to stakeholders at the 2016 Cotton Conference at the Gold Coast.

Outcomes

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

The project application was built around two planned outcomes:

- (a) Growers and crop managers gain sufficient knowledge to implement IPM friendly pest management programs
- (b) Growers with knowledge of effective insecticide options for MB control; Reduced reliance on broad-spectrum insecticides

Although CMB is a global pest of cotton, there were no examples of an effective chemical control strategy that is compatible with modern IPM based crop protection systems anywhere in the world prior to the outputs of this project. The CMB management package delivered by this project contributes to both planned outcomes listed above. The management package includes clearly defined chemical control options for a range of CMB abundance and distribution patterns typically encountered in the

field. The curative options are anchored within a comprehensive decision support framework which includes appropriate insecticide/product choice recommendations in relation to crop development stage, product efficacy and incidence of key non-target pests. This will enable growers and cotton agronomists to target a range of crop protection scenarios with confidence and in a cost-effective manner. Knowledge of what insecticidal products with efficacy on CMB are capable of delivering will enable cotton growers and agronomists to make informed product choice decisions, reduce reliance on broad-spectrum chemicals and achieve better environmental outcomes.

6. Please describe any:-

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

Conclusion

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

The most important likely impact of this project's outputs for the cotton industry is greater confidence in managing one of the most feared insect pests of cotton in the GM era, and by proxy, generally more effective, IPM based management of other pests such as mirids, SLW and aphids. The fearful reputation of this insect pest stems partly from the fact that prior to the outputs of this project, effective IPM compatible insecticides capable of delivering commercially acceptable ($\geq 90\%$) control of the target population had not been successfully identified from previous research. This key gap in capability has been addressed by the outputs of this project.

The experience of the last five or more years has shown that inappropriate use of broad-spectrum products to target CMB, largely resulting from an inadequate understanding of its biology, ecology and IPM principles, has typically served only to flare populations not only of this pest but non-target species as well by decimating beneficial insect communities. The presence of healthy and vibrant beneficial insect communities is the corner stone of cost effective and sustainable pest management in GM cotton crops. Widespread dissemination of the outputs of this project will serve to minimise the negative CMB management experiences of the past and contribute to more informed and effective decision making with regard to protection of cotton crops.

Not enough is known about natural biological control of CMB in crops such as cotton other than observations on the genera of natural enemies present in different areas/regions and seasons and their temporal incidence. Detailed knowledge of the chemical susceptibilities and pest population regulation contribution made by different genera/species would assist the cotton industry in making more effective conservation and product choice decisions.

Take home messages:

Good farm hygiene is the cornerstone of CMB management - Minimise on-farm sources of mealybug survival and carryover.

Conserve beneficial insect communities within the crop – avoid flaring MB when managing for other pests.

Consider release of commercially available beneficial insects - Cryptolaemus, lacewings and/or ladybird beetles - in CMB hotspots.

Put into practice the industry's Come-Clean-Go-Clean protocols.

Chemical control should be the option of last resort for CMB control; use chemical insecticides primarily to target “hot spots” so as to minimise the overall impact on the production system.

Adhere closely to the IPM guidelines developed from this project’s outputs and endorsed by the cotton industry peak bodies.

Extension Opportunities

- 8. Detail a plan for the activities or other steps that may be taken:**
 - (a) to further develop or to exploit the project technology.**
 - (b) for the future presentation and dissemination of the project outcomes.**
 - (c) for future research.**

- 9. A. List the publications arising from the research project and/or a publication plan.**
(NB: Where possible, please provide a copy of any publication/s)
 - B. Have you developed any online resources and what is the website address?**

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

The cotton mealybug (CMB), *Phenacoccus solenopsis*, also known as the solenopsis mealybug, is a highly polyphagous and invasive global insect pest of cotton. In Australia, economically damaging outbreaks of CMB on cotton were reported from the Burdekin and Emerald regions of Central Queensland in the 2009-10 cotton season; it has since continued to spread further south, having been found in southern regions of Queensland and northern New South Wales in 2016 and 2017, respectively.

CMB is easily controlled by naturally occurring arthropods (predators and a parasitic wasp) in agroecosystems where beneficial arthropod populations are not exposed to the detrimental effects of broad-spectrum insecticides. CMB is not easily controlled by chemical insecticides. Most newly developed “soft” (selective) chemical insecticides currently approved for use within cotton production systems in Australia have been shown to vary in CMB control efficacy from ineffective to having only suppressive effects. Some older organophosphates such as methidathion (e.g. Supracide®) give effective control but are highly hazardous from an environmental and user safety perspective, and incompatible with modern integrated pest management (IPM) friendly cotton production systems due to adverse impacts on beneficial insect communities.

The aim of this project, jointly funded by The Australian Cotton Research & Development Corporation and the Queensland Department of Agriculture and Fisheries, was to enhance the knowledge base and confidence of cotton growers and crop consultants/agronomists in effective management of CMB. The main objectives were: (a) to identify IPM-friendly and cost-effective curative (insecticidal) options for CMB control, and (b) to develop an IPM strategy and decision support system which would enable integration of chemical control tactics with practical options for conserving and protecting in-crop beneficial insect populations to achieve environmentally responsible and commercially satisfactory CMB management outcomes.

The key outputs of the project include identification of effective insecticide options and usage guidelines, and an IPM strategy underpinned by a comprehensive decision support system for CMB control. Insecticide efficacy testing in the project showed that:

- spirotetramat and buprofezin offer good potential for the development of metabolically distinct (alternative) approaches to managing field populations of CMB; spirotetramat gives the highest level of adult CMB mortality of all the insecticides tested whereas buprofezin (insect growth regulator) is effective on early instars and offers useful levels of population suppression.
- sulfoxaflor gives useful levels of adult and juvenile CMB mortality and offers good potential as a strategic tool in cotton IPM systems
- emulsifiable crop oils can supplement field control of adult and juvenile CMB when used as stand-alone treatments or in combination with spirotetramat; the addition of emulsifiable crop oils to spirotetramat treatments as a tank mix partner can increase CMB mortality by 10-30%
- the use of a double knock tactic (two sequential applications of the same product 10-14 days apart) significantly improves the level of CMB control provided by the new-generation, selective insecticidal products such as spirotetramat and sulfoxaflor.

The presence of healthy and vibrant beneficial insect communities is the corner stone of cost effective and sustainable pest management in GM cotton crops. The Australian cotton industry is increasingly reliant on relatively “soft”, IPM friendly chemistry to manage key pests without adversely impacting on beneficial insect communities and flaring other, non-target pest species. Widespread dissemination of the outputs of this project will contribute to industry development by enhancing the capability and confidence of growers and crop consultants/agronomists to make informed and effective decisions with regard to IPM based management of CMB and other cotton pests.