Australian Government

Cotton Research and Development Corporation

Annual, Progress and Final Reports

REPORTS

Part 1 - Summary Details

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

CRDC Project Number: **DAN164c**

Annual Report: ☐ Due 30-September

Progress Report: ☐ Due 31-January

Final Report: ☒ Due 30-September (or within 3 months of completion of project)

Project Title: Project Title: Mechanisms of insecticide resistance in the cotton aphid Aphis gossypii

Project Commencement Date: 01/07/02 **Project Completion Date:** 30/06/04

Research Program: 3 Crop Protection

Part 2 – Contact Details

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Signature of Research Provider Representative: ____________________

Part 3.3 – Final Reports (due 3 months after completion of project)

(The points below are to be used as a guideline when completing your final report. Postgraduates please note the instructions outlined at the end of this Section.)

1. Outline the background to the project.

Since the first reported control failures at Emerald during the 1998-1999 cotton season, insecticide resistance in cotton aphid, *Aphis gossypii,* has emerged as a significant threat to the Australian cotton industry. Although once considered a late season pest, aphid populations now require targeted control earlier resulting in increased aphicide sprays and consequent selection for resistance.

Previously, the cotton aphid had been readily controlled with the IPM compatible carbamate, pirimicarb. However, by 1999-2000 pirimicarb resistance was common in southeast Queensland and New South Wales. Pirimicarb resistance also conferred cross-resistance to the unrelated organophosphate compounds dimethoate and omethoate rendering them useless. Efficacy of the remaining organophosphates is variable with some aphids also resistant to pyrethroids and endosulfan. These multiple resistant aphids can only be controlled by newer products such as diafenthiuron. The effects of resistance can be reduced with effective resistance management. Management in part requires an understanding of underlying resistance mechanisms, however, this is not well understood in Australian *A. gossypii* populations.

Consequently, the objective of this project was to better understand the underlying resistance mechanisms used by *A. gossypii*. That information will then be used to develop more robust control strategies including a field based kit to detect pirimicarb resistance.

2. List the project objectives and the extent to which these have been achieved.

To aid the develop of an effective cotton aphid resistance management strategy

- Mechanism studies supported the moving of aldicarb out of the current organophosphate / carbamate block into its own rotation group

To develop a diagnostic field kit to detect pirimicarb resistance in the cotton aphid

- A rapid field based resistance detection kit was developed and distributed to cotton IDO's for use. CGS expressed an interest in kit commercialization but as yet this has not been realized.

Determine the mechanisms of profenofos resistance in *A. gossypii*

- Profenofos resistance is due to esterase and possible insensitive acetylcholinesterase mediated metabolisms.

Determine the mechanisms of pyrethroid and endosulfan resistance in *A. gossypii*

- Results indicate that 'knock down resistance' (kdr) or esterase are not involved in pyrethroid (bifenthrin) resistance. Endosulfan resistance was not studied in detail due to the rarity of resistant aphids but a preliminary data indicated that an insensitive AChE was unlikely.

Potential resistance mechanisms to newer control agents such as imidacloprid and diafenthiuron.

- Esterase mediated resistance to imidacloprid has been detected in other pest species and highly resistant aphid clones possess huge amounts of esterase. The light activated form of diafenthiuron was inhibited by esterase possibly implicating future esterase mediated resistance.

Investigate the genetics of profenofos, pyrethroid and endosulfan resistance in *A. gossypii*.

- Not achieved due to early project termination.

3. Detail the methodology and justify the methodology used.

Total esterase assay

Samples from each aphid population were homogenised in 0.02M pH 7.0 phosphate buffer containing 0.05% Triton X-100. Total esterase activity was determined on 10μL aliquots. The reaction was initiated by adding 240μL of 0.2M pH 6.0 phosphate buffer with 0.6% Fast Blue RR Salt and 1.86% 1-naphthyl acetate. Kinetic assays were performed at 25°C, using a Bio-Rad 3550 microplate reader and Kinetic Collector 2.0 software run on an Apple Macintosh SE computer. The assay was run for 15 minutes, taking absorbance readings (450 nm) at 14 second intervals. Linear regressions were performed by the computer. The kinetic velocity was calculated by the computer as the slope of the fitted regression line.

Model substrates are used extensively in detecting enzyme activity, as they give products which are readily assayed, usually spectrophotometrically (Devonshire, 1990). *In vitro*, when insect homogenates possessing an esterase mediated resistance mechanism towards an insecticide are incubated with that insecticide and assayed for activity, then the enzyme-insecticide complex prevents substrate binding, thus lowering activity.

Acetylcholinesterase assay

Acetylcholinesterase activity was measured by the hydrolysis of the substrate analogue acetylthiocholine iodide (ATChI), which is measured colourimetrically in a microplate reader by the absorbance of 2-nitro-5-thiobenzoate at 405nm, after the reaction of 5-5'-dithio-bis(-2-nitrobenzoate) (DTNB) (Sigma-Aldrich) with the liberated thiocholine.

Aphids were homogenised in 0.1M pH 7.5 phosphate buffer containing 0.1% Triton X-100. Aliquots (10μL) were pipetted into a 96 well microplate. ATChI and DTNB solutions were dissolved in buffer (0.1M pH 7.5 phosphate buffer containing 0.1% Triton X-100), and 100μL of each added, to give final concentrations of 0.5μM and 0.05mM, respectively. The microplate was placed in a Bio-Rad microplate reader (Bio-Rad Laboratories) and read at 405nm for 30 minutes at 14 second intervals, utilising Kinetic Collector software to fit linear regressions to the kinetic plots.

Basic Electrophoresis Method

Polyacrylamide gel electrophoresis method used where individual adult aphids were homogenised in 20μL of 1.6% Triton X-100 (especially purified for membrane research containing 10% sucrose and a few grains of bromocresol purple. Aliquots of 15μL homogenate (0.75 insect equivalent) were pipetted into wells of polyacrylamide gels. Gels containing 7.5% polyacrylamide, but to achieve optimum resolution, the triton X-100 concentration was 0.20% in the stacking gel and 0.05% in the resolving gel with specially designed gel combs that cast wells with 4.5mm spacing in the stacking gel.

The gels were run at 250V maximum current for 90 minutes at 5°C (Gel Electrophoresis Apparatus, GE-2/4). Gels were stained for esterase activity, using 0.5mM α-naphthyl acetate (Sigma-Aldrich) and 0.2% Fast Blue RR in 0.02M phosphate buffer pH 6.0. Gels were fixed in 5% acetic acid. Electrophoretic mobilities (R_m) ratios were measured as the distance that esterase bands travelled down the gel, relative to the buffer interface.

Detection of Knockdown resistance (Kdr)

Techniques used to investigate the possibility of kdr resistance in the cotton aphid included genomic DNA extraction, PCR and DNA sequencing.

4. Detail and discuss the results including the statistical analysis of results.

Resistance detection kits

The kit identifies pirimicarb resistant aphids by a simple colour change. Briefly, aphids are collected from the field and individual aphids are squashed in a microplate well. Solutions are added as per the included instructions. The results can be read accurately by eye after 10-20 minutes and give the proportion of aphids resistant to pirimicarb; if they are resistant, the solution turns yellow. If any of the aphids tested are resistant, it is recommended not to use pirimicarb, dimethoate or omethoate.

Kits were distributed to all Cotton Industry Development Officers. Several kits were given to Mark Hickman, to be used in the IPM workshops. Feedback was used to further simplify and improve the kit. These resistance detection kits show an enormous potential to assist cotton farmers to make informed decisions before pirimicarb spray is applied against *A. gossypii.*

Mechanisms

Diafenthiuron

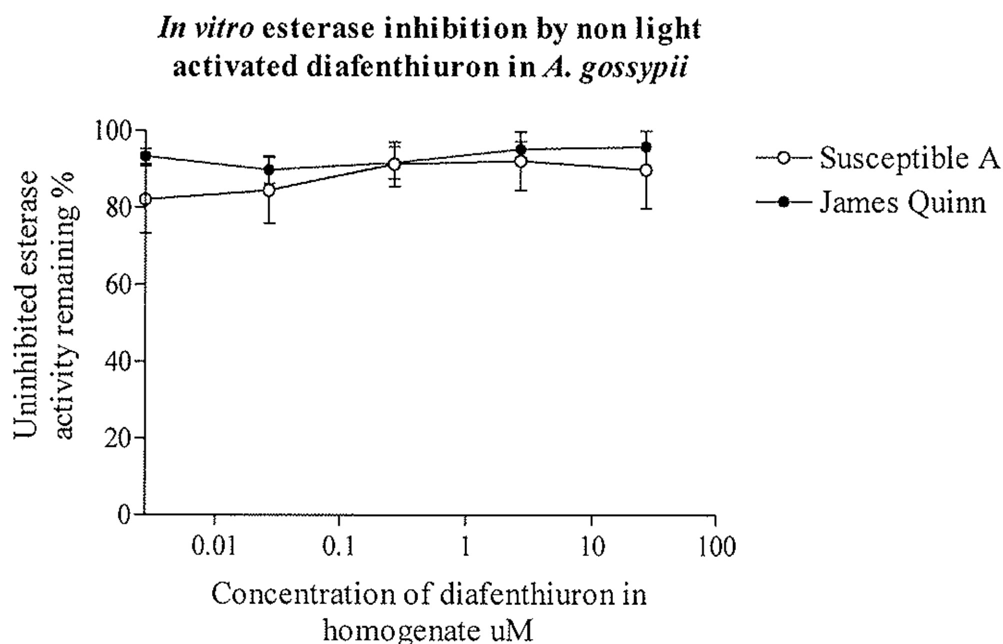


Figure 4.1 *In vitro* esterase inhibition by non light activated diafenthiuron in susceptible and multiple resistant (James Quinn) *A. gossypii*

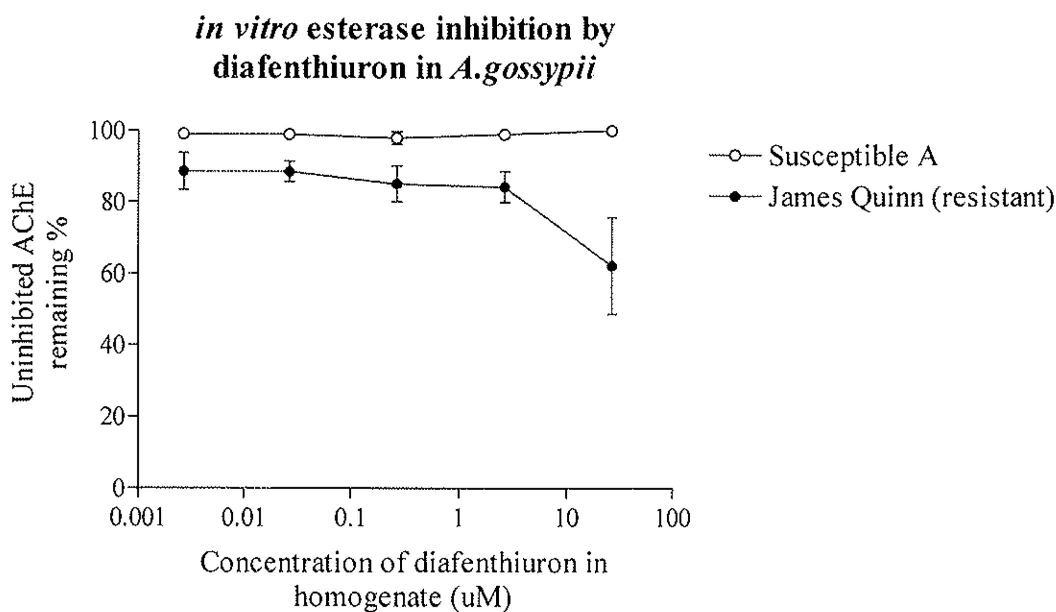


Figure 4.2 *In vitro* esterase inhibition by light activated diafenthiuron in susceptible and multiple resistant (James Quinn) *A. gossypii*

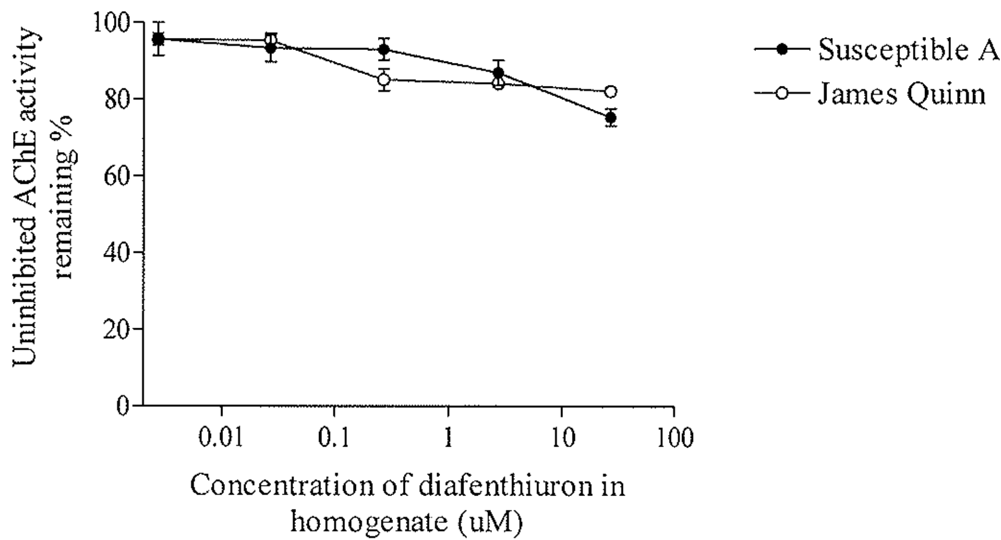


Figure 4.3 *In vitro* AChE inhibition by non light activated diafenthiuron in susceptible and multiple resistant (James Quinn) *A. gossypii*

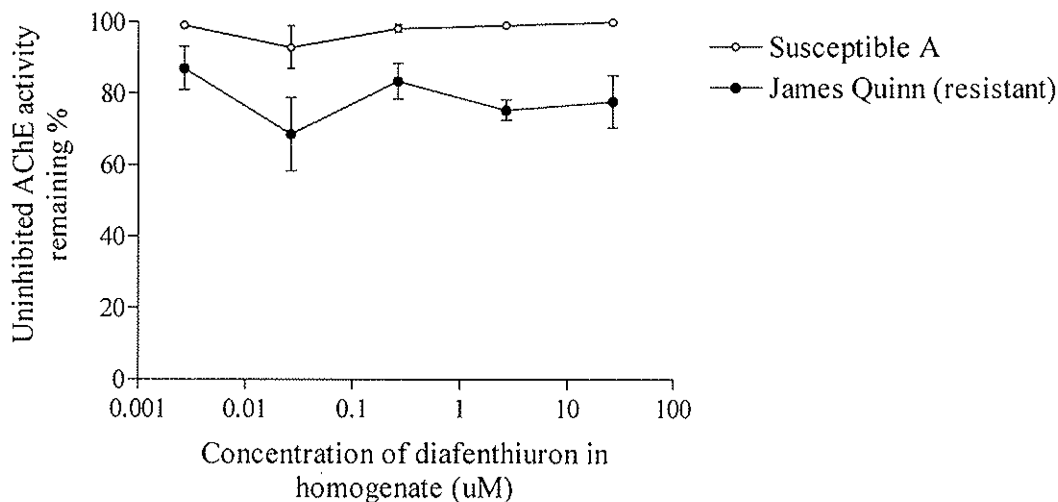


Figure 4.4 : *In vitro* AChE inhibition by diafenthiuron in susceptible and multiple resistant (James Quinn) *A. gossypii*

No esterase or AChE inhibition was observed in either strain when incubated with non light activated diafenthiuron (Figures 4.1 and 4.3). When light activated diafenthiuron was used, the James Quinn strain showed esterase inhibition up to approximately 40% (Figure 4.2), whereas the susceptible A strain showed no inhibition at all. Similarly, AChE inhibition was also observed in the multiple resistant James Quinn aphids (Figure 4.4) This may suggest if and when diafenthiuron resistance is detected esterase and or AChE mediated resistance may well be involved.

Chlorpyrifos-methyl

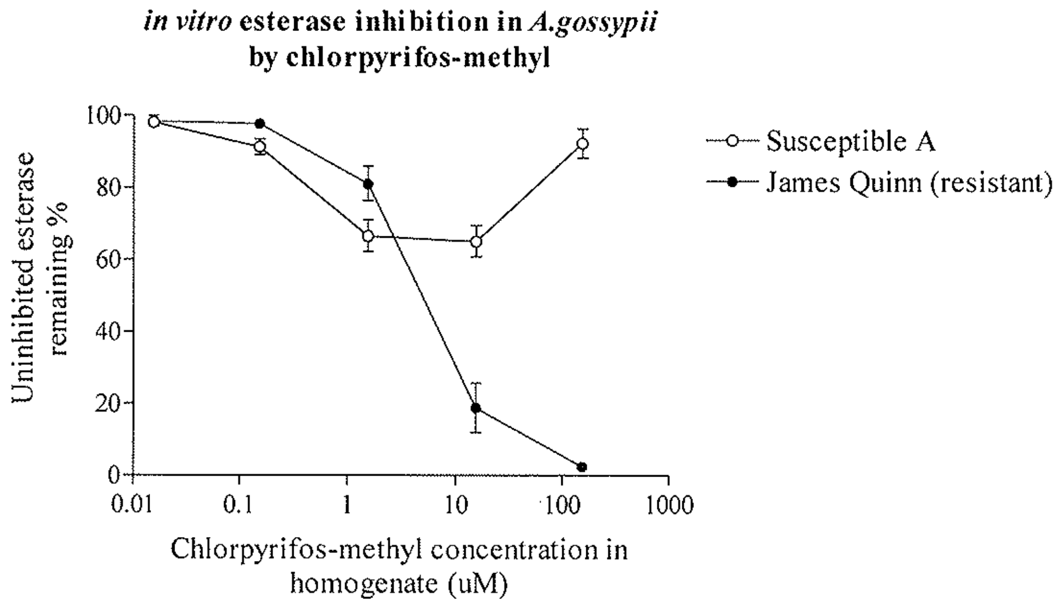


Figure 4.5 *In vitro* esterase inhibition by diafenthiuron in susceptible and multiple resistant (James Quinn) *A. gossypii*

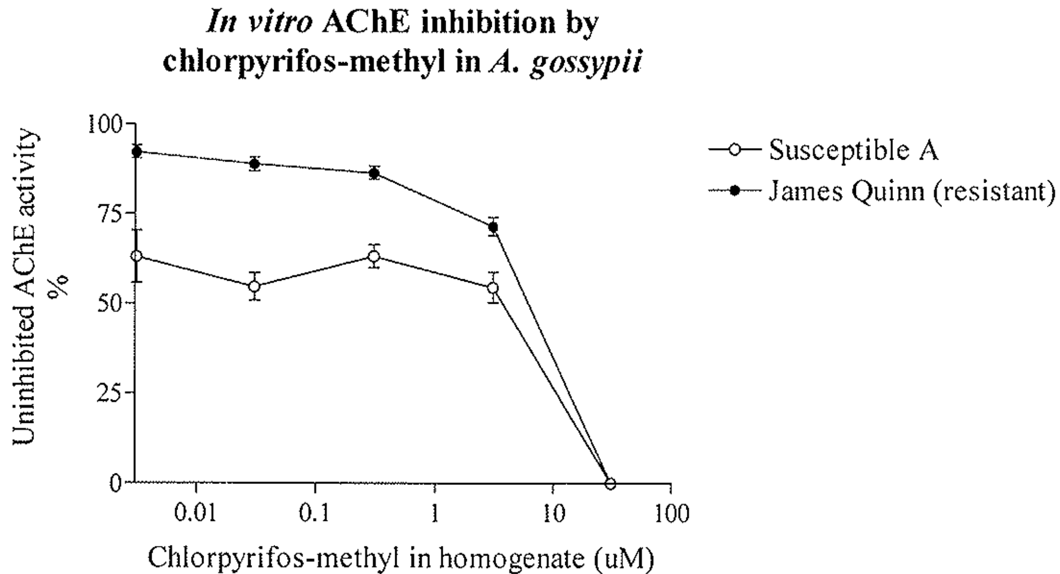


Figure 4.6 *In vitro* AChE inhibition by chlorpyrifos-methyl in susceptible and multiple resistant (James Quinn) *A. gossypii.*

Chlorpyrifos-methyl is an acytlcholinesterase inhibiting organophosphate insecticide (Figure 4.6). Resistance appears esterase mediated with nearly 100% of esterase inhibited by chlorpyrifos methyl in the highly resistant James Quinn strain (Figure 4.5).

Bifenthrin

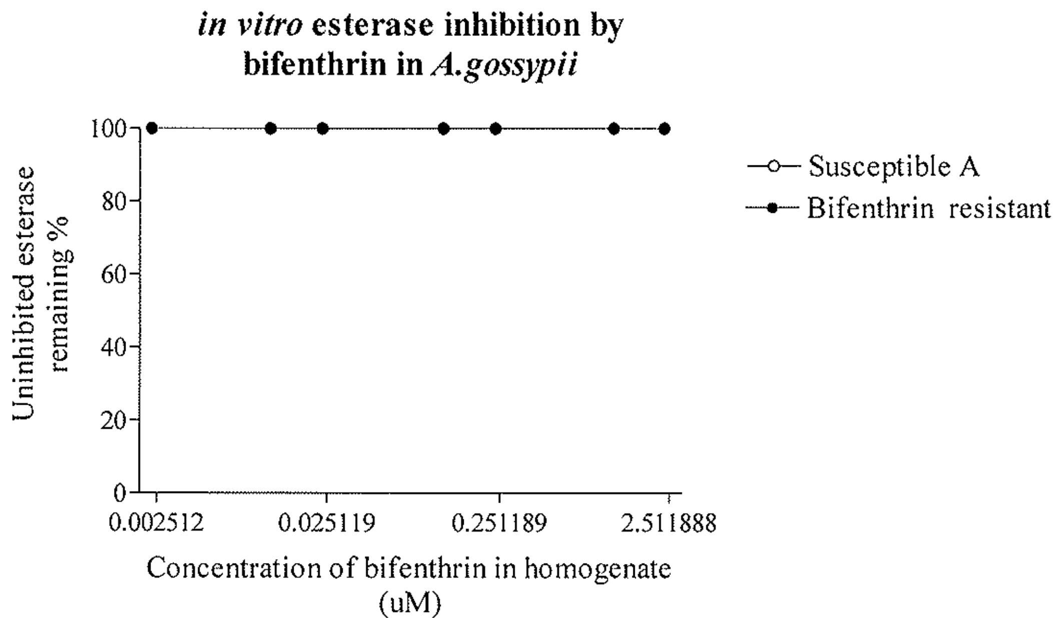


Figure 4.7 *In vitro* esterase inhibition by bifenthrin in susceptible and multiple resistant (James Quinn) *A. gossypii*

No esterase inhibition was shown in the Susceptible A or bifenthrin resistant strain (Figure 4.7).

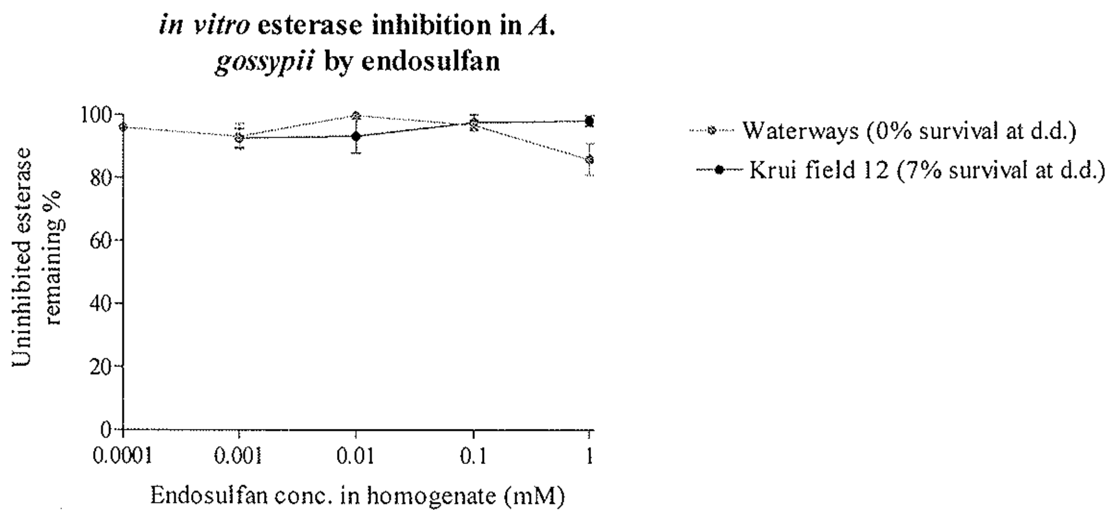


Figure 4.8 *In vitro* esterase inhibition by endosulfan in susceptible and low level resistant (Kruifield 12) *A. gossypii*

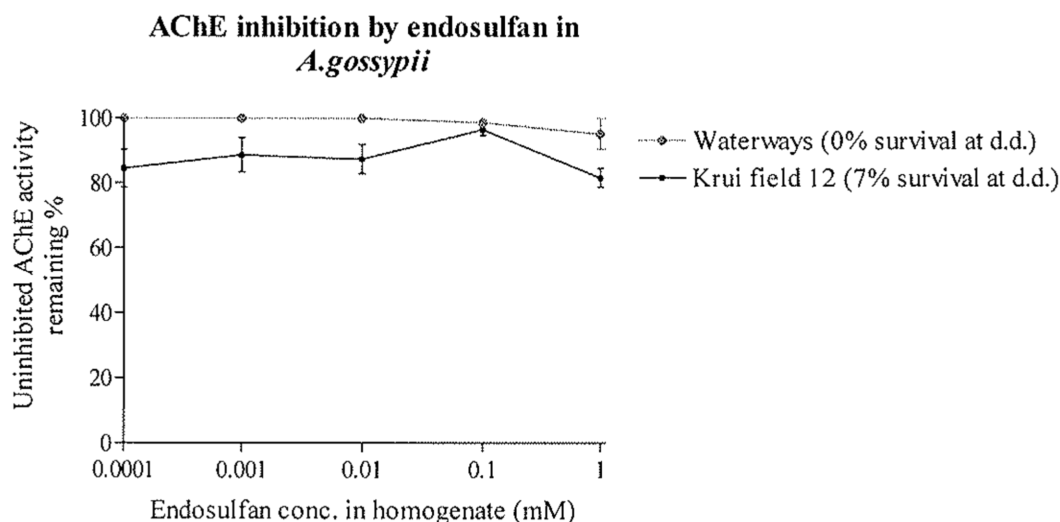


Figure 4.9 *In vitro* AChE inhibition by endosulfan in susceptible and low level resistant (Kruifield 12) *A. gossypii*

The low level endosulfan resistant strain Kruifield 12 showed little difference in esterase or AChE inhibition when compared to susceptible Waterways (Figures 4.8 and 4.9). This implies that these two mechanism may not be involved in endosulfan resistance, however, it must be remembered that the Kruifield 12 population does not have a high proportion of resistant clones.

Imidacloprid

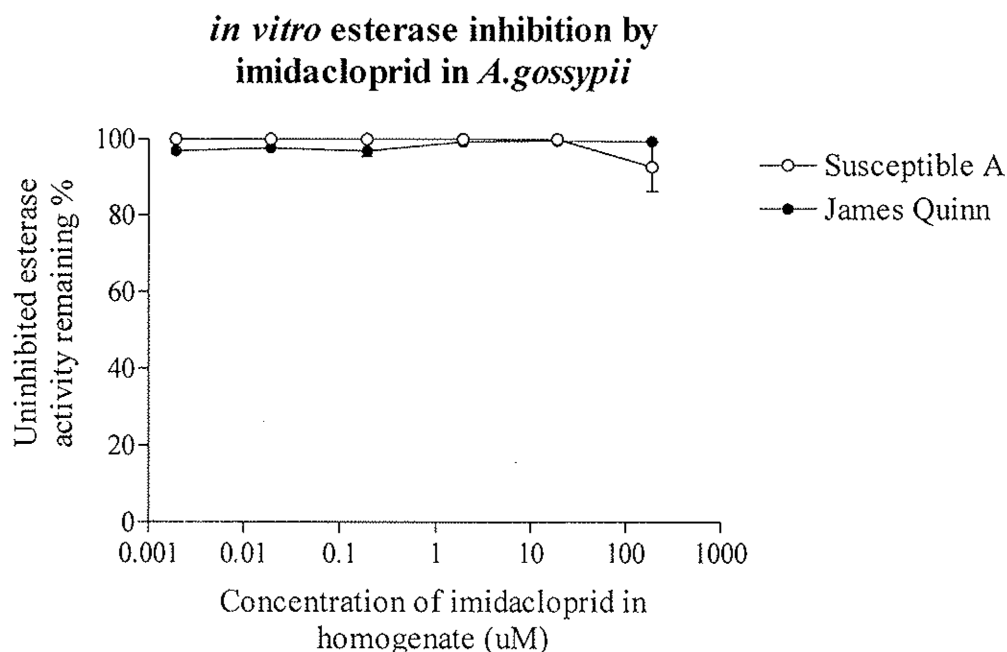


Figure 4.10 *In vitro* esterase inhibition by imidacloprid in susceptible and multiple resistant (James Quin) *A. gossypii*

Neither showed esterase inhibition implying that esterase mediated resistance may not be involved in imidacloprid resistance (Figure 4.10). This result is in contrast to esterase mediated resistance in silver leaf whitefly, *Bemisia tabaci*.

Carbosulfan

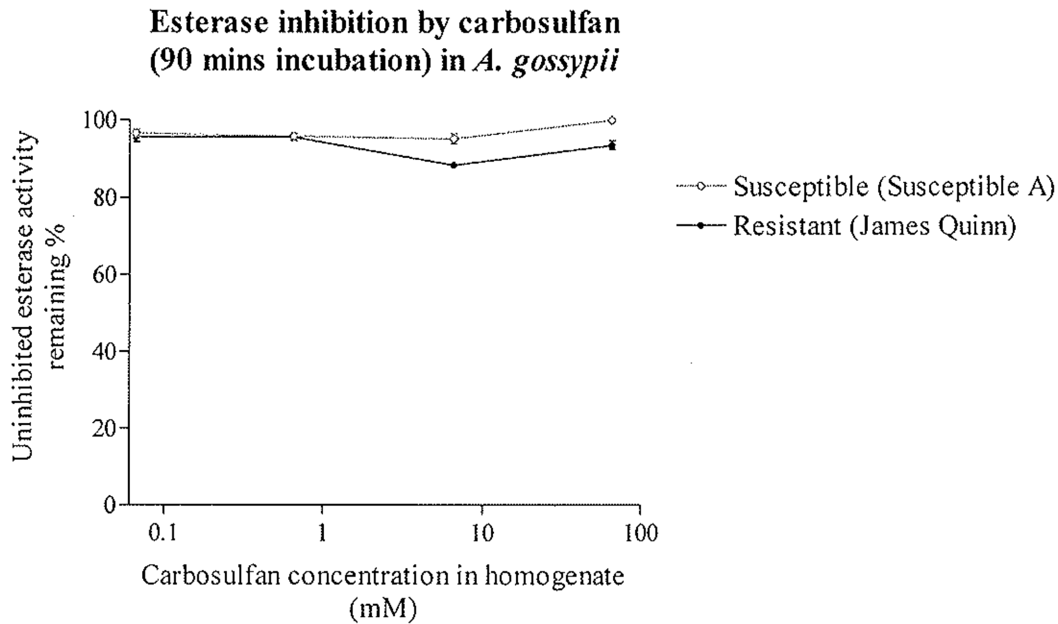


Figure 4.11 *In vitro* ninety minute esterase inhibition by carbosulfan in susceptible and multiple resistant (James Quin) *A. gossypii*

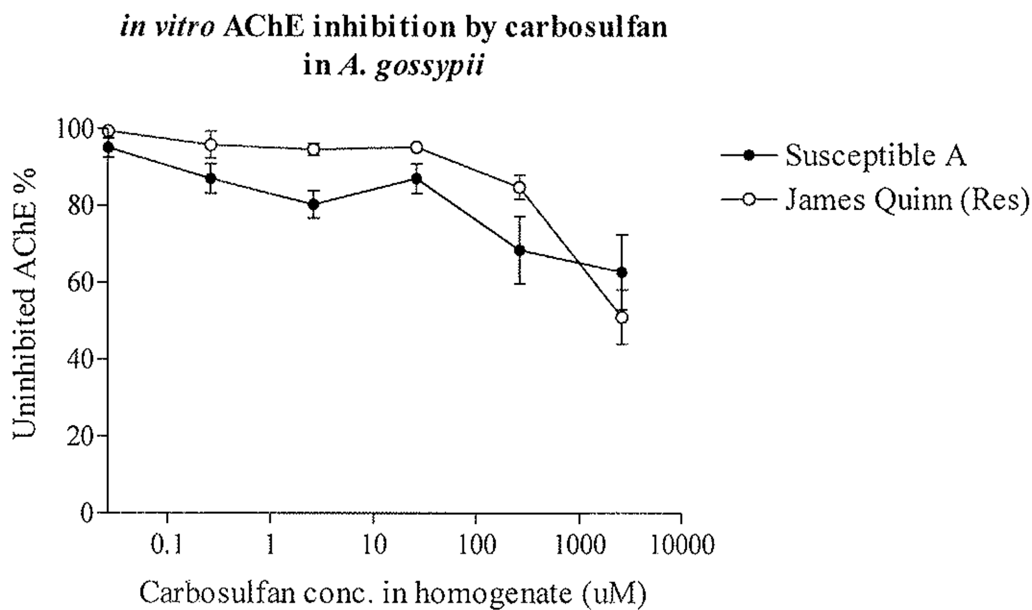


Figure 4.12 *In vitro* AChE inhibition by carbosulfan in susceptible and multiple resistant (James Quinn) *A. gossypii.*

There was only a slight inhibition of esterase by carbosulfan in the James Quinn strain after incubation for 90 minutes (Figure 4.11) suggesting esterase mediated resistance is unlikely. There was no significant difference in the response of AChE to inhibition with carbosulfan at the highest concentration tested (Figure 4.12) suggesting that the presence of an insensitive AChE is also unlikely.

Aldicarb

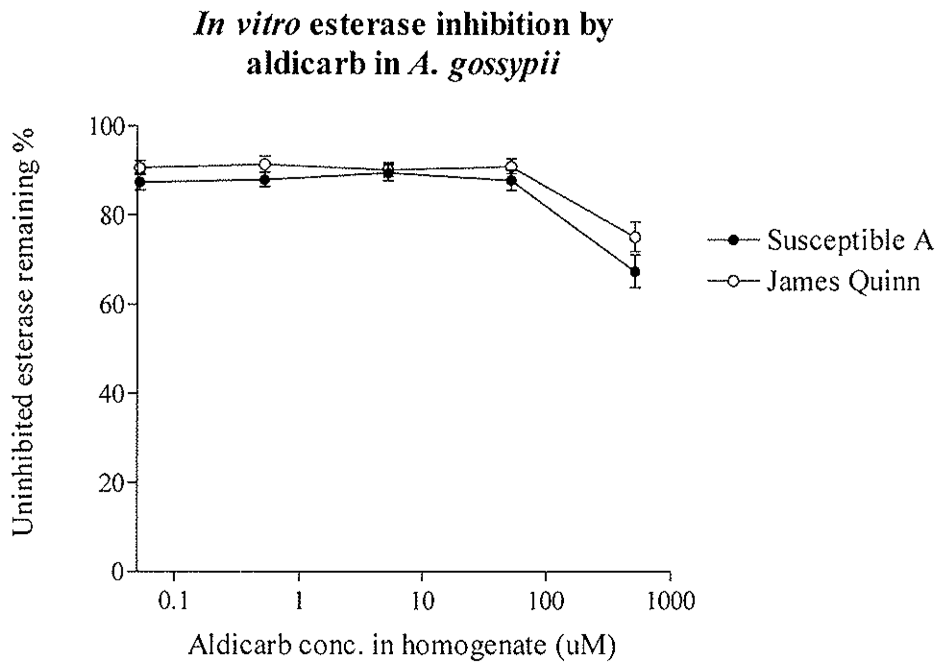


Figure 4.13 *In vitro* esterase inhibition by aldicarb in susceptible and multiple resistant (James Quin) *A. gossypii*

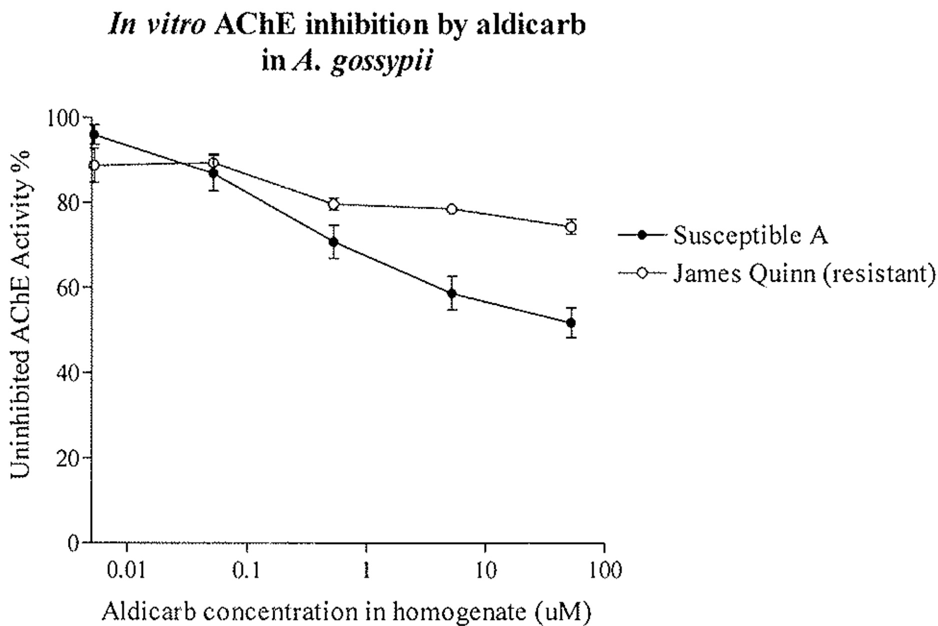


Figure 4.14 *In vitro* AChE inhibition by aldicarb in susceptible and multiple resistant (James Quinn) *A. gossypii*.

Inhibition with aldicarb showed if there is a modified AChE associated with resistance, it is only partially modified (Figure 4.14). At the highest three concentrations tested, there is approximately 20% more inhibition shown in the susceptible A strain as compared to the James Quinn. Esterase inhibition was shown at the highest concentration of aldicarb tested (525 uM in homogenate) but as there was no significant difference between the responses of the two strains (Figure 4.13); it is unlikely that an esterase mediated resistance mechanism is involved.

It appears that the pirimicarb/omethoate/dimethoate insensitive AChE does not confer resistance to aldicarb. There does not appear to be cross-resistance between aldicarb and chlorpyrifos methyl. This supports the concept of moving aldicarb out into its own rotation group independent from the current organophosphate/carbamate rotation group. Although aldicarb can succumb to an aldicarb specific AChE the product is only registered as a furrow treatment at planting. This will restrict use to once a season reducing so selection on the aldicarb specific AChE mechanism.

<u>Aphid AChE Category</u>

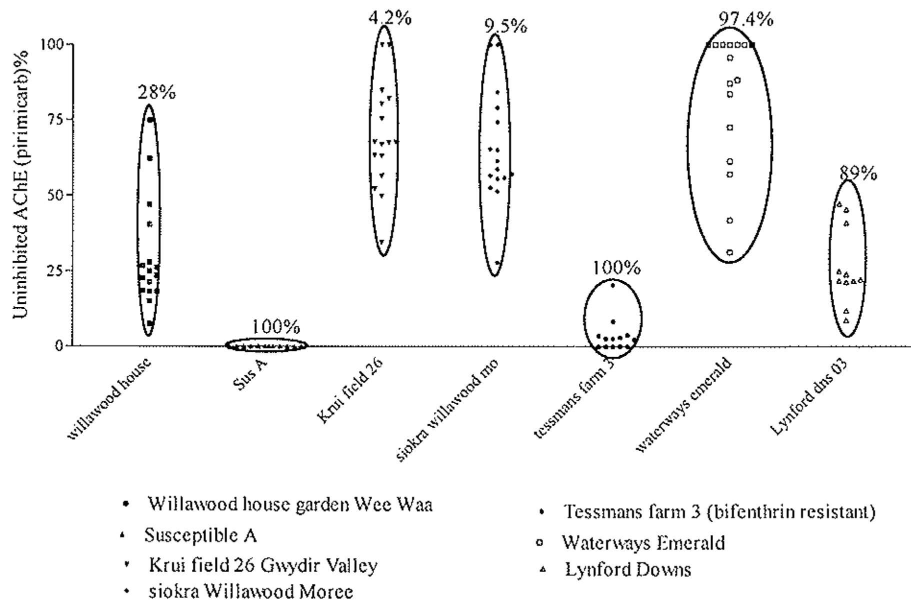
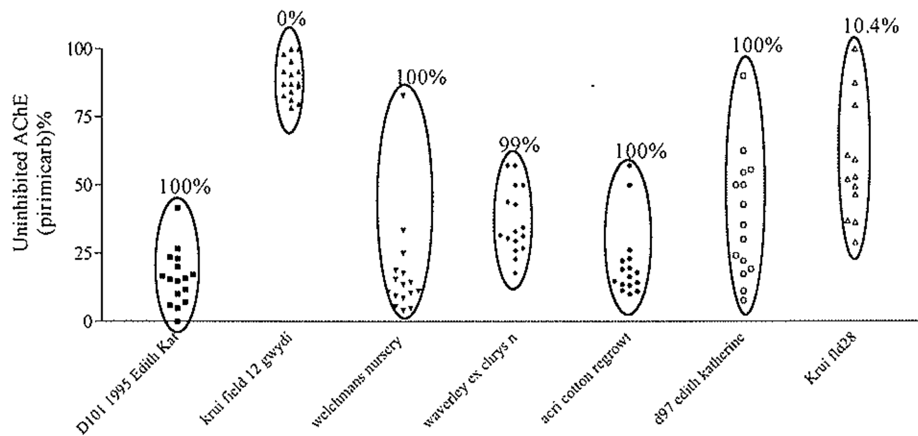


Figure 4.15 Percent uninhibited AChE shown by aphids from a variety of populations with various (percentage) levels of pirimicarb mortality at the discriminating dose



- D101 1995 Edith Katherine
- Krui field 12 Gwydir Valley
- Welchmans nursery
- ACRI Narrabri cotton regrowth
- Waverley ex chrysanthemum Narrabri
- D97 Edith Katherine NT
- Krui field 28

Figure 4.16 Percent uninhibited AChE shown by aphids from a variety of population with various (percentage) levels of pirimicarb mortality at the discriminating dose

Susceptible A, as the baseline susceptible strain, is a homogenous population, with the AChE of all aphids tested being 100% inhibited by pirimicarb (Figure 4.15). In most cases, the variation of aphid clone types within collection is high (Figures 4.15 and 4.16). This supports the notion pirimicarb resistance found in cotton growing areas of Australia will be highly. These results highlight the need for a field kit to test for pirimicarb resistance on a paddock by paddock basis because the variability of resistance levels of different clones within a field population is high.

5. Provide a conclusion as to research outcomes compared with objectives. What are the "take home messages"?

To aid the develop of an effective cotton aphid resistance management strategy

- Mechanism studies were successful and supported the moving of aldicarb out of the current organophosphate / carbamate block into its own rotation group. This has now been included into the current 2004-2005 management strategy published in the Cotton Pest Management Guide.

IRAC Group	Chemical	Sold as
	Aldicarb	Temik
1A	Carbamate	Foliar: Pirimor, Aphidex
1B	Organophosphates	At planting or side dress: Thimet

		Foliar: Dimethoate, omethoate, chlorpyrifos, chlorpyrifos-methyl, parathion-methyl profenofos
4A	Neonicotinoids	Seed treatments: Gaucho, Amparo, Cruiser Foliar: Confidor, Intruder, Actara
12B	Diafenthiuron	Pegasus
2A	Endosulfan	Thiodan *etc*
9A	Pymetrozine	Fulfil

- A rapid field based resistance detection kit continues to provide growers with a reliable method to delineate pirimicarb susceptible and resistant populations. This is particularly important because pirimicarb resistance needs to be assessed on a paddock by paddock basis due to high resistance variability.

6. Detail how your research has addressed the Corporation's three Outputs - Economic, Environmental and Social?

Economic Aphids have the potential to cause substantial loss of yield and lint quality. The capacity to effectively manage these sucking pests is critical to the profitability and competitiveness of cotton production. This mechanism study has increased available chemical rotation groups for aphid control so increasing the capacity for ongoing effective insecticidal control.

Environmental Field control failures caused by resistance invariably lead to an increase in pesticide use as growers struggle for field control. Such sprays may well be inappropriate with potential for adverse environmental impact. The ability to effectively detect and consequently manage resistance in aphids will prevent the unnecessary application of ineffective controls.

Social The key element in resistance management is minimisation of pesticide use. This in turn minimises pesticide residues in food and fibre products ensuring MRLs are not exceeded.

7. Provide a summary of the project ensuring the following areas are addressed:

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.)

b) other information developed from research (eg discoveries in methodology, equipment design, etc.)

c) are changes to the Intellectual Property register required?

Cotton aphid (*Aphis gossypii*) is emerging as a significant problem to the Australian cotton industry. In the past, the cotton aphid has been readily controlled with all products but since

the 1988-2000 season resistance has caused control failures. Resistance is caused by underlying biochemical resistance mechanisms but little was known about resistance mechanisms in Australian cotton aphid populations.

This study found the mechanism of resistance to pirimicarb in the cotton aphid to be a modification of the target site acetylcholinesterase (AChE). As a consequence of identifying the resistance mechanism it was then possible to develop a field based resistance detection kit. This was successfully achieved and involves aphid homogenization and the addition of specific kit ingredients. After some 10-20 minutes a simple colour change may develop; if resistant the test solution turns yellow, if it stays clear the aphid was susceptible. By testing many aphids the proportion of resistant aphids in any population can be determined and appropriate spray decisions made. CGS has expressed interest in commercializing the kit.

Resistance mechanisms to a variety of aphicides were investigated however the result for aldicarb proved most stimulating. The mechanism study found is a partially modified AChE associated with resistance that was different to all others ie aldicarb specific. For this reason aldicarb could be removed from the current carbamate / organophosphate group to its own independent group. So the mechanism study has directly increased the effectiveness of the aphid resistance management strategy.

No changes to the intellectual property register are required.

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.

Further research using synergists to break esterase mediated resistance mechanisms could be undertaken. For example, in future years, if and when current novel chemicals such as diafenthiuron succumb to resistance, and that resistance is proved to be esterase mediated, effective control may be restored by novel use of synergists.

9. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

<ins>Refereed</ins>

Cottage, E.L.A. & Gunning, R.V. (in press). Buprofezin and novaluron inhibit acetylcholinesterase activity in B-biotype *Bemisia tabaci* in Australia. In Fisher, A & Soreq, H (eds) *Cholinergic Mechanisms.*

Cottage, E.L.A. & Gunning, R.V. 2002. Insect growth regulators inhibit acetylcholinesterase activity in B-biotype Bemisia tabaci. In Proceedings of the Brighton Crop Protection Conference – Pests and Diseases.

<ins>Conferences</ins>

Herron,G., Cottage, E., Wilson, L. & Gunning, R. 2004 . Insecticide resistance in cotton aphid (*Aphis gossypii*) : Results and management options after seasons 2002-2003 and 2003-2004, *Proceedings of the 12th Australian Cotton Conference*, August 10 –12, Gold Coast.

Cottage, E.L.A., Gunning, R.V., Balfe, M.E., Herron, G. A. & Moores, G.D. 2003. Field based pirimicarb resistance detection kit for cotton aphid in Australia, *Proceedings of the World Cotton Research Conference - 3, March 9-13, Capetown.*

Cottage, E.L.A. & Gunning, R.V. 2002. Insect growth regulators inhibit acetylcholinesterase activity in B-biotype *Bemisia tabaci*. *Boden Conference: Applied Genomics of Insect Pests in Agriculture, 4th-6th July, Brisbane, Australia.*

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

No

11. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry or the Australian community.

This research will have an immediate and significant impact on the Australian cotton industry. Cotton growers' would normally wait many years and industry spends millions of dollars to bring new chemistry for aphid control to market. This project has within two years and for very little money done just that; it has delivered the cotton industry an additional rotation group for aphid control and the bonus resistance detection kit for field use.