Part 1 - Summary Details

CRDC Project Number: **DAN162**

Project Title: Insecticide resistance management in *Bemisia tabaci*

Project Commencement Date: 1/07/2002 **Project Completion Date:** 30/06/2005

CRDC Program: On-Farm

Part 2 – Contact Details

Administrator: Mr Graham Denny
Organisation: NSW Department of Primary Industries
Postal Address: LMB 21 Orange, NSW 2800
Ph: (02) 6391 3219 **Fax:** (02) 6391 3327 **E-mail:** graham.denny@dpi.nsw.gov.au

Principal Researcher: Dr Robin Gunning
Organisation: NSW Department of Primary Industries
Postal Address: Tamworth Agricultural Institute, 4 Marsden Park Road, Calala, NSW 2340
Ph: (02) 6763 1128 **Fax:** (02) 67 63 1222 **E-mail:** robin.gunning@dpi.nsw.gov.au

Supervisor: Dr Bob Martin
Organisation: NSW Department of Primary Industries
Postal Address: Tamworth Agricultural Institute, 4 Marsden Park Road, Calala, NSW 2340
Ph: (02) 6763 1128 **Fax:** (02) 67 63 1222 **E-mail:** bob.martin@dpi.nsw.gov.au

Signature of Research Provider Representative: ______________________________

Part 3 – Final Report Guide (due 31 October 2008)

Background

The cotton whitefly *Bemisia tabaci* is a serious pest of fibre, horticultural and ornamental crops world -wide. When present in sufficient numbers, it can cause extensive damage through direct feeding, the production of large quantities of honeydew and as a vector of many viruses. Comparatively recently, a new biotype was identified in the USA, known as the B-type or poinsettia strain. Overseas, the B-type *B, tabaci* is a primary pest on cotton, other vegetable crops (curcubits, tomatoes, rock melons) and ornamentals. This strain is extremely virulent, highly insecticide resistant, adapts to temperate climates and has a host range of over 500 plants.

B-type *B. tabaci* was detected (by Robin Gunning, NSW DPI) for the first time in Australia in October 1994. This whitefly is widely distributed over eastern Queensland and NSW and the Darwin area of the NT and is a major pest of horticultural crops. B-biotype *B. tabaci* has been progressively increasing on NSW and Queensland cotton since 1995 . In the 2001/2002 season, silverleaf whitefly exploded on cotton at Emerald in Queensland with primary pest status with other cotton areas in Qld. and NSW at risk Insecticides are a major weapon against this insect in Australia, however, insecticide resistance is a major problem in the silverleaf whitefly. It is essential that the use of all chemicals is carefully managed to minimise or avoid resistance problems. This can only be achieved by establishing effective resistance detection and monitoring techniques and understanding the underlying mechanisms of resistance.

Objectives

Objectives Year 1:

To establish the insecticide resistance status of *B. tabaci* populations on cotton and to put into place a cotton resistance management strategy for B-biotype *B. tabaci*. *Achieved*

- To study in detail, the rate of insecticide resistance selection with a strategy which involves rotation of a number of insecticides with differing modes of action. *Achieved with insect growth regulators*
- To complete studies on insecticide resistance in endosulfan and bifenthrin. *Achieved with bifenthrin,, recent restrictions on endosulfan use made it impractical for whitefly control*.
- To monitor *B. tabaci* population numbers on cotton. *Achieved*

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Objectives Year 2:

- To monitor the insecticide resistance status of *B. tabaci* populations on cotton and to refine the resistance management strategy for cotton populations. *Achieved*
- To investigate the efficacy of piperonyl butoxide as an insecticide synergist in the management of resistant B-biotype *B. tabaci*. *Achieved*
- To complete studies on cross-resistance between insect growth regulators in B- iotype *B. tabaci. Achieved*
- To monitor *B. tabaci* population numbers on cotton. *Achieved*

Objectives Year 3:

- To monitor the insecticide resistance status of *B. tabaci* populations on cotton and to refine the resistance management strategy for cotton populations. *Achieved*

- To complete studies of amitraz resistance in B-biotype *B. tabaci*. *Workload of whitefly identifications has delayed resistance mechanism work, however, amitraz resistance has been identified in field populations of silverleaf whitefly.*
- • To investigate synergists to counteract B-biotype *B. tabaci* resistance to insect growth
- regulator. *Achieved*
- • To monitor *B. tabaci* population numbers on cotton. *Achieved*

Methods

4.1 Whitefly Identification

The project used established electrophoretic techniques to distinguish between *B. Tabaci* biotypes. The method exploited biotype distinctive esterase ectomorphs (see, for example, Brown, J. K. *et al* (1996) – The relevance of variability within the *Bemisia tabaci* complex…*In* Gerling, D. & Mayer, R.T. *Bemisia 1995: Taxonomy, biology, damage, control and management,* Intercept, Andover.). Esterase ectomorphs separated by polyacrylamide gel electrophoresis and visualised by traditional biochemical esterase staining method (Fig.1). The detection of this esterase protein polymorphism provided a valuable marker to facilitate tracking of *B. biotype B. tabaci* as it spread round the world (Brown, J. K. *et al* (1996). (While DNA level poymorphism can be also detected between the biotypes, gene probes cannot approach the ease, economy, speed and throughput of the electrophoretic methods).

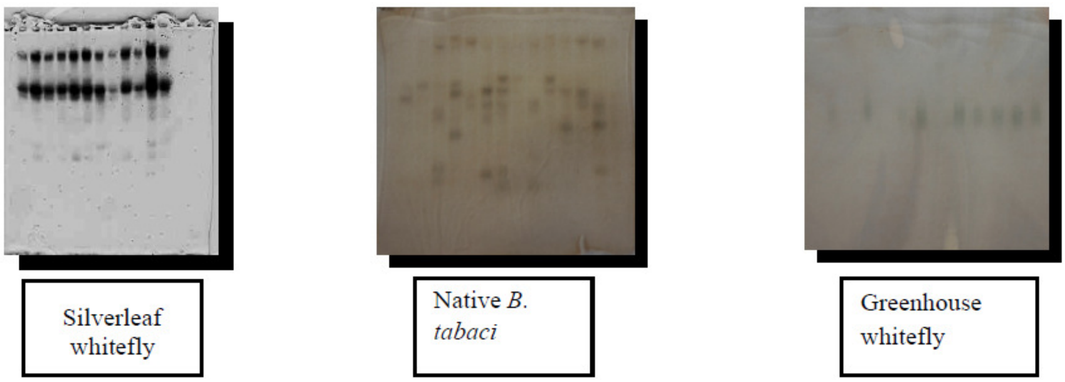

***Figure** 1 Polyacrylamide gel electrophoresis of whitefly species (each track represents 0.75 whitefly).*

A polyacrylamide gel electrophoresis method for esterase was used to identify *B. Tabaci* biotypes. Individual adult or immature whiteflies 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 pipette 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. Specially designed gel combs that cast wells with 4.5mm spacing in the stacking gel were used.

The gels were run at 250V maximum current for 90 minutes at 5°C . Gels were stained for esterase activity, using 0.5mM α-naphthyl butyrate and 0.2% Fast Blue RR in 0.02M phosphate buffer pH 6.0. Gels were fixed in 5% acetic acid. Electrophoretic mobility's (R_m)

ratios were measured as the distance that esterase bands travelled down the gel, relative to the buffer interface.

4.2 Total esterase assay

Samples from each whitefly population containing 50 adult *B. tabaci* were homogenised in 1mL of 0.02M pH 7.0 phosphate buffer containing 0.05% Triton X-100 (20µL/whitefly). 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 butyrate. Kinetic assays were performed at 25°C, using a microplate reader and Kinetic Collector 2.0 software. 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.

4.3 Leaf dip" bioassay technique for contact insecticides

The leaf dip bioassay method used for testing contact insecticides against *B. tabaci* was similar to that used at Rothamsted Research. Cotton plants were grown in the glasshouse without any exposure to insecticides. Leaf discs were cut and dipped into aqueous solutions of insecticide containing 0.01% Agral® surfactant and allowed to dry at 25ºC. Control leaves were dipped in Agral and distilled water only. Leaf squares were placed adaxial side down on a bed of agar gel (0.5%) in plastic bioassay trays.

Female adult whiteflies of required strains were captured using an aspirator, temporarily anaesthetised with carbon dioxide and placed on the cotton leaf squares. Twenty whiteflies were placed on each cotton leaf square and bioassay trays were sealed with a breathable clear plastic cover (BIO-CV-4, C-D International, Inc.). The whiteflies were allowed to feed on the leaf squares and were assessed at maximum mortality (24 or 48 hours).

4.4 Bioassay technique for insect growth regulators

Small leaf cages, containing 20 adult *B. tabaci*, were attached to cotton leaves of plants in glasshouse cages. Leaf cages were used to encourage oviposition in a concentrated area of the leaf and give a better distribution of eggs. After oviposition, the whitefly in the leaf cages were removed so that no more eggs would be laid, thus ensuring that resultant nymphs were at the same developmental stage. Immature *B. tabaci* on leaves were counted and then the leaves were dipped into formulated insecticide and Agral® solutions (to ensure wetting).

Mortality was assessed 20 days after oviposition, by counting the number of living nymphs.

4.5 Field Studies

Field experiments comprise small scale, replicated, random block, spray trials.

Results

5.1 Insecticide Resistance in the Silverleaf whitefly

Populations of whiteflies were collected from Emerald, during cotton season bioassayed with insecticides that the whiteflies had been exposed to. Resistance data, 2002 – 2005, are plotted as resistance factors in Figure 2. (Resistance factors were calculated with reference to a native strain of *B. tabaci* collected from Darwin, NT). Data showed that there was resistance to all the insecticides tested, with exception of pymetrozine and chlorpyrifos. In some, namely the pyrethroids and the insect growth regulator buprofezin, resistance factors were several hundred fold.

Previous studies (of Robin Gunning, Mathew Cahill and Emma Cottage), have shown that the silverleaf whitefly entered Australia with pre-existing resistance to many insecticides. Insecticide use against the silverleaf whitefly on horticultural crops in Queensland selected for resistance to insecticides to which the silverleaf whitefly had been originally been susceptible. As the data from this project show, resistance limitation in the silverleaf whitefly at Emerald has proved to be difficult.

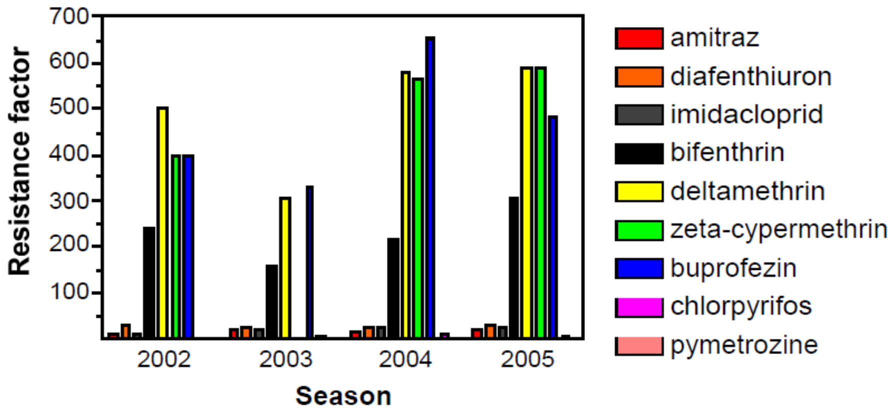

Figure 2*. Insecticide resistance in silverleaf whitefly populations from Emerald.*

5.2 Detailed Studies of Insect Growth Regulator Resistance

The basis of successful silverleaf whitefly on cotton overseas (Israel and USA) has been the use of insect growth regulators against early season silverleaf whitefly, preventing the explosive population build-ups that are so damaging to cotton lint. Two insect growth regulators, with differing modes of action, have been used against the silverleaf whitefly on cotton in central Queensland, first, buprofezin and then pyriproxyfen. (Buprofezin was withdrawn from use by Dow because of resistance concerns). Research by Robin Gunning and Mathew Cahill in 1995, demonstrated that silverleaf whitefly had entered Australia with a low level of resistance (5 fold) to buprofezin. Emma Cottage and Robin Gunning later confirmed a 5-10 fold resistance in field populations of the silverleaf whitefly that had never been treated with insect growth regulators. Given resistance, albeit at a low level, the first priority was to monitor insect growth regular resistance at Emerald.

Silverleaf whiteflies were collected from Emerald, in during cotton seasons before and after insect growth regulator use. Whiteflies were cultured on cotton and pyriproxyfen was bioassayed against immature whiteflies. Initial studies during the 2002/3 season, showed that even with very limited use of buprofezin there had a strong selective effect (Fig 3). In addition resistance was also resistance detected to pyriproxyfen although there had been no use of pyriproxyfen (Fig. 4).

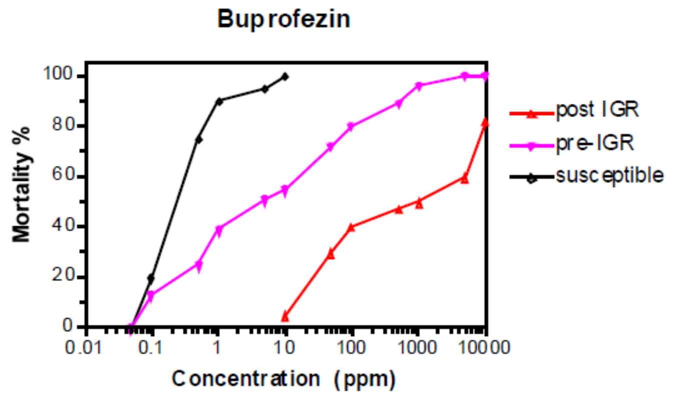

Figure 3. *Effect of field selection with buprofezin on buprofezin resistance levels in populations of silverleaf whitefly at Emerald 2002*

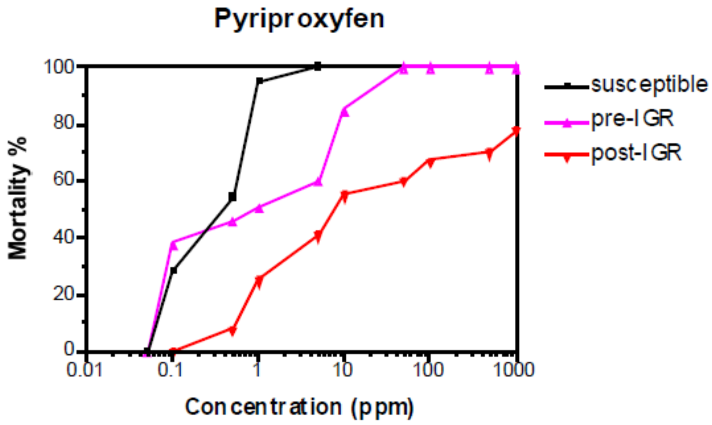

***Figure** 4 Effect of field selection with buprofezin on pyriproxyfen resistance levels in populations of silverleaf whitefly at Emerald 2002.*

These data (Figs 3 and 4), and Emma Cottage's PhD studies, suggested that there was crossresistance between the two insect growth regulators (buprofezin and pyriproxyfen), in the silverleaf whitefly. Data from buprofezin and pyriproxyfen sprayed field populations in 2003 (Figs 5 and 6, Tables 1 and 2) also indicated that exposure from one insect growth regulator selected for resistance to the other.

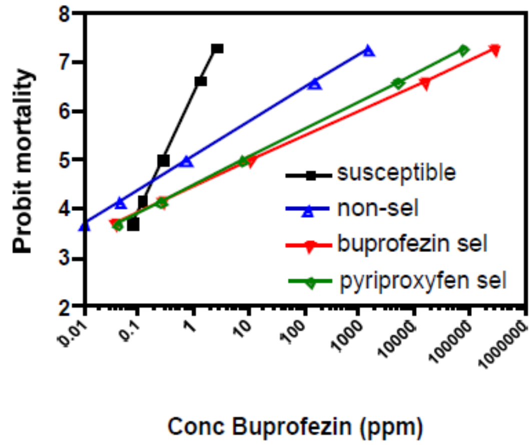

Figure *5 Response off Emerald silverleaf whitefly (unselected, field buprofezin selected and field pyriproxyfen selected) to buprofezin 2003*

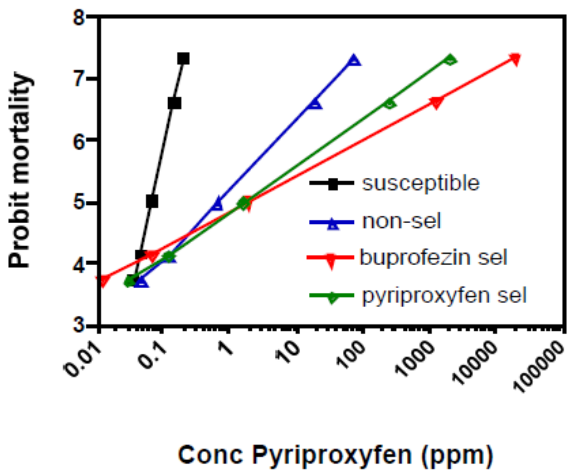

Figure *6 Response of Emerald silverleaf whitefly (unselected, buprofezin selected and field pyriproxyfen selected) to pyriproxyfen,2003.*

Table *1 Response of Emerald silveleaf whitefly to Pyriproxyfen after field selection with Buprofezin or Pyriproxyfen*

Strain	Slope	X^2	LC_{50} ppm (95% fiducial limits)	Resistance factor* LC_{50}	LC_{99} ppm (95% fiducial limits)	Resistance factor* LC_{99}
susceptible		0.001	0.066 (0.057 – 0.070)	1	0.197 (0.120 – 0.320)	1
Field Non-selec ted	1.3	12.6	0.64 (0.50 – 1.1)	7.7	73.8 (17 –315)	375
Field buprofezin sel.	0.60	38	1.89 (1.3 – 4.2)	28.7	19056 (1755 – 206469)	96730
Field pyriproxyfen sel	0.7	4.6	1.6 (1.3 – 3.2)	24.2	2024 (339 – 12079)	10274

Table 2 Response of Emerald silveleaf whitefly to Buprofezin n after field selection with Buprofezin or Pyriproxyfen.

Strain	Slope	X^2	LC_{50} ppm (95% fiducial limits)	Resistance factor* LC_{50}	LC_{99} ppm (95% fiducial limits)	Resistance factor* LC_{99}
susceptible	2.5	6.7	0.26 (0.23 – 0.31)	1	2.3 (1.7 – 3.2)	1
Field Non-selec ted	0.7	14.2	0.74 (0.23 – 3.3)	2.9	1492 (86 - 2561)	649
Field buprofezin sel.	0.52	41	11.3 (5.4 – 23.7)	43.5	310663	51750
Field pyriproxyfen sel	0.6	45	7.6 (3.7 – 15.2)	29.3	792 50 (10607 – 992106)	34456

Definitive proof, however, was obtained in the laboratory, by selecting populations that had never been exposed to insect growth regulators. The B-biotype *B. tabaci* were selected with two consecutive sprays of pyriproxyfen or buprofezin (at the LC_{80} level). Surviving whiteflies were bred to form buprofezin or pyriproxyfen selected populations and bioassayed.

Data (Figs. 5 and 6 and Tables 3 and 4), indicate that selection with either insect growth regulator, produced high levels of resistance to both buprofezin and pyriproxyfen. Thus, cross-resistance was confirmed. There were no significant differences in selecting pressure between buprofezin and pyriproxyfen.

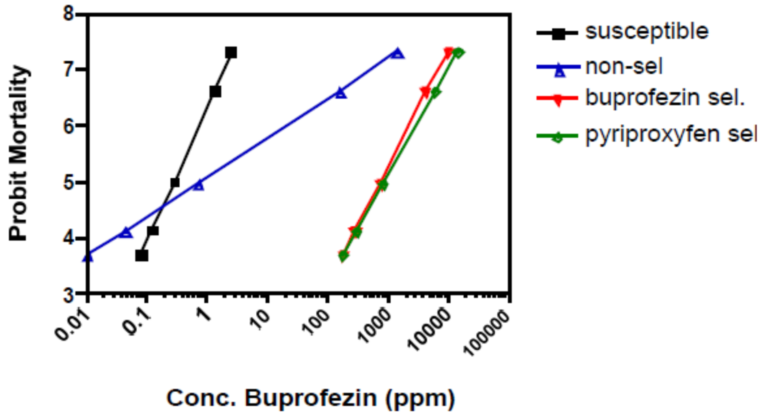

Figure 7 Response of silverleaf whitefly to Buprofezin after laboratory selection by buprofezin or pyriproxyfen.

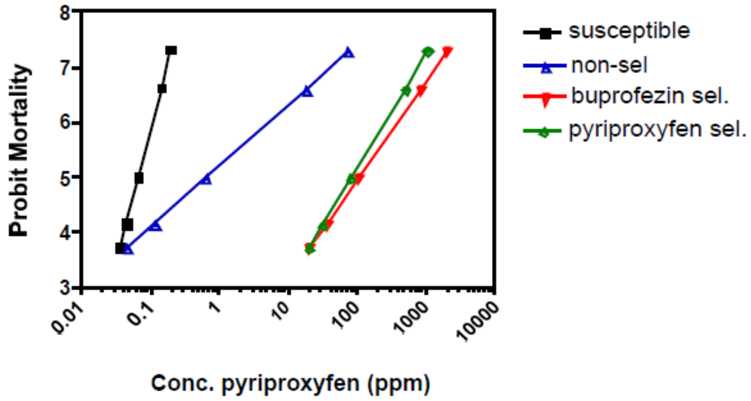

Figure 8 Response of silverleaf whitefly to Pyriproxyfen after laboratory selection by buprofezin or pyriproxyfen.

Table 3 *Response of silverleaf whitefly to Pyriproxyfen after laboratory selection with Buprofezin or Pyriproxyfen*

Strain	Slope	X^2	LC_{50} ppm (95% fiducial limits)	Resistance factor* LC_{50}	LC_{99} ppm (95% fiducial limits)	Resistance factor* LC_{99}
susceptible	4.9	0.001	0.066 (0.057 – 0.070)	1	0.197 (0.120 – 0.320)	1
Field Non-selec ted	1.3	12.6	0.64 (0.50 – 1.1)	2.8	73.8 (17 –315)	27.1
lab buprofezin sel.	1.8	23.6	106.1 (68.7– 163.8)	393	2035 (708 - 5847)	748
Lab pyriproxyfen sel	2.0	4.9	82.4(69.8 – 97.2)	305	1071 (719 – 1595)	394

Table 4 *Response of silverleaf whitefly to Buprofezinn after laboratory selection with Buprofezin or Pyriproxyfen*

Strain	Slope	X^2	LC_{50} ppm (95% fiducial limits)	Resistance factor* LC_{50}	LC_{99} ppm (95% fiducial limits)	Resistance factor* LC_{99}
susceptible	2.5	6.7	0.26 (0.23 – 0.31)	1	2.3 (1.7 – 3.2)	1
Field Non-selec ted	0.7	14.2	0.74 (0.23 – 3.3)	2.8	1492 (86 - 2561)	649
Lab buprofezin sel.	2.1	21.6	766 (519 - 1130)	2946	10358 (4085 – 26264)	4503
Lab pyriproxyfen sel	1.9	9.6	836.3 (657 - 1132)	3216	14834 (7737 - 28457)	6450

Buprofezin and pyriproxyfen cross-resistance is as a result of a common resistance mechanism, namely, sequestration by esterase iso-enzymes (this mechanism was discovered by Emma Cottage in her PhD studies). Given that IGR's are considered vital for management of silverleaf whitefly, it will be important to determine whether cross-resistance extends to other insect growth regulators.

5.3 Pyriproxyfen Resistance Monitoring

Following the withdrawal of Buprofezin from field use, insect growth regulator resistance monitoring was restricted to pyriproxyfen. Data (Fig. 9, Table 5) showed that each year, only one use of pyriproxyfen per season, at produced a very strong selective effect on the surviving generation, increasing resistance, compared to pre-spray levels. The post insect growth regulator use resistance levels were so high, as to suggest that consecutive generations of silverleaf whitefly were receiving pyriproxyfen selection. Thus, is likely that the threshold based initiation of insect growth use is leading to a longer use period at Emerald, than is desirable, is compromising area-wide management It is, however, very fortunate that between cotton seasons, insect growth regulator resistance levels decline markedly, perhaps indicating lack of fitness. In 2004, after insect growth regulator use, pyriproxyfen resistance was extreme and coincided with reports of very poor control at Emerald with pyriproxyfen.

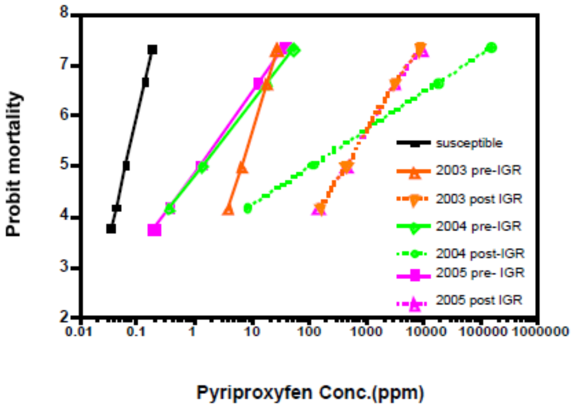

Figure 9 *Response of Emerald silverleaf whitefly to pyriproxyfen 2002/3 –2004/5*

Table 5 *Response of Emerald Silverleaf whitefly to pyriproxyfen, pre and post IGR use, 2003 - 2005*

Strain	Slope	X^2	LC_{50} ppm (95% fiducial limits)	Resistance factor* LC_{50}	LC_{99} ppm (95% fiducial limits)	Resistance factor* LC_{99}
susceptible	4.9	0.00 1	0.066 (0.057 – 0.070)	1	0.197 (0.120 – 0.320)	1
Field pre-IGR 2003	3.8	0.02	6.9 (5.4 – 8.6)	105	28.3 (11.4 – 70.7)	147
Field post-IGR 2003	1.8	16	475 (296– 759)	7197	8920 (2390 - 33285)	44600
Field pre-IGR 2004	1.5	7.8	1.5 (1.0 – 2.1)	22.8	57.9 (23 –145)	290
Field post- IGR 2004	0.7	4.2	118 (54 – 257)	1787	157316	786580
Field pre- IGR 2005	1.6	4.3	3.8 (0.9 – 1.8)	57.6	39 (15 – 100)	195
Field post-IGR 2005	1.8	11.1	475 (296– 759)	7196	8920 (2390 - 33285)	44600

5.4 Silverleaf Whitefly Resistance Management Strategy

Based on the insecticide resistance data, a resistance management strategy was formulated, in collaboration with the central Queensland cotton industry. Key features of the strategy are a "windows approach for insect growth regulators" to chemical use and an area-wide flexible start date (based on thresholds). There is "one spray only" use of insect growth regulators after the whiteflies reach spray thresholds. Other guidelines are:

- All insecticides to be used with the context of the current *Helicoverpa* and aphid and mite resistance management strategies.
- Resistance management needs to be undertaken on an area-wide basis.
- Avoid use of broad spectrum pesticides in the early season.
- Do not use consecutive applications of any chemistry against silverleaf whitefly

As noted above, the flexible start date to the insect growth use period may be leading to a too long a use period and selection of more than one generation of the silverleaf whitefly with pyriproxyfen.

5.5 Bifenthrin Resistance in the Silverleaf Whitefly

When silverleaf whitefly first entered Australia, while resistant to most pyrethroids, they were susceptible to bifenthrin. Resistance, however, was rapidly selected for on horticultural crops. Our project studies show, that bifenthrin resistance in the silverleaf whitefly appears primarily due to hydrolysis and sequestration by esterase isoenzymes (Figure 6), with strong binding of esterase to bifenthrin in resistant silverleaf whitefly.

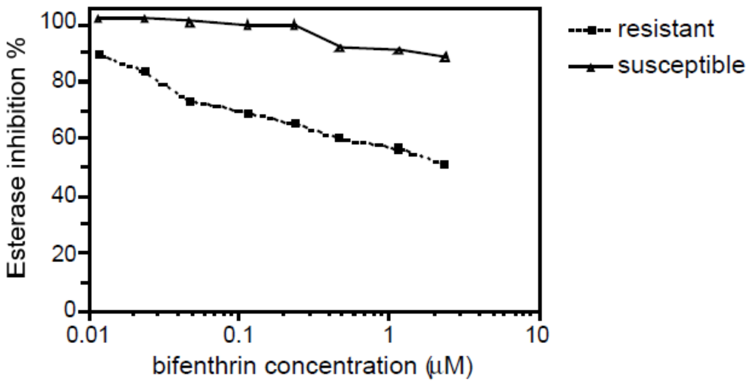

Figure 10 *In vitro inhibition of silverleaf whitefly esterase by bifenthrin in resistant and susceptible strains.*

5.6 Whitefly surveys on Cotton

Control of the silverleaf whitefly is dependent on early detection of any increase, in whitefly numbers. All cotton growing areas in Australia are at risk from the silverleaf whitefly. As a consequence, whitefly numbers were monitored on cotton in most cotton growing areas (with the exception of central Queensland, where Silverleaf whitefly, continues to be abundant).

Randomly selected cotton leaves (collected by cotton industry personnel) were sent to Tamworth for whitefly counts and whitefly identification. Over the course of the project some 25,000 whiteflies have been identified and this has become a major use of the project resources.

In 2002/3, while actual whitefly numbers relatively low, in terms of the whitefly population dynamics, the proportion of silverleaf whitefly on cotton was increasing at the expense of native *B. tabaci* and the greenhouse whitefly (Figures 11 and 12). Areas of particular concern were the Macintrye Valley, the Burnett and Namoi Valley. In the Macintyre Valley the mean number of silverleaf whitefly/leaf exceeded 1 per leaf.

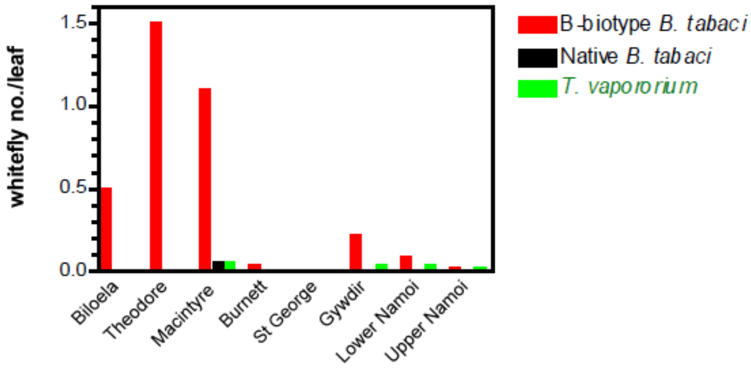

Figure 11. *Whitefly abundance on cotton 2002/3*

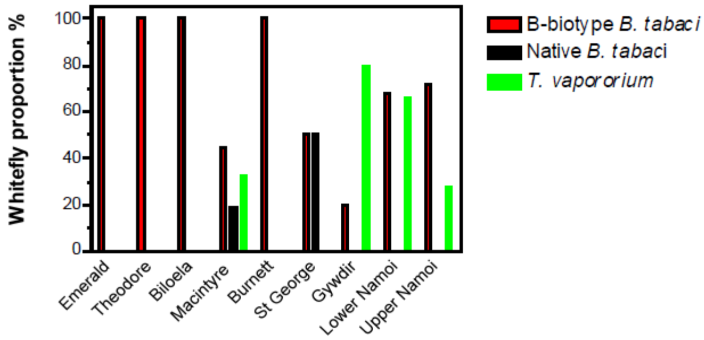

Figure 12 *Species composition of whitefly population on cotton 2002/3*

The 2003/2004cotton season, was distinguished by considerable whitefly pressure on cotton in districts, that had not previously been troubled by whiteflies. In some areas (Gwydir and Macintyre Valleys), whitefly control was considered necessary data in Fig. 13, show that B-type *Bemisia tabaci* (the silverleaf whitefly) was the predominant (50 – 80% of the whitefly population) whitefly species found on cotton in the Gwydir, Macintyre, Upper Namoi, Macquarie and St George in early 2004.
In the Lower Namoi Valley*, Trialerodes vapororium*, the greenhouse whitefly, was the dominant species, with silverleaf whitefly comprising only ~ 25% of the population. There were insufficient whiteflies sent to for identification to make an accurate estimate of species composition in other areas. Numbers of all whitefly types (Fig. 14) found on cotton, were greatly increased over previous years. Areas with particularly high numbers were the Gwydir, Macintrye and Upper Namoi.

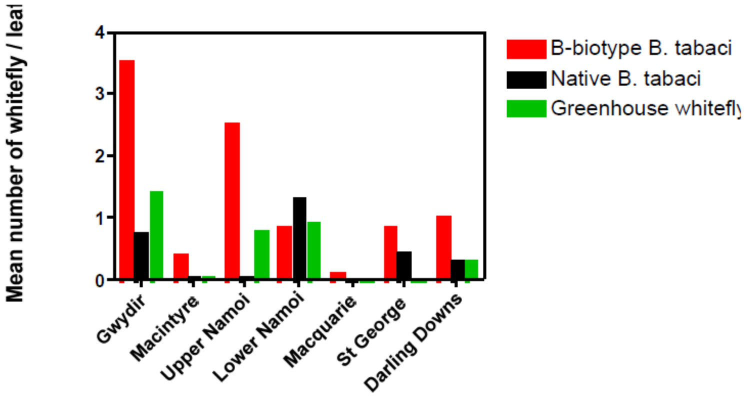

Figure 13. *Number of whiteflies during the 2003/2004, cotton season in Queensland and New South Wales*

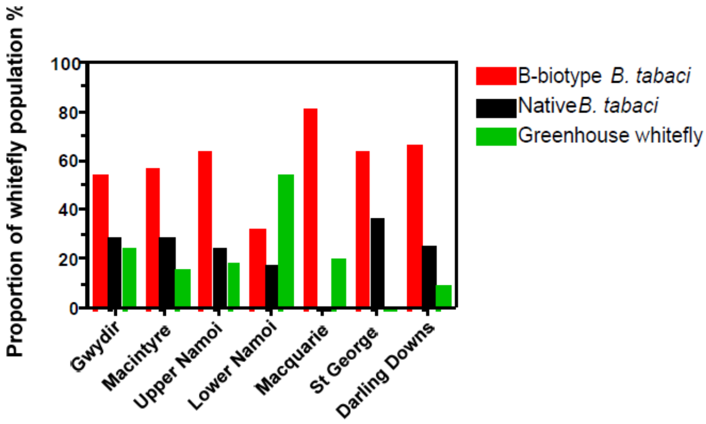

Figure 14 *Whitefly species composition on cotton in Queensland and New South Wales during the 2003/2004 cotton season*

In 2004/05, with the exception of the Macintyre valley, very few collections of whiteflies were made. Data (Figs. 15 and 16) show that silverleaf whitefly numbers were exceeded by native *B. tabaci* and the Greenhouse whitefly in the Macintyre, Upper and lower Namoi and Gwydir Valleys.

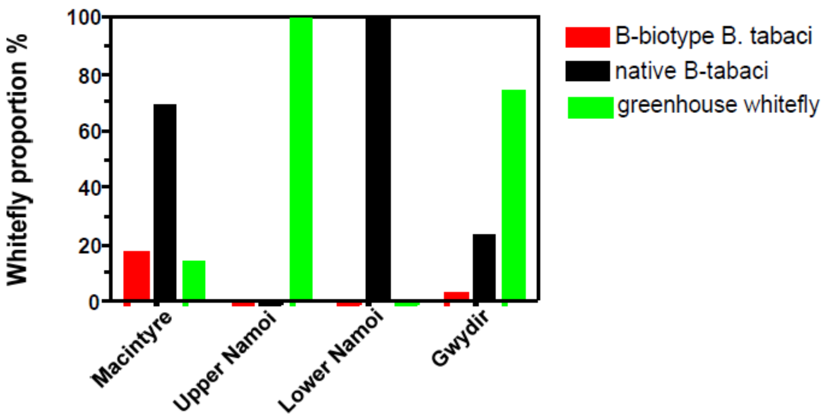

Figure 15 *Whitefly species composition on cotton 2004/2005*

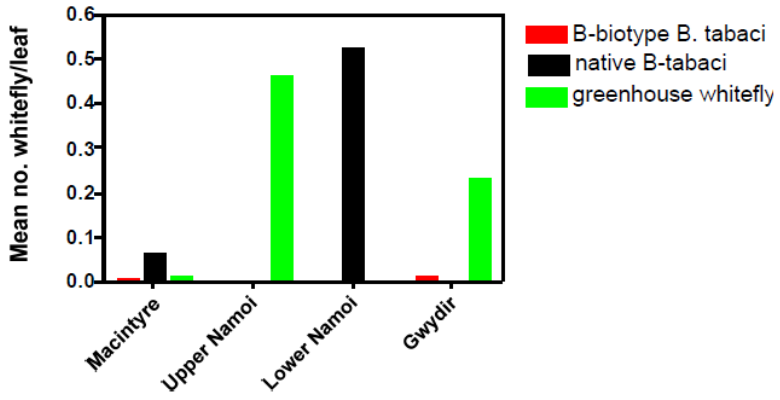

Figure 16. *Whitefly numbers on cotton 2004/2005*

5.7 In Depth Analysis of whitefly populations in the Macintyre IPM Area
Weekly sampling of cotton leaves for whitefly analysis in the Macintyre IPM area enabled a more detailed study of whitefly population dynamics in this area (Figs .17, 18, 19 and 20).

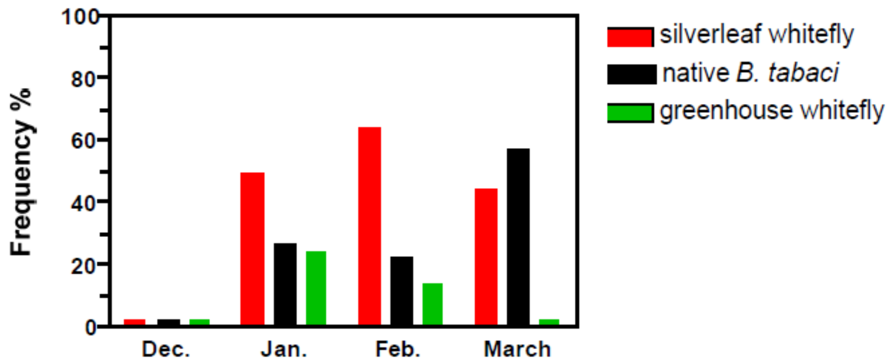

Figure 17. *Analysis of whitefly species composition in the Macintyre Valley 2003/2004*

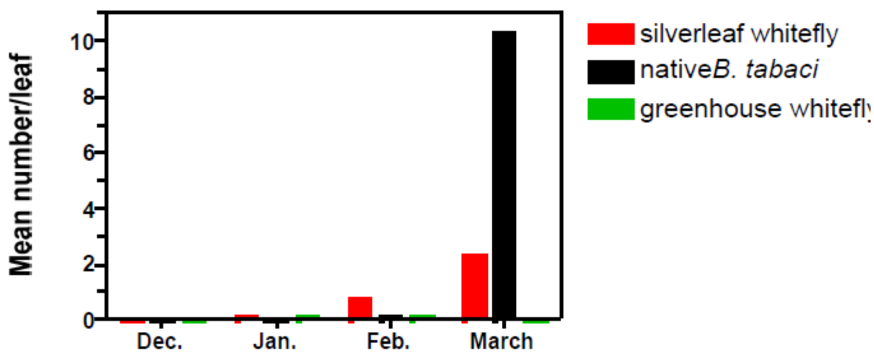

Figure 18. *Whitefly numbers in the Macintyre Valley 2003/2004*

In the 2003/2004 cotton season (Figs. 17 and 18), in the Macintyre Valley, whiteflies were detected in very low frequencies during December and January but the dominant whitefly was the silverleaf whitefly (~50%), . Native *B. tabaci* and green house whitefly comprised the remainder of the population. During February, the proportion of silverleaf whitefly increased to 70% (~ 1 whitefly/leaf) and in March, silverleaf whitefly numbers rose, exceeding 2 silverleaf whitefly/leaf (45% of population). The remainder of the large whitefly population was native *B. tabaci*. Late season, *B. tabaci* numbers sometimes exceeded thresholds and control was considered necessary.

The 2004/2005 season (Figs 19 and 20), saw reduced whitefly numbers in the Macintyre Valley with native *B. tabaci* being the main whitefly type detected.

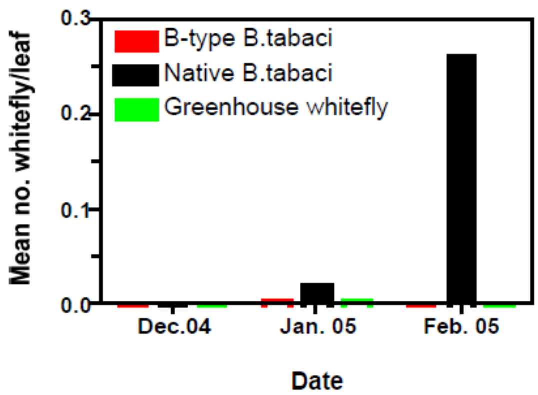

Figure 19. *Whitefly numbers in the Macintyre 2004/2005*

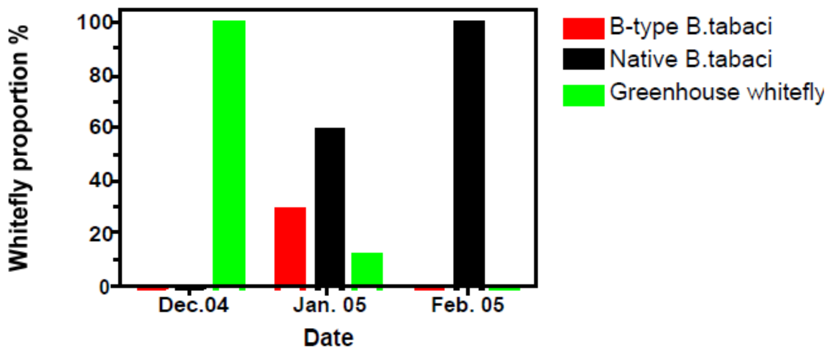

Figure 20. *Analysis of whitefly species composition in the Macintyre Valley 2004/2005*

The Macintyre Valley data show that Greenhouse whitefly, silverleaf whitefly and native *B. tabaci* can co-exist on cotton. Rapid population expansions of silverleaf whitefly numbers is normally triggered by use of broad spectrum pesticides which kill predator and parasites and the other whitefly the other, insecticide susceptible whitefly types. Given that silverleaf whitefly was less evident on cotton in 2004/2005, compared to other whitefly types, it is likely that non-sprayed Bollgard II cotton has had an impact on whitefly population dynamics. The data, however, show that numbers of silverleaf whitefly are variable and these may also be influenced by climatic conditions. It is therefore essential that monitoring of whitefly populations on cotton be continued.

5.8 Investigation of the efficacy of piperonyl butoxide as an insecticide synergist in the management of pyrethroid resistant B-biotype B. tabaci

Studies in collaboration with Dr Graham Moores (Rothamsted Research), have resulted in significant improvements in the efficacy of piperonyl butoxide (PBO), as an insecticide synergist against silverleaf whitefly.

Pyrethroid resistance in *B. tabaci* is caused by metabolism by esterase iso-enzymes. PBO is an esterase inhibitor in the silverleaf whitefly, however, inhibition takes some hours and this prevents significant when PBO is used in tank mixes with pyrethroids. Previous work showed that split PBO/insecticide application (with prior application of PBO) in the field, gave control of highly pyrethroid resistant B-biotype *B. tabaci*. However, spraying twice (especially by air), is not cost effective. A solution, which would allow effective synergism in PBO/insecticide tank mixes, is microencapsulation of insecticides, to delay release, to allow the factors causing resistance to be effectively inhibited, prior to insecticide release. Control of resistant silverleaf whitefly, can achieved by microencapsulation of insecticides to delay release until the synergist has effectively suppressed the factors causing resistance, (thus, allowing insecticide use in a tank mix with PBO). This concept has been patented by NSWDPI and Rothamsted Researh (UK Patent No. 0309773.0, PCT/GB2003/001861).

In collaboration with Endura SpA, Italy, some experimental, microencapsulated pyrethroids were developed; giving delayed, burst release of pyrethroid insecticides. Microencapsulated alapha cypermethrin and lambdacyhalothrin were tested in a mix with PBO in the field at Emerald, against highly, pyrethroid resistant silverleaf whitefly. Outstanding control was achieved (Figs. 21 and 22). A process is underway to commercialise this technology with a commercial partner.

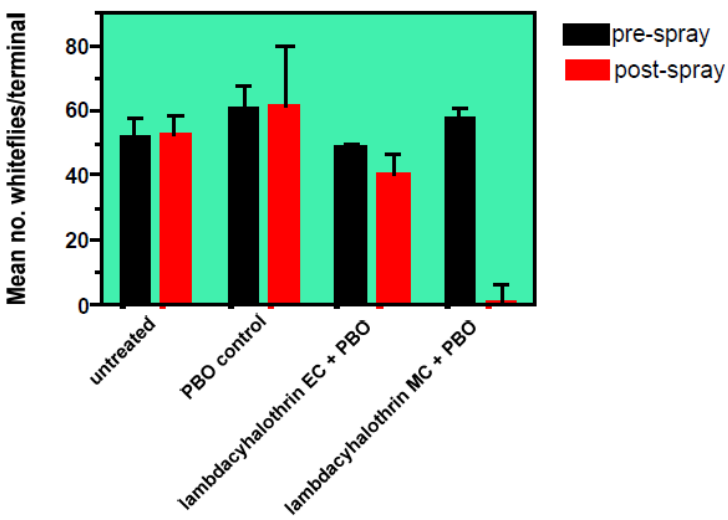

Figure 21. *Lambdacyhalothrin MC (5h delay in release)/PBO mix against pyrethroid resistant adult silverleaf whitefly on cotton at Emerald, compared to a conventional mix of immediately available lambdacyhalothrin EC plus PBO.*

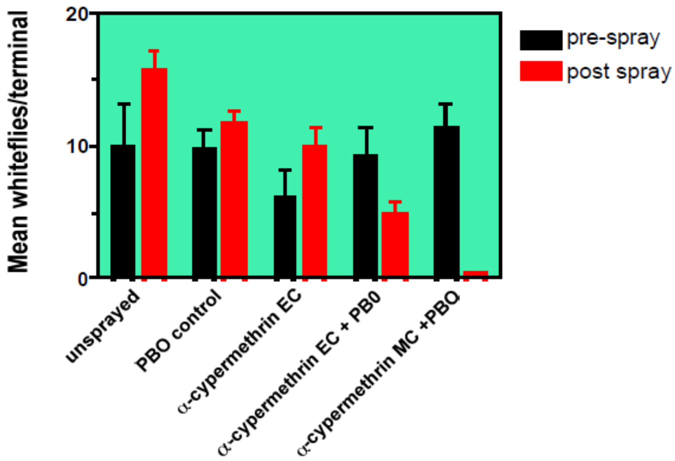

Fig. 22. *Alpha-cypermethrin MC (5h delay in release)/PBO mix against pyrethroid resistant adult silverleaf whitefly on cotton at Emerald, compared to a conventional mix of immediately available Alpha-cypermethrin EC plus PBO.*

5.9. Synergism Of Insect Growth Regulators To Manage Resistance In The Silverleaf Whitefly

Emma Cottage's PhD studies indicated that resistance to the insect growth regulator buprofezin was due metabolism by esterase-isoenzymes and that the resistance mechanism conferred cross-resistance to pyriproxyfen. This project has confirmed the cross-resistance. Since the successful use IGR pyriproxyfen is considered vital for the control of the silverleaf whitefly on cotton, and that pyriproxyfen resistance is affecting field performance of pyriproxyfen, we investigated synergism as a means to overcome resistance. Piperonyl butoxide was chosen as the synergist because we have shown, in previous work, that it inhibits esterase in the silverleaf whitefly.

Emerald pyriproxyfen resistant Silverleaf whitefly nymphs were bioassayed with pyriproxyfen after exposure (by leaf dip) to formulated piperonyl butoxide (0.1%, which is within registered rates on cotton). Data (Fig. 23, Table 6) indicated that PBO completely synergised pyriproxyfen and suppressed resistance. The use of microencapsulated formulation, to delay pyriproxyfen release, until PBO inhibited the enzyme causing resistance is being pursued in collaboration with Endura SpA. Application of the technolgy to pyriproxyfen resistance problems could give effective control of the silverleaf whitefly and, as well, add considerable value to the microencapsulation patent.

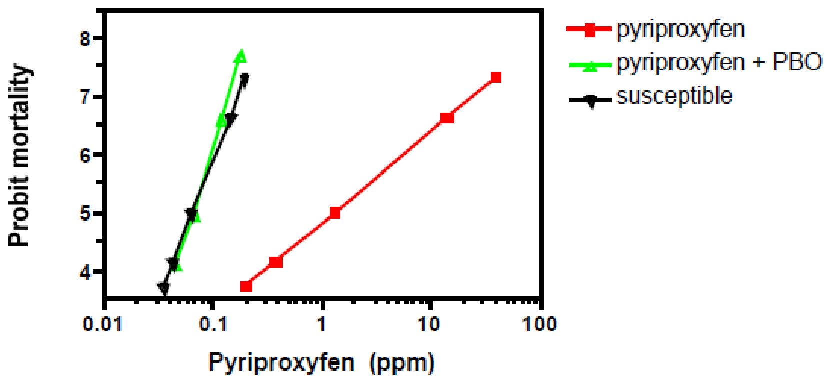

Figure *23 Response of pyriproxyfen resistant silverleaf whitefly to pyriproxyfen and PBO plus pyriproxyfen.*

Table 6. *Response of pyriproxyfen resistant silverleaf whitefly to pyriproxyfen and PBO plus pyriproxyfen.*

Strain	Slope	X^2	LC_{50} ppm (95% fiducial limits)	Resistance factor* LC_{50}	LC_{99} ppm (95% fiducial limits)	Resistance factor* LC_{99}
susceptible	4.9	0.001	0.066 (0.057 – 0.070)	1	0.197 (0.120 – 0.320)	1
Field resistant	1.6	4.3	1.3 (0.9 – 1.8)	19.7	39 (15 - 100)	198
Field resistant +PBO	5.2	0.005	0.065 (0.056 – 0.071)	1	0.18 (0.12 – 0.28)	1

Outcomes

All aims of this project have been met:

- • To use toxicological, biochemical molecular and genetic techniques to continue investigate insecticide resistance mechanisms in B-biotype *B. tabaci*. *Resistance mechanism studies and studies into the mechanisms of synergism in the silverleaf whitefly have provided the means to overcome most resistances in the silverleaf whitefly.*
- To continue a resistance monitoring programme for B- biotype, and native non-B biotype *B. tabaci*. *Resistance monitoring has exposed serious resistance problems in the silverleaf whitefly to most insecticides, including the insect growth regulators. The project studies have demonstrated cross-resistance between two insect growth regulators*.
- To devise and test resistance insecticide management strategies for management of Bemisia tabaci in Australia. *The results of this research project have been used to develop, modify and expose potential problems the central Queensland, silverleaf whitefly resistance management strategy*
- To monitor whitefly species distribution on cotton. *The project has tracked the expansion of the silverleaf whitefly into most cotton areas of New South Wales and Queensland*.

The silverleaf whitefly is a great threat to the economic production and sustainability of cotton in Australia. This research project addressed all three CRDC outputs (economic, social and environment): by tracking the spread of the silverleaf whitefly in cotton, establishing insecticide resistance status, providing the scientific basis for a resistance management strategy, as well new technology to control resistant silverleaf whitefly.

Resistance in the silverleaf whitefly is one of the greatest threats to the sustainability of the cotton industry because insect pest control is a major cost factor. Effective resistance management in the silverleaf whitefly reduce the need for insecticide use, protecting natural resources, and the community.

Technical Advances

This project has developed more effective insecticide synergism for control of resistant silverleaf whitefly. Using the patented concept of microencapsulation of insecticides, to delay insecticide release until piperonyl butoxide has effectively inhibited the factors causing resistance, we have been able to overcome resistance. The technology has been patented, NSW DPI, Rothamsted Research and CRDC are currently negotiating commercialisation options.

Gunning, R. V and Moores, G. D. *Method and Composition for Combating Pesticide Resistance* UK Patent no. 0309773.0, PCT/GB2003/001861

Conclusion

Results of this project have shown that the silverleaf whitefly is expanding into most cotton areas in Australia. Some of these outbreaks have required insecticidal control in the Macintyre and Gwydir Valleys. Rapid population expansions of silverleaf whitefly numbers is normally triggered by use of broad spectrum pesticides which kill predator and parasites and the other whitefly the other, insecticide susceptible whitefly types. However, Greenhouse whitefly, silverleaf whitefly and native *B. tabaci* can co-exist on cotton. Given that silverleaf whitefly was less evident in 2004/2005, compared to other whitefly types, it is likely that non-sprayed Bollgard II cotton has had an impact on whitefly population dynamics. The whitefly distribution data show, nonetheless, that numbers of silverleaf whitefly are variable and this may also be influenced by climatic conditions. It is therefore essential that monitoring of whitefly populations on cotton be continued.

Insecticide resistance levels in the silverleaf whitefly can be very high and resistance is easily selected for, reinforcing the need to rotate chemistry in control strategies. Resistance to the insect growth regulator pyriproxyfen, is of particular concern because of the central Queensland cotton industry's dependence on pyriproxyfen for prevention of population build-ups. The post insect growth regulator resistance levels are so high, as to suggest that consecutive generations of silverleaf whitefly are receiving pyriproxyfen and it is likely that the threshold based initiation of IGR use is leading to a longer a longer use period in the area than is desirable. This problem, however, may be counterbalanced by the fact, that between cotton seasons resistance levels decline markedly, perhaps indicating lack of fitness in the resistant whiteflies. In 2004, after IGR use, pyriproxyfen resistance was extreme and coincided with some reports of poor control with pyriproxyfen at Emerald. Laboratory bioassays with the insecticide synergist piperonyl butoxide, showed complete suppression of pyriproxyfen resistance and this may be a very valuable tool in the field, in gaining control of resistance and prolonging the life of pyriproxyfen.

This project has also developed more effective insecticide synergism for control of resistant silverleaf whitefly. Using the patented concept of microencapsulation of insecticides to delay insecticide release, until piperonyl butoxide has effectively inhibited the factors causing resistance, we have been able to overcome resistance. Field trials demonstrated outstanding control with pyrethroids, against highly resistant silverleaf whitefly. This technology has the potential to be applied to a number of esterase mediated resistances in the silverleaf whitefly such insect growth regulators, imidacloprid, the pyrethroids and will lead to greatly improved control options for Australian cotton growers.

Publications

The results of this project have been progressively disseminated to the cotton industry via the TIMS Resistance Roadshow in 2003 and 2004, the CRDC Resistance Forum, the Cotton Consultants Association AGM in 2004, the Macintyre Valley Research Reviews 2004 and 2005 and in the *Australian Cotton Grower*. Contributions, of whitefly distribution and insecticide resistance data have been made, to publications of the Industry Development Officers.

Results have also been presented to the international scientific community, at scientific meetings:

Gunning, R. V. (2003) – Insecticide resistance in Australian *Bemisia tabaci*, International *Bemisia* Workshop, Barcelona, Spain. March 2003.

Moores, G. D., Gunning, R. V *et al* (2004) – Overcoming pyrethroid resistance in *Bemisia tabaci* by the use of piperonyl butoxide. *2nd European Whitefly Symposium*, Cavtat, Croatia, September, 2004.

Gunning, Young, S, Bingham, G. V. and Moores, G. D (2004) – Novel technologies for the control of silverleaf whitefly in Australia. *2nd European Whitefly Symposium*, Cavtat, Croatia, September, 2004.

Gunning, R. V (2005) – Novel technologies for the control of insecticide resistant pests. *International Symposium of Crop Protection,* Ghent, Belgium, May 2005.

Other articles

Gunning, R. V and Moores, G. D. *Method and Composition for Combating Pesticide Resistance* UK Patent no. 0309773.0, PCT/GB2003/001861

Moores, G. D., Bingham, G. V. and Gunning, R. V (2005) – Use of temporal synergism to overcome insecticide resistance. *Outlooks on Pest Management* **16,** 7 – 9.

Gunning, R.V., Young, S., Bingham, G.and Moores, G.D. (2004) – Synergists turn back the clock on insecticide resistance. *The Australian Cotton Grower*, **25** (4): 10 – 11.

Further data will be progressively published in scientific journals.

Online Resources

A number of whitefly extension publications, to which this project has contributed, are available on-line, at the Cotton CRC web-site (www.cotton.crc.org.com).

Part 4 – Final Report Executive Summary

The silverleaf whitefly, B-biotype *Bemisia tabaci*, first detected in Australia in 1999, has become a major pest on cotton in central Queensland. The silverleaf whitefly is also found, albeit in low numbers, in most other cotton districts in Australia. The silverleaf whitefly represents a considerable threat to cotton production in Australia.
The objectives of this project were:

- *To monitor whitefly species distribution on cotton.*
- *To use toxicological, biochemical molecular and genetic techniques to continue investigate insecticide resistance mechanisms in B-biotype B. tabaci.*
- *To continue a resistance monitoring programme for B- biotype, B. tabaci.*
- *To devise and test resistance insecticide management strategies for management of Bemisia tabaci in Australia.*

The project has tracked the expansion of silverleaf whitefly into most cotton areas of New South Wales and Queensland. Resistance monitoring has exposed serious insecticide resistance problems in the silverleaf whitefly, including resistance the insect growth regulators. Studies have demonstrated cross-resistance between the two insect growth regulators that have been used on cotton in Australia. Resistance mechanism and synergism studies, however, have provided the means to overcome most resistances in the silverleaf whitefly. The research project has been an instrumental part of the central Queensland, silverleaf whitefly, resistance management strategy, which, so far, has prevented "sticky cotton" problems.

Major Outputs of this project have been:

- Results of this project have given knowledge of the distribution and abundance of whitefly species on cotton in Queensland and New South Wales. While the silverleaf whitefly has expanded into most cotton areas, data show that Greenhouse whitefly, silverleaf whitefly and native *B. tabaci* can co-exist on cotton.
- Insecticide resistance levels in the silverleaf whitefly can be very high and resistance is easily selected for, thus emphasising the need to rotate chemistry. Resistance to the insect growth regulator pyriproxyfen is of particular concern, because of the industry's dependence on use early season, to prevent silverleaf whitefly population build-ups Post insect growth regulator use, pyriproxyfen resistance levels are so high, as to suggest that consecutive generations of silverleaf whitefly are receiving pyriproxyfen. It is therefore likely that the threshold based initiation of pyriproxyfen use, is giving a longer use period in the area than is desirable. In 2004, post insect growth regulator use, pyriproxyfen resistance was extreme and coincided with reports of poor control at Emerald.. Piperonyl butoxide synergism studies with pyriproxyfen, however, showed complete suppression of resistance and piperonyl butoxide synergism may become a very valuable tool in prolonging the life of pyriproxyfen on cotton.
- This project has also developed technology for more effective insecticide synergism in the field, for the control of resistant silverleaf whitefly. Using our patented concept (Gunning, R. V and Moores, G. D. *Method and Composition for Combating Pesticide Resistance* UK Patent no. 0309773.0, PCT/GB2003/001861),of microencapsulation of insecticides to delay insecticide release until piperonyl butoxide has effectively inhibited the esterase enzymes causing resistance, we have been able to overcome resistance. Field trials demonstrated outstanding control with pyrethroids, against highly resistant silverleaf whitefly. This resistance control technology can be applied to a number of esterase mediated resistances in the silverleaf whitefly, such as insect growth regulators, imidacloprid, the pyrethroids will lead to greatly improved control options for the silverleaf whitefly..