If you are participating in the presentations this year, please provide a written report and a copy of your final report presentation by 31 October. If not, please provide a written report by 30 September.

Part 1 - Summary Details

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

CRDC Project Number: **03DAQ006**

Project Title: Silverleaf whitefly insecticide resistance monitoring 2007-2010

Project Commencement Date: 1 July 2007 **Project Completion Date:** 30 June 2010

CRDC Program: Crop Protection

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

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

Background

Silverleaf whitefly (SLW), *Bemisia tabaci B* biotype (Gennadius), is a major insect pest of cotton and horticultural industries. In cotton it is a pest because it produces sugary exudates (honeydew) that contaminate cotton lint and cause problems during textile processing. Honeydew contaminated lint may receive price penalties or in extreme cases may be rejected from sale. Insecticides are an important SLW management tool, but SLW has the propensity to rapidly develop resistance to many insecticide groups including synthetic pyrethroids, organophosphates and carbamates. Currently, the cotton industry is highly reliant on a limited number of insecticides for SLW control. Due to the pressure on these insecticides for management of SLW, monitoring for changes in resistance levels is critical to ensure the longevity of these products and conserve the Australian cotton industry's reputation as a high quality fibre producer. Pyriproxyfen (Admiral®) is currently considered the most important insecticide for SLW management because it has excellent efficacy against high density infestations. Overseas, resistance to Admiral® has been reported for SLW (Horowitz et al., 1994, Li et al., 2003). The heavy reliance on Admiral® for management of SLW in Australia combined with the high resistance risk has meant that resistance monitoring is critical as part of an insecticide resistance management strategy (IRMS).

Objectives

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

1.1 Insecticide testing laboratory fully functional to meet project requirements.

The laboratories were equipped to perform bioassays, sterilise lab-ware and maintain bioassays at constant temperature, lighting and humidity. Stereo-microscopes, glassware and insecticides were readily available to perform experiments as needed. Glassware was sterilised by immersing in bleach baths for 24 hours. Two controlled temperate (CT) rooms were available for maintaining bioassays under controlled conditions. All work was carried out in accordance with current work place health and safety requirements.

1.2 Staff appointed and appropriately trained

Zara Ludgate was appointed as the principal researcher on the project as a graduate entomologist on 1 February 2008. Zara has rapidly attained skills in collecting whitefly samples, conducting bioassays, analysing and interpreting data. Training opportunities for Zara included a 10 day study tour to the USA to learn more about data analysis and future directions in resistance monitoring. Mentoring has been provided by senior research and experimentalist staff within DEEDI (David Murray, Melina Miles and Richard Lloyd). Training in parasitoid identification was kindly provided by Verni Sivasubramanium (DEEDI).Training has also been provided by Pat Collins (DEEDI) and Sharon Downes (CSIRO) in resistance theory; Paul De Barro (CSIRO), Richard Sequeira and Paul Grundy (DEEDI) in SLW ecology; Matthew Cahill (IACR) in bioassay methodology and Susan Fletcher (DEEDI) in experimental design and statistical analysis.

Richard Lloyd was appointed as the principal experimentalist on the project at its commencement and is competent at developing methodologies and equipment for bioassays, conducting bioassays and maintaining SLW colonies. Training opportunities for Richard have included a 10 day study tour to the USA to learn more about developing methodologies and equipment for resistance monitoring.

Tracey Shatte was appointed as the experimentalist conducting molecular based whitefly diagnostics from 2007-09. Tracey is competent in the development and implementation of molecular based diagnostics. Training opportunities for Tracey included a three day bioinformatics course run by Sydney University learning how to use bioinformatics software and the associated tools to visualise and analyse data as well as being able to design primers.

Raechelle Grams was appointed as the molecular scientist on a casual basis in 2009-10 to perform molecular diagnostics for the project. Raechelle has completed a PhD that involved molecular diagnostics and is competent at developing molecular based techniques for identification of whitefly and performing routine diagnostics for whitefly.

2.1 Colonies of silverleaf whitefly (SLW) successfully maintained on caged, potted plants in a glasshouse.

Sufficient numbers of whitefly were collected by project staff and collaborators to perform dose response assays, test species composition and parasitism levels (Table 1).

Table 1. The number of whitefly collections for three cotton seasons between 2007-10 for purposes of resistance monitoring, species identification and parasitism levels.

	Year		
Number of whitefly collections	**2007-08**	**2008-09**	**2008-09**
Resistance monitoring	7	16	11
Species identification	22	185	66
Parasitism levels	16	38	35

Insect-proof emergence cages and colony cages were designed and built to maintain insect colonies and increase efficiency of bioassays while insuring the integrity of individual colonies. Bioassays were completed within four generations in culture to ensure the results of bioassays reflected the resistance levels in the field.

2.2 Identification of B biotype confirmed

Identification of B biotype was confirmed by the molecular test, Polymerase Chain Reaction (PCR) using rapd and microsatellite primers using positive controls for *Bemisia tabaci* B biotype, Q biotype and Australian native and Greenhouse whitefly. The molecular based diagnostics complemented morphological based identification to separate the *Bemisia tabaci* species complex from Greenhouse whitefly by the presence or absence of a fringe of waxy rays. From 2007 until 2010, 229 samples were processed for purposes of species identification using a combination of molecular and morphological skills. This became

particularly important during the suspected identification of the exotic Q biotype in Australia by NSW I&I staff in 2009.

A method for identification of Q biotype was developed as a pre-emptive action prior to Q biotype's identification in Australia. In response to the suspected detection of Q biotype in Australia, DEEDI staff performed widespread monitoring using PCR techniques. Ninety-five samples consisting of 2497 individual whitefly were tested as part of the Q biotype distribution program and no Q biotype were detected in any samples. The International Plant Protection Convention (IPPC) changed the status of Q biotype in Australia to absent on 28 April 2010 citing 'an error in the technical process' (Rymer, 2010a).

3.1 Conduct bioassays of selected insecticides against SLW using appropriate methodologies. Recurring milestone.

Full dose response assays were conducted annually against field collections of SLW. Collections were made when whitefly were available from all cotton growing regions in QLD and NSW encompassing Emerald, Biloela, the Burdekin, St George, Darling Downs, Moree, and Narrabri. Field collections were tested for resistance to pyriproxyfen (Admiral®), diafenthiuron (Pegasus®), bifenthrin (Talstar®) and spirotetramat (Movento®). Three bioassay techniques were developed targeting each of the different insect life stages (egg, nymph and adult). All techniques provided repeatable results indicating that the tests were reliable.

4.1 Dosage response curves provided for key compounds. Recurring milestone.

The results of the full dose response assays were used to calculate the lethal dose to kill 50 percent and 90 percent of field populations using probit analysis. These data were then compared to a susceptible strain (SU07-1) to calculate a resistance factor (RF) for each of the field strains. The RF for collections taken from cotton for pyriproxyfen were susceptible in most cases excluding samples from St George and the Burdekin in 2007-08, where elevated RF were recorded and in horticultural production areas in Bowen, the Burdekin (QLD) and Warburn (NSW) between 2008 and 2010 where elevated RF were recorded in cucurbits. Resistance factors for diafenthiuron and bifenthrin were elevated in many field strains with RF ranging from 1-11 between 2007-10, however there were no observable changes in RF over time. This information was provided to the Cotton Research and Development Corporation (CRDC) in half yearly reports and to the Transgenic Insecticide Management Strategies (TIMS) panel annually to form part of the annual cotton IRMS.

4.2 SLW insecticide resistance information circulated to relevant interest groups. Recurring milestone.

The resistance monitoring data was circulated to key industry groups including CRDC, TIMS, growers, consultants, insecticide companies and other industry representatives.
This information was made available through half yearly CRDC progress reports, 10 DEEDI entomology blog site articles (the Beat sheet blog), presentations at grower and consultant meetings (e.g. the Resistance Road show), 2 conference papers in the biannual cotton conference proceedings and hands on displays (e.g. Moree Trade Show, Cotton Conference hands-on session).

4.3 IRMS for SLW developed and implemented. Recurring milestone.

Information gained from resistance testing was provided to the TIMS panel annually. This information was used to develop the IRMS for whitefly for each cotton season and was included in the annual Cotton Pest Management Guide. The current insecticide resistance management strategy (IRMS) is working effectively, particularly the IRMS guideline of a maximum of one application of pyriproxyfen per season which is a valuable resistance prevention tool.

Methods

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

SPECIES IDENTIFICATION AND PARASITISM ASSESSMENT

Morphological based diagnostics

Project staff, growers and consultants collected whitefly infested leaves from cotton fields throughout the season. Leaves were collected by moving at least 20 metres into the field and collecting whitefly infested leaves randomly throughout the field. Leaves were brought back to the laboratory and inspected for whitefly species composition and parasitism levels using a stereo microscope. A minimum of 30 leaves and 50 fourth instar whitefly were inspected looking at half the leaf from the edge of the leaf to the mid-vein on the abaxial leaf surface.

Whitefly morphology was used to differentiate greenhouse whitefly (GHW) *Trialeurodes vaporariorum* (Westwood) and the *B. tabaci* species complex. Fourth instar nymphs of GHW were identified by a fringe of waxy rays encircling the body while *Bemisia tabaci* was identified by an absence of these waxy rays (Figure 1). The morphology of parasitised whitefly was used to identify *Eretmocerus* sp. and *Encarsia* sp. Parasitised fourth instar whitefly were identified by morphological characteristics that included the asymmetric position of the mycetomes, opaque appearance of the whitefly nymph and round exit hole in the empty nymph case (Figure 2). Adult *Encarsia spp*. were identified by a dark thorax and *Eretmocerus spp*. were identified by a yellow thorax. *Eretmocerus hayati* was distinguished from *E. mundus* by the presence of high numbers of males in the population (identified by hairy antennae) compare to very low numbers of males in the *E. mundus* population.

Figure 1 . Greenhouse whitefly nymphs (left) have a fringe of waxy rays circling the body while Silverleaf whitefly nymphs (right) are absent of these waxy rays.

Figure 2. Parasitised silverleaf whitefly nymphs showing an *Encarsia* spp. parasitoid (left) and early and late stage *Eretmocerus* spp. parasitoids (middle and right).

The species and parasitism composition was calculated as a percentage of the total number of whitefly tested and information was relayed back to the relevant growers and consultants within three working days of receiving the sample.

Molecular based diagnostics

A polymerase chain reaction (PCR) based method optimised from existing methodologies using operon primers OPA10 and OPH16 was used to distinguish Australian native *B. tabaci* (AN) and B biotype (Boukhatem et al., 2007, DeBarro and Driver, 1997). A microsatellite method using primers Bem23F and Bem23R was enhanced from established methodologies to distinguish B and Q biotypes using positive controls of Q biotype from Israel, USA and Spain (Chu et al., 2009) (Figure 3).

The operon primers, OPA10 and OPH16 were not reliable for identification of Q biotype. Both the OPA10 and the OPH16 primers identified variations within Q biotype which were possibly haplotypes of the biotype. Microsatellite were used for identification of Q biotype because it produced a strong band for Q biotype from different regions including Spain, USA and Israel. Thank you to Rami Horowitz (Gilat Research Centre, Israel), Paul De Barro (CSIRO) Xiachun Li (UA) Iain Kay (DEEDI) and the team at Biological services, Loxton, Australia, who kindly provided positive controls for use in the molecular tests

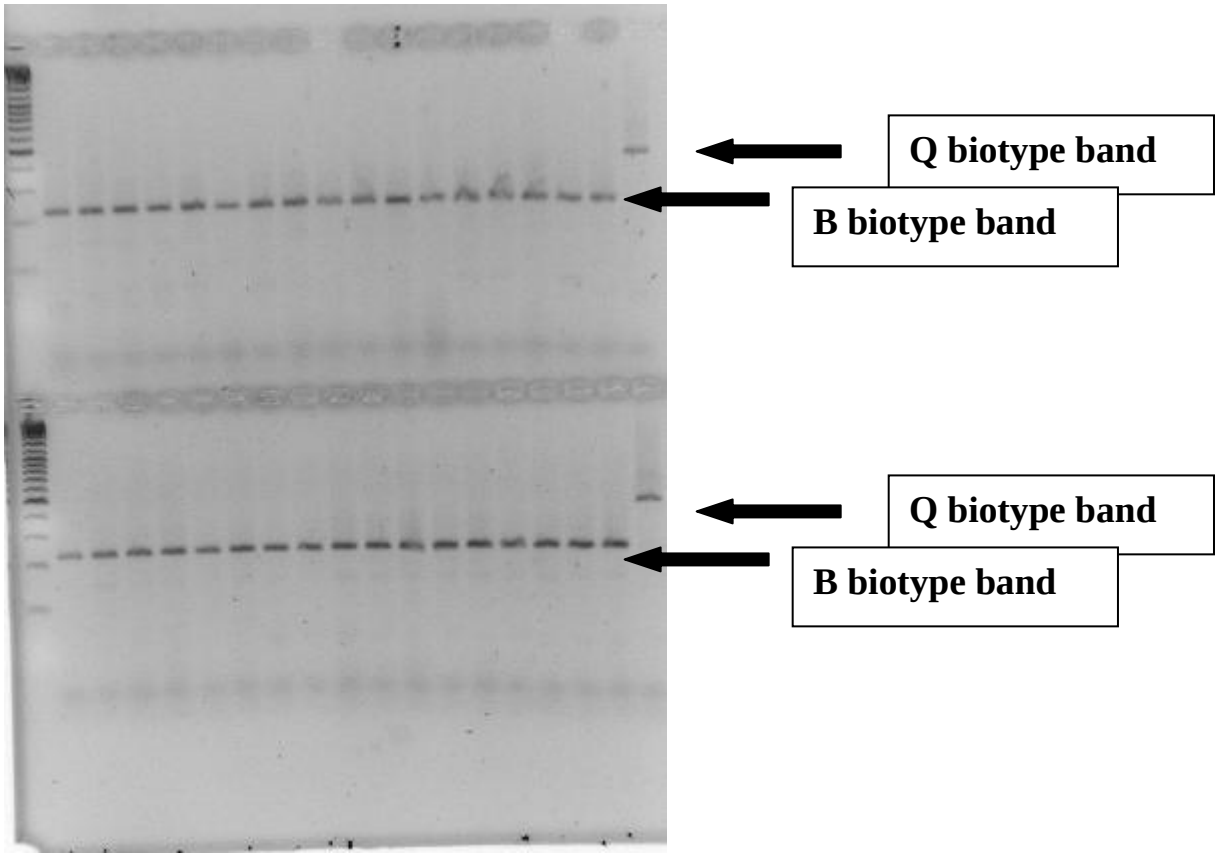

Figure 3. Showing gel image from PCR using microsatellite primers Bem23 for whitefly collected from Warburn, NSW. On the very far left is the DNA ladder, followed by the bands for the field collected whitefly which were all B biotype, and then the positive controls which were B biotype and Q biotype.

RESISTANCE MONITORING

Colony Maintenance

A susceptible strain of SLW (SU07-1) was obtained from CSIRO, Indooroopilly in 2007 where it had been in culture since 1995. The SU07-1 strain is not a true susceptible strain as it has had prior exposure to or risk of exposure to insecticides. It is believed that SLW entered Australia already possessing resistance to insecticides (Gunning et al., 1995). Further, the SU07-1 strain has been exposed to gene flow from the addition of wild type SLW of unknown origin prior to 2005 and was exposed to Malathion prior to 2007.

Field collections of whitefly were taken from cotton fields in central Queensland, the Burdekin, St George, Darling Downs, Gwydir and Lower Namoi when numbers were adequate. Whitefly were collected by removing cotton leaves infested with late fourth instar whitefly and transporting them back to the laboratory. Whitefly infested leaves were placed in emergence cages and newly emerged adults were aspirated from the cage for use in bioassays or transferred to insect rearing cages to establish colonies.
Silverleaf whitefly colonies were reared on six week old cotton plants. Whitefly were allowed to oviposit on cotton plants for 48 hours before being removed with the aid of a

modified vacuum cleaner. Plants were kept in insect proof cages under glasshouse conditions until adult emergence for use in bioassays.

The emergence boxes (Figure 4) were a unique design developed for the purpose of the SLW project. Rectangular boxes were painted black on the inside with a clear glass lid. Whitefly display a positive phototaxis response and adults could be easily aspirated from the top of the cage and transferred to colony cages or used in bioassays. Placing infested leaves in emergence cages and then transferring them to colony cages reduced the risk of pest insects including thrips, mites and spiders destroying colonies.

Figure 4. The emergence boxes were painted black inside with a clear glass top so that the whitefly would be attracted to the top of the cage where they could be easily removed for bioassays or be transferred to colony cages without the risk of introducing pest insects including thrips and mites.

The colony cages (figure 5) were a unique design developed for the purposes of the SLW project. Colony cages were made using a light weight aluminium frame with a solid floor and insect proof netting for walls to assist in ventilation. The top of the cage was constructed using a clear sheet of Perspex. Adults were attracted to light at the top of the cage where they could be removed from the cage by aspiration for use in bioassays or transferred to colony cages. Aspirating insects off glass minimised damage to insects as removing SLW from leaves may cause stylets to break during feeding. Further, the cage design allowed sexing to be quickly conducted for bioassays where only females were required. The larger abdomens of the females could be clearly viewed through the Perspex as the whitefly clung upside down to the roof of the cage.

Figure 5. The colony cages were designed with light weight aluminium frames for ease of transport, insect proof netting for ventilation and a Perspex top so that insects could be easily aspirated from the roof of the cage without breaking their stylets or causing other damage.

Full dose response assays

Three methodologies were developed to test the insecticides for SLW management in cotton targeting each of the life stages, egg, nymph and adult. Insecticides tested were pyriproxyfen (Admiral®), diafenthiuron (Pegasus®), bifenthrin (Talstar®) and spirotetramat (Movento®).

An egg bioassay based the Insecticide Resistance Action Committee (IRAC) method 12C (2010) was used to test the resistance status of pyriproxyfen (Figure 6). The bioassay method involved confining mated female adults in clip cages (15/cage) on leaves of 5-7 node cotton plants (Sicot 81) for 6 h to lay eggs. Adults were then removed, and eggs were counted and their position on the leaf marked using a water proof pen under a stereo microscope. Approximately 50 eggs per leaf was considered desirable. Leaves were allocated to treatments so that an even distribution of egg numbers was achieved across treatments. Five treatments with 6 infested leaves per treatment were then dipped for 20 sec in a serial dilution solution and allowed to dry. To obtain the desired concentration, the emulsifiable concentrate was diluted with distilled water containing 0.01% Agral® non-ionic surfactant. Leaves dipped in distilled water plus Agral® acted as controls. After treated leaves had dried, the petioles of leaves were then placed in water filled 20 mL glass scintillation vials and held at 27 ± 1°C and photoperiod of 16:8 (L:D) h for 10 days before assessing egg hatch. Mortality data, measured as failure of eggs to hatch were assessed by probit analysis.

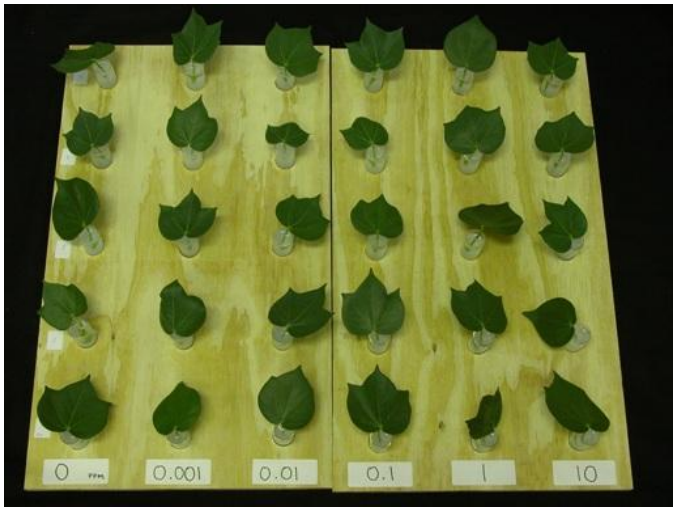

Figure 6. A bioassay for pyriproxyfen (Admiral®) in progress. After eggs are allowed to be layed on leaves, the leaves are treated with a serial dilution of insecticide to determine the lethal concentration that kills 50% and 90% of the field collection.

A nymph bioassay was used to test the resistance status for spirotetramat. This assay was based on IRAC method 12c (2010) and was similar to the egg bioassay. Clip cages were places on leaves of potted plants with 15 adults per clip cage to allow egg lay. The position of the clip cage was marked on the leaf with a waterproof pen and cages were removed from leaves. Adults were carefully removed from leaves using a laboratory vacuum line taking care to remove all adults so that the progeny were all of the same age. After 10 days in constant conditions (27 ± 1°C and photoperiod of 16:8 (L:D) h) eggs had hatched and had reached settled first instar stage. The position of nymphs was marked on the leaves with a water based pen and the total number of nymphs per leaf was recorded with the aid of a stereo-microscope. Treated leaves were assessed 10 days post treatment with proportion of survivors measured by the presence of empty nymph cases where adults had emerged subtracted from the initial population.

Marking the position of the eggs or nymphs on the leaves was a novel method that to our knowledge has not been described previously. The IRAC method entailed counting eggs and/or nymphs at the completion of the bioassay however given the small size of both eggs and nymphs and the mobility of crawlers, we found that marking the position of the eggs and nymphs on the leaf prior to treating and then counting unhatched eggs/spent pupal cases 10 days post treatment improved the accuracy and efficiency of our methodology over the method described in IRAC (2010).

An adult bioassay was used to test the resistance status for bifenthrin and diafenthiuron based on methods described in IRAC method number 8 (2010) with changes to the cage design (Steve Castle, USDA 20 September 2008). Five treatments with six leaves per treatment were dipped for 20 sec in a serial dilution solution/suspension and allowed to dry. To obtain the desired concentration, the suspension concentrate was diluted with distilled water containing 0.01% Agral® non-ionic surfactant. Leaves dipped in distilled water plus Agral® acted as controls. After treated leaves had dried, the petioles of leaves were then placed in water filled 20 mL glass scintillation vials. Fifteen adults (male and female) were aspirated from colony cages into clip cages and maintained in a CT room at 27 ± 1°C for 2 days or 3 days for

bifenthrin and diafenthiuron assays, respectively. Dead and alive adults were counted under a stereo microscope and results were subjected to probit analysis.

The clip cage design (Figure 7) was constructed following a design developed by Steve Castle's team in Arizona (Steve Castle, USDA 20 September 2008). The design was enhanced by replacing the white ventilation cloth with black cloth. The allowed SLW to be viewed clearly through the high contrast material allowing bioassays to be scored with the cages containing the SLW as opposed to the initial methodology where cages were removed and SLW counted quickly before/while the flew away.

Figure 7. Clip cages developed by Steve Castle's group and optimised by Richard Lloyd, DEEDI, were used for adult SLW bioassays.

Analysis

Mortality data were analysed using Probit 5 for Windows (Gillespie, 1995). Mortality data were adjusted using Abbott's formula to correct for any control mortality (Abbott, 1925). Probit regressions were calculated and LC_{50} and LC_{90} values were estimated. The slope of the line was also calculated. A resistance factor (RF) was calculated by dividing the LC_{50} value of the tested strain by the LC_{50} value of the susceptible strain (SU07-1) for the same insecticide. Field collections were considered significantly different to the susceptible strain if the fiducial limits did not overlap.

Results

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

IDENTIFICATION AND PARASITISM ASSESSMENT

DEEDI staff processed 229 whitefly samples for the purposes of species identification and parasitism assessment between 2007 and 2010. Samples were collected from all SLW affected cotton regions and some horticultural districts in QLD and NSW.

Silverleaf whitefly were detected in every sample processed from cotton and horticulture in QLD and NSW. Silverleaf whitefly made up the greatest proportion of whitefly present in samples collected from central QLD, the Burdekin, St George and the western Darling Downs (e.g. Norwin), accounting for >90% of all whitefly species/biotypes in most samples. In the eastern Darling Downs and the Namoi/Gwydir region, whitefly consisted of mixed populations of SLW and GHW.

In the Gwydir and Lower Namoi, B biotype reached population levels requiring insecticide control for the first time in 2008-09. In the 2009-10 cotton season fields in the Macquarie valley reached economic thresholds for the first time. While SLW is widespread in Australia, until 2008-09 the more 'marginal' southern production areas were considered too cool for populations to reach levels warranting control in cotton however a trend has been observed over the past decade with SLW progressively moving south.

The identification service was expanded in 2009 to determine the distribution of Q biotype in Australia following a suspected identification of Q biotype by Dr Robin Gunning (NSW I&I) in late 2008. A PCR based procedure was developed by DEEDI staff prior to the notification of Q biotype in Australia in preparedness for such an event. In response to the notification of Q biotype in Australia, widespread sampling was conducted by DEEDI staff and collaborators throughout QLD and NSW and tested at the DEEDI Toowoomba laboratory using PCR based diagnostic tools. Ninety five samples (2500 individual whitefly) were processed as part of the distribution study. No Q biotype were detected by DEEDI from whitefly collected in Australia. The status of Q biotype in Australia has since been changed to absent on the International Plant Protection Convention (IPCC) website citing an 'error in the technical process' (Rymer, 2010b).

GHW consistently appeared in high numbers in the eastern Darling Downs from 2007-10. Cooler temperatures and migration of GHW from spring planted sunflowers was thought to contribute to the high numbers of GHW in the population. Species composition changed as the season progressed and in some instances a predominantly GHW population early in the season was replaced with a predominantly SLW population by the end of the season. Mixed populations of GHW and SLW were often recorded in the Namoi/Gwydir regions. GHW is currently not considered a pest of cotton because it does not produce trehalulose in its honeydew.

Small group training exercises were conducted in areas where GHW are commonly encountered on the eastern Darling Downs and the Namoi/Gwydir region. The purpose of these training exercises was to up-skill growers and consultants in species identification to improve management decisions and productivity for producers. Awareness of the importance of distinguishing GHW and SLW is greater amongst consultants and growers who have attended these sessions however due to the small size of the insects; there is still some hesitation in identification, particularly from growers and consultants with poor eye sight.

Australian native (AN) was rarely encountered in cotton in QLD. It was occasionally recorded at low levels on the Darling Downs but never made up >10% of the population. In the more marginal SLW regions of Namoi-Gwydir, AN made up <10 percent of whitefly in most samples except one sample from Narrabri in 2008 where AN consisted of 35% of the population. This was prior to the 'outbreak' of SLW in Narrabri in 2008-09. High levels of AN were recorded from Bagara and Mon Repos, near Bundaberg on painted spurge (*Euphorbiaceae cyathophora)* where they made up 100% and 75% of the population, respectively, and on Papaw seedlings at an Emerald garden nursery where AN accounted for 23% of the whitefly population. The low proportion of AN on cotton is attributed to host preferences for other plants and displacement of AN by the invasive SLW through mating interference (De Barro et al., 2006).

Molecular based tools are useful in screening for exotic biotypes of *B. tabaci*, namely Q biotype. Our capacity to identify whitefly using molecular based tools was limited in the speed at which we can process samples. This was observed during the suspected Q biotype incursion event in 2009 when large numbers of samples were sent to DEEDI within a very short period of time. In the event of a similar demand for this service to respond to a biosecurity threat, additional resources would be required to respond adequately.

Whitefly parasitoids were identified in all samples taken from all cotton growing valleys. *Eretmocerus spp*. made up the greatest proportion of the parasitoid population. *Eretmocerus spp*. were not identified to species level in all samples, however, *E. hayati* was present in high levels in samples that were identified. *Encarsia spp*. were also present in the populations sampled, albeit, at much lower levels.

In the absence of disruptive insecticides, parasitism levels increased in most regions as the season progressed. Parasitism generally reached 30-50% in most valleys except Emerald where parasitoids often reached levels of >70%. In the first season of whitefly management in the Namoi/Gwydir region very limited parasitism was recorded. However, the following season parasitism levels appeared to be much greater with 50% parasitism recorded in one field in Moree.

A case study at Brigalow on the Darling Downs showed parasitism numbers built exponentially in the absence of disruptive insecticides. Parasitism levels changed over the course of three weeks from 11% in the first week to 23% in the second week and to 44% in the third week. The combined effect of environmental conditions (e.g. rainfall) and parasitism allowed the grower to avoid an insecticide application for whitefly. This highlights the importance of limiting insecticide usage and choosing selective insecticides where possible.

RESISTANCE MONITORING

Pyriproxyfen (Admiral®)

The susceptible strain had a LC_{50} of 0.07 ppm (FL50 0.11-0.05 ppm) and LC_{90} of 0.32 ppm (FL90 0.14 – 0.75 ppm) (Appendix, page 24). The slope of 1.84 was flatter then is normally desirable. This may be a reflection of the bioassay method or the susceptible strain may have been previously exposed to pyriproxyfen and contain low levels of resistant genes.

Resistance factors for pyriproxyfen (Admiral®) since monitoring commenced in 2007-08 remained low and unchanged in most valleys. Resistance factors for Emerald, Biloela, Darling Downs, Moree and Narrabri were not significantly different to the susceptible strain. There was no evidence of resistance developing to pyriproxyfen at these localities.

In St George, elevated RF were recorded in 2008-9 (RF_{50} 3, RF_{90} 17) (Table 2). Resistance factors decreased from 2008 onwards and the RF for St George in 2010 was not significantly different to the susceptible strain. There was no evidence of resistance developing to pyriproxyfen in St George, however, the number of whitefly collections taken from this valley will be increased in future seasons to provide more material to measure precise changes in RF.

Elevated RF's were recorded for pyriproxyfen in cotton in the Burdekin for the 2008-9 season. Resistance factors for 50% and 90% of the colony were 22 and 51, respectively, which was significantly different to the susceptible strain. Pyriproxyfen was not used by cotton producers in the Burdekin; however, pyriproxyfen is heavily used in horticulture. The close proximity of cotton and horticulture production and the high resistance factors recorded in horticulture are likely to be responsible for the elevated RF in cotton in the Burdekin. Insufficient SLW were available in cotton in 2009-10 to obtain colonies for testing.

Elevated resistance levels have been recorded in horticultural areas in QLD and NSW between 2008 and 2010. Resistance factors for 50% of the population of 108, 178 and 7 were obtained from Ayr, Gumlu (QLD) and Warburn (NSW), respectively, which was significantly different to the susceptible strain. Communication with local consultants from Bowen and the Burdekin suggests that the field efficacy of Admiral® has declined significantly in some regions since it was first registered for horticulture. (Pers. Comm. C. Monsour, Crop Consultant, *Peracto*, Bowen. 6.11.09).

Verbal communication from growers and consultants indicates that some producers are using more than one application of Admiral® in a season, in contravention of the IRMS recommendation of a maximum of one application in a season. This may be a result of crops that are not uniform due to environmental conditions (e.g. floods and drought), causing delays in crop maturity. There are also reports of aerial applications of below label rates of Admiral® in double skip cotton. These practises select for resistant whitefly in the population and put undue pressure on a product for which there is a high risk of resistance developing.

Table 2. Resistance status of whitefly tested for pyriproxyfen between 2007 and 2010 from cotton and other host plants. RF_{50} refers to the Resistance Factor that kills 50 percent of the population, calculated by dividing the lethal dose for 50 percent of the field strain by that of the susceptible strain.

Year	Location	Host Plant	Resistant
2007-08	Ayr	Cotton	✓ (RF_{50} 22)
	Dalby	Cotton	✗
	Emerald	Sunflower	✗
	Moura	Cotton	✗
	St George A	Cotton	✗
	St George B	Cotton	✓ (RF_{50} 3)
2008-09	Ayr	Melon	✓ (RF_{50} 108)
	Biloela	Cotton	✗
	Dalby	Cotton	✗
	Emerald	weeds	✗
	Gumlu	Melon	✓ (RF_{50} 178)
	Moree	Cotton	✗
	Namoi/Gwydir	Cotton	✗
	Namoi valley (NM09-1)	Soybean	✗
	Namoi valley (NM09-2)	Cotton	✗
	Namoi valley (NM09-4)	Cotton	✗
	St George (SG09-1)	Cotton	✗
	St George (SG09-2)	Cotton	✗
	St George (SG09-3)	Cotton	✗
	Theodore	Cotton	✗
2009-10	Biloela	cotton	✗
	Condamine	cotton	✗
	Norwin	cotton	✗
	Brigalow	cotton	✗
	Emerald (EM10-1)	cotton	✗
	Emerald (EM10-2)	cotton	✗
	Comet	cotton	✗
	Moree	cotton	✗
	St George	cotton	✗
	Warburn	rockmelon & honeydew	✓ (RF_{50} 7)

Manipulating resistance in a colony

In a lab study, a field collection of SLW collected from horticulture in the Burdekin in 2009 (AY09-1) displaying a RF_{50} of 108 was split into two sub colonies; AY09-1S and AY09-1R. AY09-1S was maintained without insecticide pressure for eight generations in culture. AY09-1R was pressured twice with a sub lethal dose (LC_{70}) over three generations. Without selection pressure, resistance declined in AY09-1S from RF_{50} 108 to RF_{50} 23 after eight generations. In AY09-1R, two selection events increased resistance from RF_{50} 108 to 409 within four generations.

The cotton IRMS guideline for a maximum of one application of Admiral® per season is a valuable resistance management tool. In the aforementioned study, two selection events under laboratory conditions increased the RF_{50} from 108 to 409 within four generations. Minimising the number of selection events in a season will slow the development of resistance. Further, SLW resistance to pyriproxyfen does not appear to have any major fitness disadvantages. In the study, resistance declined in the absence of selection pressure, however, after eight generations (12 months) RF still remained 23 times higher then the susceptible strain. It is strongly recommended that the IRMS guideline of one application Admiral® per season is practised by growers and consultants.

Diafenthiuron (Pegasus®)

The susceptible strain (SU07-1) had a LC_{50} of 18.41 ppm (13.91 – 41.42 ppm) and a LC_{90} of 41.42 ppm (27.61 – 62.14 ppm) (Appendix page 25). The slope of the line was 3.48 which is steep and indicates a population that is homogenous and made up of homozygous susceptible SLW.

In 2007-08, RF obtained from field collections from Dalby, Ayr, Moura, Emerald and one St George site were not significantly different to the susceptible strain. A field collection taken from a second St George site was significantly different to the susceptible strain (RF_{50} 2, RF_{90} 5) (Table 3).

Resistance factors remained unchanged from the susceptible strain for all field collections taken in the 2008-09 season. In 2009-10, elevated RF were recorded for field collections taken from cotton on the Darling Downs (RF_{50} 5) and at St George (RF_{50} 4) and from a horticulture collection taken at Warburn, NSW (RF_{90} 3).

The elevated RF indicates that resistant genes are present in the population. That said, there were no observable changes in RF in any regions over time. The elevated RF taken from horticulture at Warburn is unusual in that diafenthiuron is not registered in horticulture.

Diafenthiuron is not considered a product at high risk of developing resistance. It has been used in cotton in Israel for 20 years against both B and Q biotype without any noticeable changes in RF. Some field performance issues have been observed by growers in cotton areas

in Australia but this is likely to be a result of poor activation of the active ingredient due to cloudy weather conditions.

Table 3. Resistance status of whitefly tested for diafenthiuron between 2007 and 2010 from cotton and other host plants. RF_{50} refers to the Resistance Factor that kills 50 percent of the population, calculated by dividing the lethal dose for 50 percent of the field strain by that of the susceptible strain.

Year	Location	Host plant	Resistant
2007-08	Dalby	Cotton	✘
	Ayr	Cotton	✘
	Moura	Cotton	✘
	Emerald	Sunflower	✘
	St George (SG08-1)	Cotton	✘
	St George (#14)	Cotton	✓ (RF_{50} 2)
2008-09	Biloela	Cotton	✘
	Dalby	Cotton	✘
	Emerald	Weeds - various	✘
	Moree	Cotton	✓ (RF_{50} 2)
	Namoi/Gwydir	Cotton	✘
	Namoi valley (NM09-1)	Soybean	✘
	Namoi valley (NM09-2)	Cotton	✘
	Namoi valley (NM09-3)	Cotton	✘
	St George (SG09-1)	Cotton	✘
	St George (SG09-2)	Cotton	✘
	St George (SG09-3)	Cotton	✘
	Theodore	Cotton	✘
2009-10	Biloela	Cotton	✘
	Darling Downs (DD10-1)	Cotton	✘
	Darling Downs (DD10-2)	Cotton	✘
	Darling Downs (DD10-3)	Cotton	✘
	Emerald (EM10-1)	Cotton	✘
	Emerald (EM10-2)	Cotton	✘
	Emerald (EM10-4)	Cotton	✘
	Moree	Cotton	✘
	St George	Cotton	✓ (RF_{50} 4)
	Warburn	Melon	✓ (RF_{90} 3)

Bifenthrin (Talstar®)

The susceptible strain (SU07-1) had LC_{50} of 3.19 ppm (FL_{50} 2.25 - 4.54 ppm) and LC_{90} of 19.06 ppm (FL_{90} 11.78 – 30.83 ppm) (Appendix, page 27). The slope of the line was 1.57 which is flatter then is normally considered desirable for a susceptible strain. This may be a reflection of the bioassay technique or it may indicate that the population is a heterogeneous population made up of SLW that possess both susceptible and resistant genes.

Elevated RF were recorded every season for SLW collected from cotton. In the first year of monitoring, elevated RF were recorded from Ayr (RF_{50} 9) and one Dalby collection (RF_{90} 9) (Table 4). In 2008-09 elevated RF were identified in two collections from the Namoi valley and two collections from St George. In the third year of monitoring, elevated RF were recorded from Biloela, Emerald, Darling Downs and Warburn.

The elevated RF recorded each season indicates that resistance genes are present in the population. That said, there have been no observable changes in RF since monitoring commenced in 2007. Reports of poor field performance of bifenthrin for SLW control may indicate resistance in the population but it is also likely to be a reflection of poor insecticidal contact with the whitefly as bifenthrin has no translaminar activity. Use of this product in cotton is not recommended due to poor efficacy against SLW and off-target effects to beneficial insects including parasitoid wasps.

Table 4. Resistance status of whitefly tested for bifenthrin between 2007 and 2010 from cotton and other host plants. RF_{50} refers to the Resistance Factor that kills 50 percent of the population, calculated by dividing the lethal dose for 50 percent of the field strain by that of the susceptible strain.

Year	Location	Host plant	Resistant
2007-08	Emerald	Sunflower	✗
	Ayr	Cotton	✓ (RF_{50} 9)
	Dalby (DB08-1)	Cotton	✗
	Dalby (#13)	Cotton	✓ (RF_{50} 4)
2008-09	Biloela	Cotton	✗
	Dalby	Cotton	✗
	Emerald	Weeds - various	✗
	Moree	Cotton	✗
	Namoi valley (NM09-1)	Soybean	✓ (RF_{50} 2)
	Namoi valley (NM09-3)	Cotton	✓ (RF_{50} 3)
	St George (SG09-1)	Cotton	✗
	St George (SG09-2)	Cotton	✓ (RF_{50} 5)
	St George (SG09-3)	Cotton	✓ (RF_{50} 2)
	Theodore	Cotton	✗
2009-10	Biloela	Cotton	✓ (RF_{50} 7)
	Darling Downs (DD10-1)	Cotton	✗
	Darling Downs (DD10-2)	Cotton	✗
	Darling Downs (DD10-3)	Cotton	✓ (RF_{50} 2)
	Emerald (EM10-1)	Cotton	✓ (RF_{50} 6)
	Emerald (EM10-4)	Cotton	✗
	St George	Cotton	✗
	Warburn	Rockmelon, honeydew	✓ (RF_{50} 7)

Spirotetramat (Movento®)

Spirotetramat is expected to be registered in cotton in the 2010-11 season. Full dose response assays from different cropping regions between 2008 and 2010 have produced LD_{50} ranging between 3.12 and 6.4 (Appendix, pages 26 & 27). This is the first new insecticide with a unique mode of action that has been registered in cotton since monitoring commenced in 2007. The benefit of obtaining true baseline data for insecticides prior to SLW exposure is the improved accuracy in identifying changes in resistance factors over time.

Outcomes

4. 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 expected outputs for the project were:

1) Successful development of bioassay methodology
2) Screening field collections of SLW and obtaining resistance factors for all registered SLW insecticides
3) Assessment of parasitism
4) Identification of whitefly species
5) Development of diagnostic for Q biotype

Outcomes

- Output 1 & 2 support the IRMS to conserve the long term viability of a limited range of insecticides for SLW management. Further, the outcomes underpin IPM for SLW and other pest species that may impact on SLW management.
- Output 3 & 4 support industry in managing SLW to increase grower and consultant confidence in making decisions on SLW management and reduce pressure on insecticides in some instances.
- Outcome 5 supports biosecurity preparedness for the cotton industry in relation to detecting exotic whitefly biotypes (e.g. Q biotype).

5. Please describe any:-

a) Technical advances achieved (e.g. commercially significant developments, patents applied for or granted licenses, etc.);

NIL

b) other information developed from research (e.g. discoveries in methodology, equipment design, etc.); and

Advancements were made in equipment design with Richard Lloyd designing and constructing emergence boxes and colony cages that allowed efficient processing of insects from field through to bioassay. The emergence boxes were a unique design developed for the purpose of the SLW project. Rectangular boxes were painted black on the inside with a clear glass lid. The positive phototaxic response of the whitefly drove them to the top of the cage where they could be easily aspirated from the cage and transferred to colony cages or used in bioassays. Placing infested leaves in emergence cages and then transferring them to colony cages reduced the risk of pest insects including thrips, mites and spiders destroying colonies.

The colony cages were a unique design developed for the purposes of the SLW project. Colony cages were made using a light weight aluminium frame with a solid floor and insect proof netting for walls to assist in ventilation. The top of the cage was constructed using a clear sheet of Perspex. The positive phototaxic response of the SLW attracted adults to the top of the cage where they could be removed from the cage by aspiration. Aspirating insects off the top of the cage avoided damage to insects as removing SLW from leaves may damage stylets during feeding. Further, the design also simplified and sped up experiments where only females were used in experiments. The larger abdomens of the females could be clearly viewed through the Perspex as the whitefly clung upside down to the roof of the cage.

Clip cages were enhanced from existing designs of Steve Castle's and the 'new and improved' design have since been used by Steve Castle's group to improve the accuracy of scoring bioassays.

c) Required changes to the Intellectual Property register.

NIL

Conclusion

6. 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?

- Resistance to pyriproxyfen (Admiral®) has been observed in horticulture over the past three seasons due to overuse of this product. This is an early warning for the cotton industry of the dangers of overuse of this product.
- There is no evidence of resistance to pyriproxyfen in cotton dominated regions at this stage.
- The IRMS guideline of a maximum of 1 application of Admiral® in a season is a valuable resistance management strategy
- Below label rates of Admiral® will quickly select for resistant individuals within the population.
- Resistance factors for bifenthrin and diafenthiuron are elevated indicating that resistant genes are present in the population
- Resistance factors for bifenthrin and diafenthiuron have not changed over three seasons indicating that resistance frequencies have not increased markedly.
- There are no recommended changes to the cotton IRMS at this stage
- New insecticides with unique modes of action (eg spirotetramat) will assist the IRMS by providing a different insecticide to include in the rotation and reduce the pressure placed on other insecticides.
- Avoid broad spectrum insecticide use, particularly early-mid season for other pests, which kills whitefly natural enemies.
- Sample accurately for SLW and follow the threshold matrix guidelines to determine the optimum insecticide and timing of insecticide for SLW management

Extension Opportunities

7. Detail a plan for the activities or other steps that may be taken:

(a) To further develop or to exploit the project technology.

As opportunities present, the project team will collaborate with other resistance monitoring groups to share knowledge and skills in resistance monitoring with the aim to increase the efficiency of the monitoring project and further develop our knowledge and skills in resistance monitoring and management.

(b) For the future presentation and dissemination of the project outcomes.

The outcomes of the project will continue to be disseminated to industry through blog articles, discussions at grower and consultant meetings and presentations at seminars.

(c) For future research.

Further development of the project technology may include the expansion of the project to include identification of resistance frequencies and correlation of resistance frequencies with

insecticide field performance. This is important for the cotton industry at present, where there is significant pressure placed on Admiral® for management of SLW and due to the high costs to producers, it is important that field performance remains sturdy.

The project technology will be utilised further (exploited) to expand resistance monitoring into other cropping sectors, namely horticultural production. It is important that SLW management occurs on an area wide basis, particularly in areas of multi-crop use, like the Burdekin and St George.

FUTURE DIRECTIONS IN RESISTANCE MONITORING

A limitation of insecticide resistance monitoring is correlating resistance factors with field performance. The objective of resistance monitoring is to detect changes in resistance status of whitefly; however, it can not be used to establish a critical RF at which field performance would decline. Given the high reliance on Admiral® for control of SLW in cotton and the high cost of this insecticide, a valuable addition to the current SLW resistance management project would be the development of a technique to correlate RF with field performance.

Many of the insecticides used in cotton for management of SLW are also used in horticulture production and to a lesser extent, grains. The development of an Area Wide Management strategy for insecticide usage and management of SLW would assist in reducing the selection pressure on SLW for resistance and may help to alleviate any ill-feeling that may be felt between stakeholders from the different cropping sectors.

The cotton industry would benefit from increased collaboration between research teams for purposes of biosecurity preparedness. Monitoring for high risk biosecurity threats, namely Cotton leaf curl disease (CLCuD) may be improved by combining routine molecular based diagnostics for whitefly species with virus diagnostics.

8. 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)

LLOYD, R., LUDGATE, Z., SHATTE, T., MURRAY, D. & MILES, M. (2008) Development of an insecticide resistance monitoring program and identification diagnostic for silverleaf whitefly. *2008 Cotton Conference.* Gold Coast, Australian Cotton Conference.

LLOYD, R. & LUDGATE, Z. (2008) Whitefly management in cotton. *Poster.* Toowoomba, DEEDI.

LUDGATE, Z. (2010) Three years of monitoring insecticide resistance to Silverleaf whitefly in cotton *2010 Australian Cotton Conference.* Gold Coast, Australian Cotton Conference.

MAAS, S., LUDGATE, Z., WILSON, L., DE BARRO, P. J., SEQUEIRA, S., MURRAY, D. & GRUNDY, P. (2009) Silverleaf whitefly - the IPM enforcer. *The Australian cottongrower,* 30**,** 14-17.

Please see the appendix for copies of these publications (pages 28-40).

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

Ten blog articles were submitted to the DEEDI beat sheet blog http://thebeatsheet.com.au/ on whitefly management, resistance monitoring, whitefly species identification and parasitism. Between August 2009 and August 2010 there were approximately 500 views of whitefly related blogs which increased 35 % from the previous financial year.

Ludgate, Z. May 2010. Season wrap-up for whitefly in cotton. The Beat Sheet Blog.

Ludgate, Z. February 2010. How to check for parasitism in whitefly populations. The Beat Sheet Blog.

Ludgate, Z. February 2010. Dealing with chemical shortages for whitefly management. The Beat Sheet Blog.

Ludgate, Z; Murray, D. April 2009. New whitefly found! The Beat Sheet Blog.

Ludgate, Z. February 2009. Silverleaf whitefly update. The Beat Sheet Blog.

Ludgate, Z. January 2009. Silverleaf whitefly in cotton – an update. The Beat Sheet Blog. The Beat Sheet Blog.

Murray, D. February 2008. Whitefly management options. The Beat Sheet Blog.

Murray, D. February 2008. Whitefly update. The Beat Sheet Blog.

Murray, D. February 2008. Whitefly in crops this season. The Beat Sheet Blog.

Murray, D. Ausgust 2007. St George growers meet to discuss area-wide SLW management. The Beat Sheet Blog.

A fact sheet was written for DEEDI website about the newly invaded Q biotype.

http://www.dpi.qld.gov.au/documents/PlantIndustries_FieldCropsAndPasture/Q_biotype_whitefly.pdf

And similar information was printed for the DEEDI website.

'What is Q-biotype whitefly?' http://www.dpi.qld.gov.au/26_13554.htm

Part 4 – Final Report Executive Summary

Silverleaf whitefly (SLW), *Bemisia tabaci B* biotype (Gennadius), is a major insect pest of cotton and horticultural industries. In cotton it is a pest because it produces sugary exudates (honeydew) that contaminate cotton lint and cause problems during textile processing. Honeydew contaminated lint may receive price penalties or in extreme cases may be rejected from sale.

Insecticides are an important SLW management tool, but SLW has the propensity to rapidly develop resistance to many insecticide groups including synthetic pyrethroids, organophosphates and carbamates. Pyriproxyfen (Admiral®) is currently considered the most important insecticide for SLW management because it has excellent efficacy against high density infestations. Overseas, resistance to Admiral® has been reported for SLW.

Resistance monitoring for Admiral® between 2007 and 2010 indicated there was no evidence of resistance to pyriproxyfen developing in cotton dominated regions. Resistance factors in cotton dominant regions were generally not significantly different to the susceptible strain. In 2007-08 elevated resistance factors were recorded for Silverleaf whitefly collected in cotton against pyriproxyfen in St George and the Burdekin. In St George, subsequent sampling in more recent years indicated that resistance factors had returned to a susceptible level and there was no evidence of resistance developing.

In the mixed cropping zone of the Burdekin, no pyriproxyfen usage occured in cotton as part of an area wide resistance management strategy to give priority products to the dominant cropping industry. It is likely that the elevated resistance factors recorded in the Burdekin from cotton are due to close proximity between cotton and horticulture.

Elevated resistance factors were recorded for pyriproxyfen for silverleaf whitefly collected from Ayr and Gumlu, North QLD in 2008-09. Resistance factors 100 times higher then the susceptible strain were recorded for these two field collections. These elevated resistance factors are a warning to the cotton industry of the risk of resistance developing to pyriproxyfen. The cotton Insecticide Resistance Management Strategy of a maximum of one application of Admiral® per season is a valuable insecticide resistance management tool.

Elevated resistance factors were recorded for diafenthiuron and bifenthrin between 2007-10. The increased resistance factors indicate that resistant genes are present in the populations however at this stage there is no evidence that the resistance factors have increased over the three years of monitoring. Overseas, diafenthiuron is thought to be stable to resistance however it has not be used widely due to phytotoxicity issues. Bifenthrin is not recommended for use in cotton due to poor efficacy and high toxicity to beneficial insects.

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APPENDIX

Silverleaf whitefly Resistance Testing 2007-2008

Insecticide	Collection ID	Location	Host plant	LC 50	Minimum fiducial limit	Maximum fiducial limit	LC 90	Min fiducial limits	Max fiducial limits	Slope	Heterogeneity	RF50	LC90 RF
Pyriproxyfen	**SU07-1**	**Indooroopilly**		**0.07**	**0.05**	**0.11**	**0.32**	**0.14**	**0.75**	**1.84**	**2.61**	**1**	**1**
	AY08-1	Ayr	Cotton	1.52	1.06	2.18	16.17	9.89	26.51	1.19	0.85	22	51
	DB08-1	Dalby	Cotton	0.041	0.023	0.071	0.1	0.042	0.24	3.1	1.21	1	1
	EM08-1	Emerald	Sunflower	0.039	0.015	0.1	0.07	0.016	0.35	4.5	3.45	1	1
	MR08-1	Moura	Cotton	0.036	0.004	0.32	0.09	0.0077	1.08	3.07	0.85	1	1
	#14	St George A	Cotton	0.012	0.005	0.031	0.024	0.0035	0.16	0.91	11.3	1	1
	SG08-1	St George B	Cotton	0.2	0.14	0.27	5.38	3.11	9.32	0.85	9.65	3	17
Bifenthrin	**SU07-1**	**Indooroopilly**		**3.19**	**2.25**	**4.54**	**19.06**	**11.78**	**30.83**	**1.57**	**0.71**	**1**	**1**
	EM08-1	Emerald	Sunflower	0.96	0.083	11.15	5.9	0.46	75.68	1.56	0.5059	1	1
	AY08-1	Ayr	Cotton	29.91	13.38	66.91	111.83	38.63	323.74	2.14	0.73	9	6
	DB08-1	Dalby	Cotton	2.28	0.07	73.09	15.46	0.69	345.61	1.47	2.09	1	1
	#13	Dalby	Cotton	12.03	3.79	38.21	168.63	55.23	514.89	1.07	0.22	4	9
Diafenthiuron	**SU07-1**	**Indooroopilly**		**18.41**	**13.91**	**24.37**	**41.42**	**27.61**	**62.14**	**3.48**	**1.54**	**1**	**1**
	DB08-1	Dalby	Cotton	6.78	4	11.47	19.67	8.61	44.89	2.65	0.67	1	1
	AY08-1	Ayr	Cotton	2.46	1.66	3.63	9.42	5.87	15.11	2.1	7.12	1	1
	MR08-1	Moura	Cotton	<10									
	EM08-1	Emerald	Sunflower	10.34	5.98	17.87	31.05	11.95	80.67	2.56	0.64	1	1
	SG08-1	St George	Cotton	14.04	2.93	67.38	30.64	2.17	433.1	3.62	0.34	1	1
	#14	St George	Cotton	43.89	29.28	65.8	194.94	121.6	312.51	1.89	7.12	2	5

LC_{50} – Lethal Concentration that kills 50% of the population (ppm)
LC_{90} - Lethal Concentration that kills 90% of the population (ppm)
RF_{50} - Resistance factor for 50% of the population. Calculated by dividing the LC50 of the field collection by the LC50 of the susceptible strain
RF_{90} - Resistance factor for 90% of the population. Calculated by dividing the LC90 of the field collection by the LC90 of the susceptible strain

Silverleaf whitefly Resistance Testing 2008-2009

Insecticide	Collection ID	Location	Host plant	LC50	Min fiducial limit	Max fiducial limit	LC90	Min fiducial limit	Max fiducial limit	Slope	Heterogeneity	RF50	RF90
Pyriproxyfen	**SU07-1**	**Indooroopilly**		**0.07**	**0.05**	**0.11**	**0.32**	**0.14**	**0.75**	**1.84**	**2.61**	**1**	**1**
	AY09-1S - Parent	Ayr	Melon	7.56	4.94	11.55	36.93	20.18	67.6	1.78	7.1	108	115
	AY09-1S - gen 1	Ayr	Melon	0.45	0.08	2.5	5.2	1.77	15.23	1.15	3.58	6	16
	AY09-1S - gen 2	Ayr	Melon	1.98	1.32	2.99	14.34	8.82	23.32	1.43	7.56	28	45
	AY09-1S - gen 3	Ayr	Melon	0.52	0.27	0.1	3.23	1.78	5.89	1.54	15.43	7	10
	AY09-1S - gen 4	Ayr	Melon	1.19	0.58	2.45	14.65	5.62	38.19	1.12	2.53	17	46
	AY09-1S - gen 5	Ayr	Melon	1.47	1.1	1.97	8.91	5.47	14.52	1.57	2.98	21	28
	AY09-1S - gen 6	Ayr	Melon	0.8	0.65	1.01	4.49	3.27	4.49	1.64	6.68	11	14
	AY09-1S - gen 7	Ayr	Melon	3.6	2.35	5.53	32.27	17.19	60.57	1.28	1.8	51	101
	AY09-1S - gen 8	Ayr	Melon	1.64	1.23	2.2	12.27	7.7	19.55	1.4	7.81	23	38
	AY09-1R - gen 3	Ayr	Melon	6.3	4.57	8.68	36.08	25.91	50.23	1.62	2.18	90	113
	AY09-1R - gen 4	Ayr	Melon	28.6	20.34	40.22	141.82	99.34	202.53	1.76	6.9087	409	443
	AY09-1R - gen 5	Ayr	Melon	23.22	18.11	29.79	114.19	83.45	156.24	1.77	8.84	332	357
	BL09-1	Biloela	Cotton	0.01	0.009	0.016	0.034	0.023	0.048	2.8	1.59	1	1
	DB09-1	Dalby	Cotton	0.06	0.039	0.1	0.27	0.13	0.56	1.93	0.36	1	1
	EM09-1	Emerald	weeds	0.025	0.012	0.053	0.047	0.016	0.14	4.53	0.28	1	1
	GL09-1	Gumlu	Melon	12.45	3.46	44.81	40.15	5.77	279.42	2.4		178	125
	ME09-1	Moree	Cotton	0.028	0.024	0.033	0.056	0.047	0.067	4.06	15.22	1	1
	NG09-1	Namoi/Gwydir	Cotton	0.017	0.011	0.025	0.044	0.026	0.075	2.98	13.31	1	1
	NM09-1	Namoi valley	Soybean	0.008	0.002	0.03	0.036	0.002	0.031	1.88	9.23	1	1
	NM09-2	Namoi valley	Cotton	0.046	0.028	0.074	0.13	0.056	0.32	2.64	2.01	1	1
	NM09-4	Namoi valley	Cotton	0.08	0.07	0.12	0.43	0.25	0.74	1.77	18.4	1	1
	SG09-1	St George	Cotton	0.063	0.04	0.097	0.73	0.45	1.19	1.15	7.73	1	2
	SG09-2	St George	Cotton	0.08	0.05	0.13	1.13	0.57	2.24	1	3.17	1	4
	SG09-3	St George	Cotton	0.086	0.06	0.12	0.74	0.44	1.25	1.3	11.12	1	2
	TH09-1	Theodore	Cotton	0.007	0.0033	0.018	0.027	0.0083	0.087	2.26	0.44	1	1
Bifenthrin	**SU07-1**	**Indooroopilly**		**3.19**	**2.25**	**4.54**	**19.06**	**11.78**	**30.83**	**1.57**	**0.71**	**1**	**1**
	BL09-1	Biloela	Cotton	6.65	3.23	13.6	48.77	14.95	159.13	1.42	2.28	2	3
	DB09-1	Dalby	Cotton	6.22	3.96	9.77	30.18	16.67	54.62	1.79	3.03	2	2
	EM09-1	Emerald	weeds	2.42	0.31	19.09	12.53	1	156.82	1.72	0.11	1	1
	ME09-1	Moree	Cotton	4.69	2.85	7.72	16.26	10.03	26.36	2.27	10.65	1	1
	NM09-1	Namoi valley	Soybean	7.01	4.92	9.99	30.43	18.09	51.16	1.92	0.34	2	2
	NM09-3	Namoi valley	Cotton	8.26	5.58	12.21	41.35	20.94	81.64	1.75	1.9	3	2
	SG09-1	St George	Cotton	4.95	2.37	10.35	32.38	14.46	72.51	1.5	3.59	2	2
	SG09-2	St George	Cotton	17.3	7.94	37.71	138.12	43.23	441.29	1.36	4.48	5	7
	SG09-3	St George	Cotton	7.94	5.36	11.75	25.67	15.39	42.81	2.4	1.87	2	1
	TH09-1	Theodore	Cotton	3.25	1.41	7.53	18.01	5.17	62.73	1.65	0.53	1	1

Silverleaf whitefly Resistance Testing 2008-2009 (continued)

Insecticide	Collection ID	Location	Host plant	LC50	Min fiducial limit	Max fiducial limit	LC90	Min fiducial limit	Max fiducial limit	Slope	Heterogeneity	RF50	RF90
Diafenthiuron	**SU07-1**	**Indooroopilly**		**18.41**	**13.91**	**24.37**	**41.42**	**27.61**	**62.14**	**3.48**	**1.54**	**1**	**1**
	BL09-1	Biloela	Cotton	18.25	11.58	28.76	57.22	23.9	136.98	2.47	5.53	1	1
	DB09-1	Dalby	Cotton	9.56	1.5	61	26.16	1.72	398.8	2.8	0.008	1	1
	EM09-1	Emerald	weeds	19.33	7.19	51.91	35.93	11.35	113.73	4.55	0.68	1	1
	ME09-1	Moree	Cotton	35.18	26.96	45.92	79.13	48.06	130.29	3.48	8.08	2	2
	NG09-1	Namoi/Gwydir	Cotton	7.17	4.71	10.03	18.24	9.89	33.64	3.02	0.92	1	1
	NM09-1	Namoi valley	Soybean	12.01	2.31	62.44	29.8	1.7	522.2	3.1	0.01	1	1
	NM09-2	Namoi valley	Cotton	24.05	20.27	28.54	40.35	30.95	52.59	5.45	1.72	1	1
	NM09-3	Namoi valley	Cotton	14.48	9.59	21.85	39.2	21.16	72.62	2.83	3.06	1	1
	SG09-1	St George	Cotton	9.12	2.3	36.25	24.87	3.27	188.91	2.81	0.005	1	1
	SG09-2	St George	Cotton	19.45	14.12	26.79	53.01	32.63	86.08	2.81	3.49	1	1
	SG09-3	St George	Cotton	8.64	1.92	38.9	28.55	5.03	161.94	2.36	12.36	1	1
	TH09-1	Theodore	Cotton	1.18	0.35	4	2.59	0.6	11.24	3.58	1.03	1	1
Spirotetramat	BL09-1	Biloela	Cotton	11.9	8.27	17.13	64.72	38.97	107.47	1.66	5.77		
	BL09-1	Biloela	Cotton	4.09	0.42	39.4	14.19	4.82	41.78	2.26	1.75		
	BL09-1	Biloela	Cotton	3.12	2.29	4.24	8.69	5.74	13.17	2.75	7.35		
	ME09-1	Moree	Cotton	4.99	3.46	7.21	17.48	11.27	27.14	2.25	3		
	NM09-4	Namoi valley	Cotton	13.13	7.36	23.4	148.64	76.11	290.26	1.16	13.85		
	SG09-3	St George	Cotton	4.72	1.82	12.25	8.1	2.79	23.58	5.22	7		

LC_{50} – Lethal Concentration that kills 50% of the population (ppm)
LC_{90} - Lethal Concentration that kills 90% of the population (ppm)
RF_{50} - Resistance factor for 50% of the population. Calculated by dividing the LC50 of the field collection by the LC50 of the susceptible strain
RF_{90} - Resistance factor for 90% of the population. Calculated by dividing the LC90 of the field collection by the LC90 of the susceptible strain

Silverleaf whitefly Resistance Testing 2009-2010

Insecticide	Collection ID	Location	Host plant	LC50	Min fiducial limit	Max fiducial limit	LC90	Min fiducial limit	Max fiducial limit	Slope	Heterogeneity	RF50	RF90
Pyriproxyfen	**SU07-1**	**Indooroopilly**		**0.07**	**0.05**	**0.11**	**0.32**	**0.14**	**0.75**	**1.84**	**2.61**	**1**	**1**
	BL10-1	Biloela	Cotton	0.11	0.09	0.14	0.49	0.35	0.69	1.88	38.59	2	2
	DD10-1	Condamine	Cotton	0.044	0.0036	0.55	0.18	0.05	0.18	2.0	1.56	1	1
	DD10-2	Norwin	Cotton	0.026	0.015	0.047	0.17	0.086	0.33	1.52	21.52	1	1
	DD10-3	Brigalow	Cotton	0.018	0.011	0.028	0.27	0.16	0.45	1.04	17.24	1	1
	EM10-1	Emerald	Cotton	0.04	0.0037	0.45	0.14	0.045	0.43	2.28	1.53	1	1
	EM10-2	Emerald	Cotton	0.13	0.08	0.21	0.87	0.45	1.69	1.5	15.64	2	3
	EM10-4	Comet	Cotton	0.00026	0.000000028	2.45	0.011	0.00034	0.35	0.76	4.92	1	1
	ME10-1	Moree	Cotton	0.0089	0.005	0.016	0.04	0.023	0.069	1.88	6.15	1	1
	SG10-1	St George	Cotton	0.1	0.065	0.16	1.17	0.6	2.3	1.16	2.24	1	4
	WB10-1	Warburn	Melon	0.48	0.15	1.53	2.04	0.45	9.29	1.95	0.032	7	6
Bifenthrin	**SU07-1**	**Indooroopilly**		**3.19**	**2.25**	**4.54**	**19.06**	**11.78**	**30.83**	**1.57**	**0.71**	**1**	**1**
	BL10-1	Biloela	Cotton	21.77	14.24	33.28	204.47	85.69	487.93	1.26	3.31	7	11
	BL10-1	Biloela	Cotton	5.76	3.77	8.81	30.64	19.44	48.27	1.69	0.21	2	2
	DD10-1	Darling Downs	Cotton	6.52	3.92	10.86	33.7	17.48	64.99	1.72	1.48	2	2
	DD10-2	Darling Downs	Cotton	7.41	2.76	19.91	153.87	24.27	975.36	0.93	1.33	2	8
	DD10-3	Darling Downs	Cotton	6.63	4.87	9	31.13	21.67	44.71	1.82	0.27	2	2
	EM10-1	Emerald	Cotton	17.76	12.118	26.0286	75.02	40.08	140.4	1.96	0.025	6	4
	EM10-4	Emerald	Cotton	5.42	3.36	8.76	57.88	28.92	115.83	1.19	5.72	2	3
	SG10-1	St George	Cotton	9.22	1.36	62.46	49	0.41	5865.97	1.68	9.33	3	3
	WB10-1	Warburn	Melon	21.35	16.16	28.2	77.44	53.51	112.07	2.19	4.34	7	4
Diafenthiuron	**SU07-1**	**Indooroopilly**		**18.41**	**13.91**	**24.37**	**41.42**	**27.61**	**62.14**	**3.48**	**1.54**	**1**	**1**
	BL10-1	Biloela	Cotton	49.24	16.22	149.47	124	21.05	730.36	3.05	2.21	3	3
	DD10-1	Darling Downs	Cotton	23.79	17.91	31.6	70.43	47.86	103.68	2.6	4.75	1	2
	DD10-2	Darling Downs	Cotton	44.49	7.79	253.97	193.23	18.62	2004.93	1.92	5.36	2	5
	DD10-3	Darling Downs	Cotton	94.25	67.99	130.78	321.54	197.16	524.41	2.3	10.96	5	8
	EM10-1	Emerald	Cotton	23.52	2.38	232.78	67.9	3.21	1437.02	2.66	13.16	1	2
	EM10-2	Emerald	Cotton	21.64	15.13	30.97	59.31	35.57	98.9	2.8	3.93	1	1
	EM10-4	Emerald	Cotton	24.14	16.73	34.82	67.7	37.32	122.81	2.74	3.98	1	2
	ME10-1	Moree	Cotton	10.98	8.13	14.83	35.29	23.21	53.66	2.42	4.97	1	1
	SG10-1	St George	Cotton	77.66	34.85	173.06	325.43	90.89	1165.22	1.97	16.86	4	8
	WB10-1	Warburn	Melon	45.42	33.92	60.82	104.02	69.19	156.39	3.4	10.06	2	3
Movento	**BL10-1**	**Biloela**	**Cotton**	**5.79**	**4.25**	**7.89**	**14.71**	**9.11**	**23.77**	**3.03**	**2.89**		
	DD10-3	Darling Downs	Cotton	6.4	5.63	7.29	14.11	11.93	16.67	3.57	10.28		
	EM10-1	Emerald	Cotton	5.2	3.7	7.29	11.19	7.21	17.34	3.68	5.95		
	EM10-4	Emerald	Cotton	3.98	1.87	8.48	11.89	3.18	44.4	2.58	68.97		

LC_{50} – Lethal Concentration that kills 50% of the population (ppm)
LC_{90} - Lethal Concentration that kills 90% of the population (ppm)
RF_{50} - Resistance factor for 50% of the population. Calculated by dividing the LC50 of the field collection by the LC50 of the susceptible strain
RF_{90} - Resistance factor for 90% of the population. Calculated by dividing the LC90 of the field collection by the LC90 of the susceptible strain

Development of an insecticide resistance monitoring program and identification diagnostic for silverleaf whitefly

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Introduction

The silverleaf whitefly (SLW), *Bemisia tabaci* (Gennadius) biotype B, was first recorded in Australia in 1994 (Gunning *et al.* 1995) and is a serious pest of cotton and horticultural crops, both in Australia and overseas (Oliveira *et al.* 2001). Its pest status in cotton is due to its ability to rapidly develop resistance to insecticides and secretion of honeydew resulting in sticky cotton which would seriously jeopardise marketing of Australian cotton and compromise Australian's reputation as a high quality fibre producer. It is also a vector of cotton leaf curl virus, an exotic disease that poses a potentially serious threat to the Australian cotton industry.

The Australian native *B. tabaci* (ANB) and the greenhouse whitefly (GHW) *Trialeurodes vaporariorum* (Westwood) are susceptible to all currently used insecticides whereas SLW is highly resistant to several insecticide classes. Australian native *B. tabaci* and SLW cannot be distinguished by morphological characteristics. Therefore, a diagnostic test using DNA markers is necessary to distinguish the different biotypes. Accurate identification is important if effective control is to be achieved by choosing the appropriate insecticide for the target whitefly species.

Pyriproxyfen (Admiral®), diafenthiuron (Pegasus®) and bifenthrin (Talstar®) are currently registered for whitefly control in cotton. Monitoring the resistance status of these insecticides in controlling SLW is critical if their usefulness is to be sustained. Resistance monitoring is also important in evaluating the relevance of the Insecticide Resistance Management Strategy (IRMS) guidelines and providing input into developing solutions for looming control issues before they impact on grower productivity. This paper outlines our initial course of action and preliminary results in developing a resistance monitoring program and whitefly identification service.

Materials and Methods

Whitefly collections

Whitefly samples were obtained from all the tropical and warm temperate cotton growing districts of Queensland, namely the Central Highlands, St. George, Dawson-Callide, Darling Downs and Burdekin where SLW is deemed to be a major pest. Samples were either collected as adults using a portable motorised aspirator and transferred directly onto a caged, potted cotton plant or as red-eyed nymphs on leaves and transferred to the Toowoomba laboratory within 24 h where they were placed

in an emergence cage with a potted cotton plant. Caged samples were maintained in a glasshouse at ambient temperature. A reference susceptible colony was obtained from CSIRO at Indooroopilly in 2007 and maintained on hibiscus, *Hibiscus rosa-sinensis*. This source colony was started in 1995 with small injections of new stock, the last being in 2004 and had only been exposed to malathion once in the previous two years to control the parasitoid, *Eretmocerus hayati* Zolnerowich and Rose.

Bioassays

The primary focus of the project in the first year has been handling and rearing whiteflies, refining bioassay techniques and initial range-finding tests. Where possible, bioassays were adapted from the Insecticide Resistance Action Committee (IRAC) susceptibility test methods series - version: 2 (http://www.irac-online.org/).

The bioassay protocol for pyriproxyfen involved confining 10 mated female adults in clip cages on leaves of 5-7 node cotton plants (Sicot 71) for 24 h to lay eggs. Five leaves (>20 eggs/leaf) per treatment were then dipped for 20 sec in a serial dilution emulsion. Individual leaves were maintained in 20 mL glass scintillation vials containing 1.5% agar and topped up with a weak soluble fertiliser solution. Bioassays were held in these vials for 10 days when egg hatch was assessed.

A leaf disc bioassay technique was used for both diafenthiuron and bifenthrin. This involved dipping five 38 mm leaf discs per treatment for 20 sec in a serial dilution emulsion. After drying, leaf discs were then placed on a 1.5% agar bed in a ventilated plastic Petri dish. Twenty female whiteflies (< 2 days old) were confined in each dish for 48 h (bifenthrin) and 72 h (diafenthiuron) after which time mortality was assessed. Diafenthiuron requires a longer time span as it is a pro-insecticide, which has first to be converted to its active form in the insect.

A minimum of five concentrations were used in all bioassays and were held at 27 ± 1°C, 50 ± 5% RH and photoperiod of 16:8 (L:D) h until assessment. Mortality data from all dose response bioassays were analysed using Probit 5 for Windows (Gillespie 1995). Probit regressions were calculated and LC_{50} values estimated.

Biotype identifications and parasitism monitoring

Field collections were initially examined under a microscope to segregate and quantify the proportion of *Bemisia* biotypes and GHW in the samples as well as percentage parasitism of the nymphs. A randomly amplified polymorphic DNA polymerase chain reaction (RAPD PCR) test based on optimising techniques described by De Barro & Driver (1997) was used to determine the biotype identifications in the samples. Molecular markers were used to identify minute differences in the genetic code of the whitefly biotypes.

Results and Discussion

Resistance monitoring

Toxicity data for the SLW strains tested to date against pyriproxyfen, diafenthiuron and bifenthrin in 2007-08 are shown in Table 1.

Table 1. Dose responses of several strains of SLW to three insecticides in comparison to the susceptible laboratory strain.

Insecticide	Strain	LC_{50} (ppm)	95% Confidence Limits	Slope	Resistance Factor #
Pyriproxyfen	Susceptible	0.023	0.001-0.44	1.47	-
	St. George A	0.043	0.01-0.13	0.091	1.9
	St. George B	0.78	0.47-1.30	0.84	33.9
	Dalby	0.012	0.025-0.059	1.37	0.5
	Ayr	1.52	1.06-2.18	1.19	66.1
	Moura	0.036	0.004-0.32	3.07	1.6
	Emerald*	0.039	0.015-0.104	4.51	1.6
Diafenthiuron	Susceptible	17.02	11.94-24.3	2.52	-
	St. George	43.9	29.3-65.8	1.89	2.6
Bifenthrin	Susceptible	13.01	9.53-17.78	1.99	-
	Dalby	12.03	3.79-38.21	1.07	0.9

* off late season sunflower in the Emerald Irrigation Area

LC_{50} of tested strain/LC_{50} of susceptible strain for the same insecticide

Preliminary data for the 2007-08 season indicate no major increases in the resistance levels in any of the populations tested when compared to the susceptible strain. However, there are early signs that resistant SLW individuals are present in some populations. The flat slopes in dose response assays are evidence of populations being made up of both susceptible and resistant individuals. This result demonstrates that there is potential to select for resistance through poor management of insecticides, particularly repeat applications of any product during the season. An area-wide approach to SLW insecticide resistance management is extremely important to ensure the long-term efficacy of available chemistry. Where cotton is grown alongside horticultural and grain crops, this will necessitate a collaborative cross-industry management strategy.

Biotype Identifications and parasitism levels

Results from the RAPD PCR diagnostics show that SLW predominates in the hotter regions (Central Qld, Burdekin and St. George). In the more temperate region of the Darling Downs, GHW, ANB and SLW were all present. Samples from the hotter areas west of Dalby were mainly SLW, and the cooler areas of the eastern and southern Downs were mainly GHW. A sample from the Namoi Valley near Narrabri was predominately GHW with low numbers of ANB and SLW. An example of a typical RAPD PCR profile depicting the different banding patterns for GHW, ANB and SLW is shown in Figure 1.

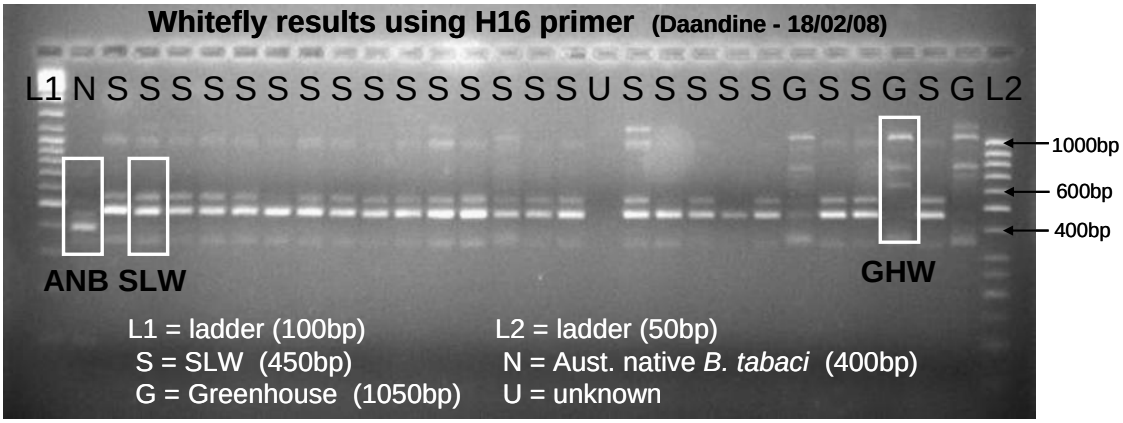

Figure 1. Gel image showing the base pair banding used to differentiate the different whitefly species and biotypes.

Very low levels of parasitoid activity were detected from all samples processed despite reports of field parasitism levels of up to 60% in some regions.

Acknowledgments

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THREE YEARS OF MONITORING INSECTICIDE RESISTANCE TO SILVERLEAF WHITEFLY IN COTTON

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KEY POINTS

- Bioassays developed and extensive screening undertaken between 2007-10
- No changes in resistance factors in cotton from 2007 to 2010
- Elevated resistance factors recorded in horticultural regions for pyriproxyfen (Admiral®) emphasise the importance of the cotton IRMS and a maximum one application Admiral® per season
- No changes recommended to current insecticide resistance management practices in cotton
- Registration of spirotetramat (Movento®) in the 2010-11 season with a unique mode of action will assist the insecticide resistance management strategy in cotton
- Future directions in resistance monitoring need to address correlating resistance factors with field performance

INTRODUCTION

Silverleaf whitefly (SLW), *Bemisia tabaci* (Gennadius), B biotype is an annual insect pest in cotton in the warmer production areas of Queensland and northern New South Wales. Silverleaf whitefly contaminate cotton lint with sugary exudates (honeydew) which reduces fibre quality and increases the cleaning frequency in spinning mills. Management of this pest is complicated by SLW's propensity to develop resistance to many insecticides. This has put considerable strain on the limited number of insecticides that remain efficacious against SLW. The registered SLW insecticides have been monitored over the past three cotton seasons (2007-10) for changes in resistance factors. The results of the resistance monitoring program are documented in this paper.

METHODOLOGY

A 'susceptible' strain of SLW (SU07-1) was obtained from CSIRO, Indooroopilly in 2007 where it had been in culture since 1995. The SU07-1 strain is not a true susceptible strain as it has had prior exposure to or risk of exposure to insecticides. It is believed that SLW entered Australia already possessing resistance to insecticides (Gunning et al., 1995). Further, the

SU07-1 strain has been exposed to gene flow from the addition of wild type SLW of unknown origin prior to 2005 and was exposed to malathion once prior to 2007.
Field collections of whitefly were taken from cotton in QLD and northern NSW. *Bemisia tabaci* biotype identification was determined using a molecular based microsatellite technique using primers Bem23F and Bem23R to distinguish between Australian native, SLW and Q biotype.

For pyriproxyfen bioassays, adults were allowed to oviposit for 16 hours on excised cotton leaves. Leaves were dipped in a serial dilution of insecticide containing Agral® (600g/L) surfactant and maintained under controlled conditions at 27°C and 60% humidity for ten days to allow egg hatch. Treatments were scored for mortality (measured as failure of eggs to hatch).

For bifenthrin (Talstar®) and diafenthiuron (Pegasus®) assays, leaves were dipped in a serial dilution of insecticide containing Agral® (600g/L) surfactant. Adult SLW were aspirated into clip cages on treated leaves and maintained under controlled conditions for two and three days for the bifenthrin and diafenthiuron assays respectively. Bioassays were scored for mortality (measured as an absence of any movement of adults after tapping the cage lightly against the bench top).

Results were analysed using Probit 5 for Windows (Gillespie, 1995) and correcting for control mortality using Abbott's formula. Resistance factors (RF) were calculated by dividing the lethal concentration (LC) for 50 percent and 90 percent mortality of field strains by the corresponding LC value for the susceptible strain (SU07-1). Strain responses were considered significantly different if the fiducial limits did not overlap.

RESULTS AND DISCUSSION

Pyriproxyfen (Admiral®)

Between 2007 and 2010, most field collections of SLW were susceptible to pyriproxyfen (table 1). In 2007-08 elevated RF were recorded in Ayr in the Burdekin (22 fold higher then the susceptible strain) and at St George (3 fold higher then the susceptible strain) from SLW collected off cotton. In 2008-09, resistance was detected at Ayr (108 fold higher then the susceptible strain) and Gumlu (178 fold higher then the susceptible strain) from SLW

collected off melons. And in 2009-10, resistance was detected at Warburn (7 fold higher then the susceptible strain) for SLW collected off rockmelon and honeydew.

The results indicate that resistance to pyriproxyfen in SLW is not an immediate concern in cotton at this stage. The elevated resistance levels recorded in cotton from St George in 2007-08 were not recorded in subsequent years with resistance factors declining to a susceptible level between 2008-10. Elevated RF detected in cotton in the Burdekin may have been a result of close proximity between cotton and horticulture production as pyriproxyfen was not used in cotton in the Burdekin.

The high RF recorded for SLW in horticulture regions indicate that there was a high proportion of resistant genes in the population, and with continual selection pressure, resistance to pyriproxyfen is likely to result in reduced field performance or field failure. In cotton-dominant areas, high RF in horticulture is likely to be diluted by the low RF in cotton and should help to conserve the efficacy of these products.

Diafenthiuron (Pegasus®)

Between 2007-10 most field collections of SLW were susceptible (table 2). In 2007-08, SLW from St George showed elevated resistance factors (2 fold higher then the susceptible strain). In 2008-09, SLW from Moree (ME09-1) had elevated resistance factors (2 fold higher then the susceptible strain). In 2009-10, a collection from St George (SG10-1) was 4 fold more resistant then the susceptible strain.

The elevated RF indicate that resistance genes are present in the population. That said, there has been no marked shift in RF since monitoring commenced in 2007. Furthermore, the elevated RF may be attributed to natural variability as no true baseline data was collected prior to registration of diafenthiuron in Australia. At this stage, there are no recommended changes to the insecticide resistance management strategy (IRMS).

Bifenthrin (Talstar®)

Between 2007-10, several field collections exhibited higher tolerance to bifenthrin then the susceptible strain (table 3). In 2007-08, field collections from Dalby (#13) and Ayr were 4 and 9 fold more resistant then the susceptible strain. In 2008-09, field collections from the lower Namoi (NM09-1 and NM09-3) and St George (SG09-2 and SG09-3) were 2, 3, 5 and 2 fold higher then the susceptible strain, respectively. In 2009-10, field collections from Biloela,

Darling Downs (DD10-3), Emerald (EM10-1) and Warburn were more resistant then the susceptible strain (7, 2, 6, and 7 fold more resistant then the susceptible strain respectively).

While RF were elevated compared to the susceptible strain, they did not increase markedly over the three years of monitoring. Because of the limitations in correlating resistance factors to field performance, it is difficult to say if the observed RF are high enough to cause reduced field performance. Personal communication with growers and consultants indicates that multiple applications of bifenthrin within a season tend to be associated with reduced performance which would indicate that resistance is at a level that is affecting field performance. It is not recommended that bifenthrin is used as a 'first line of defence', primarily due to its disruptiveness to beneficial insects and also because of concerns of resistance developing.

Future Directions

Resistance monitoring for SLW in cotton will continue through until at least 2013 with joint funding from DEEDI and CRDC. The suite of insecticides tested will be extended to include new insecticides as they are registered in cotton. Testing methodologies have been developed and baseline data has been generated for spirotetramat (Movento®) which is scheduled for registration in cotton in 2010-11. The registration of new insecticides with unique modes of action will greatly assist the IRMS by reducing the pressure placed on a limited number of insecticides for management of SLW.

Future directions in resistance monitoring require studies in correlating RF with field performance. Resistance monitoring, as it currently stands, can provide information on the changing status of RF however, it can not be used to predict when performance of an insecticide will decline in the field. Study of SLW populations in the field, pre- and post-insecticide spray combined with studies of SLW genotypes would complement existing resistance monitoring to develop a better IRMS for cotton.

SUMMARY

Three years of resistance monitoring from 2007-10 have been conducted for insecticides registered for SLW control in cotton. Elevated RF were recorded in some field collections for every insecticide tested, indicating that resistance genes are present in the population. While elevated RF were recorded in diafenthiuron and bifenthrin, there was no marked shift in RF over the three years of monitoring and there are no immediate concerns of resistance developing to these products. Elevated RF for pyriproxyfen were recorded in cotton and

horticulture in the Burdekin between 2008-09 and there is concern that this product will lose efficacy against SLW under the current insecticide use regime in horticulture in the Burdekin. In other regions, RF for pyriproxyfen were at susceptible levels and there are no immediate concerns of resistance developing to this product in cotton dominant regions. No changes to the IRMS are recommended for any products registered in cotton for SLW control at this time.

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KEY WORDS

Bemisia tabaci. Silverleaf whitefly. Resistance monitoring.

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APPENDIX

Table 1. Resistance factors (RF) for pyriproxyfen for SLW from 2007-10

Year	Location	Host Plant	Resistant*
2007-08	Ayr	Cotton	✓(RF 22)
	Dalby	Cotton	×
	Emerald	Sunflower	×
	Moura	Cotton	×
	St George A	Cotton	×
	St George B	Cotton	✓ (RF 3)
2008-09	Ayr	Melon	✓ (RF 108)
	Biloela	Cotton	×
	Dalby	Cotton	×
	Emerald	weeds	×
	Gumlu	Melon	✓ (RF 178)
	Moree	Cotton	×
	Namoi/Gwydir	Cotton	×
	Namoi valley (NM09-1)	Soybean	×
	Namoi valley (NM09-2)	Cotton	×
	Namoi valley (NM09-4)	Cotton	×
	St George (SG09-1)	Cotton	×
	St George (SG09-2)	Cotton	×
	St George (SG09-3)	Cotton	×
	Theodore	Cotton	×
2009-10	Biloela	cotton	×
	Condamine	cotton	×
	Norwin	cotton	×
	Brigalow	cotton	×
	Emerald (EM10-1)	cotton	×
	Emerald (EM10-2)	cotton	×
	Comet	cotton	×
	Moree	cotton	×
	St George	cotton	×
	Warburn	rockmelon & honeydew	✓ (RF 7)

* ✓ indicates resistance factors are significantly different to the susceptible strain (fiducial limits do not overlap)

Table 2. Resistance factors (RF) for diafenthiuron for SLW from 2007-10

Year	Location	Host plant	Resistant
2007-08	Dalby	Cotton	✗
	Ayr	Cotton	✗
	Moura	Cotton	✗
	Emerald	Sunflower	✗
	St George (SG08-1)	Cotton	✗
	St George (#14)	Cotton	✓ (RF 2)
2008-09	Biloela	Cotton	✗
	Dalby	Cotton	✗
	Emerald	Weeds - various	✗
	Moree	Cotton	✓ (RF 2)
	Namoi/Gwydir	Cotton	✗
	Namoi valley (NM09-1)	Soybean	✗
	Namoi valley (NM09-2)	Cotton	✗
	Namoi valley (NM09-3)	Cotton	✗
	St George (SG09-1)	Cotton	✗
	St George (SG09-2)	Cotton	✗
	St George (SG09-3)	Cotton	✗
	Theodore	Cotton	✗
2009-10	Biloela	Cotton	✗
	Darling Downs (DD10-1)	Cotton	✗
	Darling Downs (DD10-2)	Cotton	✗
	Darling Downs (DD10-3)	Cotton	✗
	Emerald (EM10-1)	Cotton	✗
	Emerald (EM10-2)	Cotton	✗
	Emerald (EM10-4)	Cotton	✗
	Moree	Cotton	✗
	St George	Cotton	✓ (RF 4)
	Warburn	Melon	✗

* ✓ indicates resistance factors are significantly different to the susceptible strain (fiducial limits do not overlap)

Table 3. Resistance factors (RF) for bifenthrin for SLW from 2007-10

Year	Location	Host plant	Resistant
2007-08	Emerald	Sunflower	×
	Ayr	Cotton	✓ (RF 9)
	Dalby (DB08-1)	Cotton	×
	Dalby (#13)	Cotton	✓ (RF 4)
2008-09	Biloela	Cotton	×
	Dalby	Cotton	×
	Emerald	Weeds - various	×
	Moree	Cotton	×
	Namoi valley (NM09-1)	Soybean	✓ (RF 2)
	Namoi valley (NM09-3)	Cotton	✓ (RF 3)
	St George (SG09-1)	Cotton	×
	St George (SG09-2)	Cotton	✓ (RF 5)
	St George (SG09-3)	Cotton	✓ (RF 2)
	Theodore	Cotton	×
2009-10	Biloela	Cotton	✓ (RF 7)
	Darling Downs (DD10-1)	Cotton	×
	Darling Downs (DD10-2)	Cotton	×
	Darling Downs (DD10-3)	Cotton	✓ (RF 2)
	Emerald (EM10-1)	Cotton	✓ (RF 6)
	Emerald (EM10-4)	Cotton	×
	St George	Cotton	×
	Warburn	Rockmelon, honeydew	✓ (RF 7)

*** ✓ indicates resistance factors are significantly different to the susceptible strain (fiducial limits do not overlap)**

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